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THE NUTRITIONAL & BIOLOGICAL SIGNIFICANCE OF SAPONINS

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ABSTRACT

The association between saponin intake and cholesterol metabolism is reviewed in this article. Consumption of foods with high saponin concentrations by humans and its potential danger, is also discussed. Saponins have been shown to demonstrate hemolytic action towards red blood cells, and can be toxic if given intravenously but not orally. The majority of saponins form insoluble complexes with 3-β-hydroxysteroids and are known to interact with and form large ixed micelles with bile acids and cholesterol. Thus in rats, saponin intake has been correlated with a hypocholesterolaemic effect by some authors, whilst others have disputed this claim. Saponins are also known to form insoluble saponin-mineral complexes, with iron, zinc and calcium. The beneficial effects on plasma cholesterol may be somewhat speculative in humans. Intakes up to 500 mg/day have not had particularly marked effects on plasma cholesterol although alteration to the enterohepatic circulation of bile acids and neutral sterol excretion may be of benefit. Part of the reasons for these minimal effects may be the lack of accurate characterisation of "*saponins*" from various sources. The potential hazards related to mineral complexing is an under-researched area and with the recent development of a synthetic saponin for use as an oral hypocholesterolaemic agent the risk/benefits need to be carefully analysed. Research needs to be undertaken to characterise dietary sources so that their potential usefulness can be better determined.

KEY WORDS: Saponins, Hypocholesterolaemia, Alfalfa, Medicagenic acid, Bile acids, Neutral sterols.

INTRODUCTION

Saponins are naturally occurring chemical compounds found in a wide variety of food, forage plants and a few marine animals. They exhibit characteristics such as a strong

foaming power in aqueous solutions, from which the name saponin was derived. They also demonstrate hemolytic action towards red blood cells, and can be toxic if given intravenously (1). Their deadly effect on cold blooded animals, especially fish and snails is well documented in Australian history, due to their use as fish poisons, by Aboriginal cultures (1). They also inhibit the growth of mould and their presence in the shoots and bark of trees, reinforces the view that they help protect the plant from insect attack (2). The majority of saponins form insoluble complexes with 3-β-hydroxysteroids and are known to interact with and form large mixed micelles with bile acids and cholesterol (1). Saponins exist in reasonably high quantities in many food plants (1), and due to the increased popularity of bean and legume sprout/shoots in the diet, their biological and nutritional effects are the subject of this review.

Ruminant Bloat

Research during the 1950's and 60's focused on the anti-nutritional properties of saponins, such as growth impairment and ruminant bloat. During these years soyabeans (*Glycine max*) and lucerne (*Medicago sativa)* also called alfalfa, were used as stock fodder and work by Lindahl *et al* (3) established a connection between saponins and ruminant bloat. Two mechanisms were noted, a physiological effect on gut contractibility and a reduction in surface tension. In some cases, bloat symptoms in sheep resulted from the physiological activity of alfalfa saponins, whilst in others, the surface tension properties of saponins were primarily instrumental. Both legume press juices and alfalfa saponins reduced ruminal motility with the reduction in the strength of contractions of the reticulum and reticulo-ruminal fold contributing to difficulty in clearing the cardia of frothy ingesta. The reduction in surface tension which causes froth formation, interferes with eructation, allowing the build up of rumen gas and eventual death of the animal. Administration of alfalfa saponins were found to be 50 to 60 times more toxic intravenously, than when given orally (3). Results from recent work, show that saponins constitute only one of a combination of additional factors in the pathogenesis of pasture bloat (1).

Growth & Cholesterol Binding

In 1960 Coulson & Evans (4) (Table 1), investigated *Quillaja* saponin and saponin white from *Gypsophila spp* and found both depressed weight gain in rats, but the addition of cholesterol to the diet reversed this effect. They suggested that this may be due to the formation of insoluble non-absorbable complexes of saponins with 3-β-hydroxysteroids. Substitution of β-sitosterol for cholesterol in the diet of rats (4) and chicks (5), did not prevent the weight loss, confirming the necessity of the presence of cholesterol. Not all saponin preparations act alike and there may be species specificity. Coulson & Evans (4) noted that much higher concentrations of saponins were required to produce the effect in rats, compared to chickens. Ishaaya et al (6) showed that, soyabean saponins were not as effective on growth impairment in chicks even at higher concentrations, whereas the detrimental effect of lucerne saponins rose with increasing saponin concentration in the diet, and could be reversed by dietary cholesterol supplementation (Table 1). Small structural differences between soyabean and lucerne saponins (Figure 1), result in considerable variation in cholesterol binding capacity (1,6,7,8).

Both soyabean and alfalfa (lucerne) saponins contain soya sapogenins A-F, but alfalfa also contains the aglycones, medicagenic acid, hederagenin (Figure 1) and lucernic acid (8). Recently Oleszek *et al* (9), has identified the latter as a zahnic acid tridesmoside (Figure 2). These naturally occurring zahnic acid glycosides are present in alfalfa tops and in some

varieties are the main saponin component (9). The greater polarity of medicagenic acid (two carboxylic acids groups) results in greater biological activity and anti-nutritional properties (6-10).

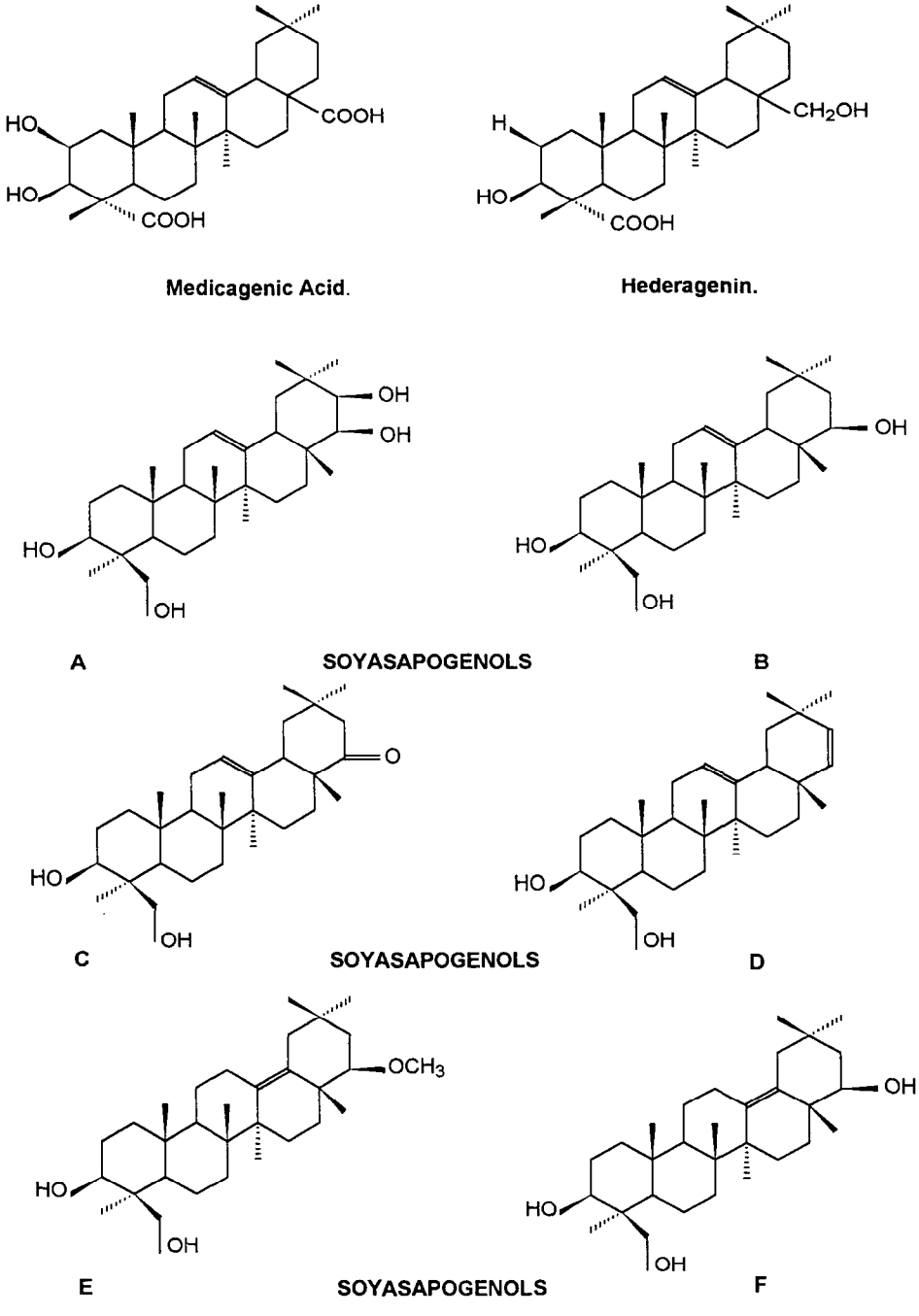

FIG. 1 Chemical Structure of the Major Alfalfa (lucerne) Aglycones & Soyasapogenols A-F

Oakenfull suggested that the positions of attachment of the sugars and other polar groups of the aglycone might be a determining factor and that the chemical structure of the aglycone moiety is important in its interaction with cholesterol. (1).

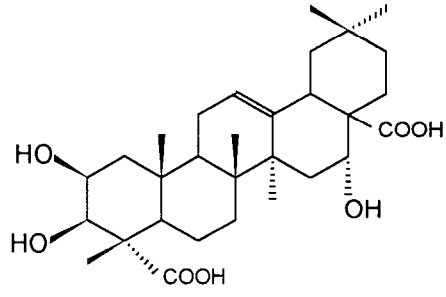

FIG. 2 Zahnic Acid.

Hypocholesterolaemic Effects

The effects of feeding alfalfa, on serum cholesterol and in modifying or preventing cholesterol induced atherosclerosis in rabbits, was studied by Cookson *et al* (11,12). They found that daily administration of large doses of cholesterol, did not elevate serum cholesterol values above normal levels in alfalfa fed rabbits and there was no evidence of atherosclerosis. A bulk effect of undigested alfalfa fibre preventing cholesterol absorption, was dismissed because there was no relationship between dose of cholesterol and dose of alfalfa. They proposed there was some component in alfalfa which blocked the absorptive mechanism for cholesterol in the intestinal mucosa. Barichello & Fedoroff (13), found that cholesterol fed rabbits with ileal bypass required less alfalfa to prevent serum cholesterol elevation, than rabbits with normal gut length. They related this to the reduced intestinal absorptive surface area, supporting the hypothesis of some form of complex formation. However, the component in alfalfa responsible for this action, was not identified.

That the hypocholesterolaemic effect of alfalfa is associated with its saponin content, was demonstrated by Malinow *et al* (14). They found the ability to decrease intestinal absorption of cholesterol, was related to the type of alfalfa saponin. Mild acid hydrolysis of the saponins increased the effectiveness by a factor of five, although the authors did not rule out an additive effect of other components of alfalfa. The complexing of bile acids with fibre components and their subsequent excretion, as a contributing factor in the hypocholesterolaemic effect of saponins was addressed by Oakenfull & Fenwick (15). Kritchevsky (16), had earlier suggested that the general mechanism for the cholesterol lowering action of various foods and plant fibres was due to intraluminal binding of cholesterol or bile acids and their subsequent loss from the body. The consequent reduced enterohepatic bile acid circulation, was offset by an enhanced conversion of cholesterol to bile acids by the liver. Oakenfull and Fenwick (15) showed that only fibre from lucerne, soya beans, mung beans, chick peas, spinach and sunflower seeds, adsorbed bile acids sufficiently, for a physiological effect. This conflicted with the theory (16) that dietary fibre *per se* (lignin etc) was the responsible agent and suggested that adsorption of bile acids depended upon the presence of saponins, perhaps bound to the fibre.

Extensive experimentation by Malinow *et al* (Table 1), with respect to alfalfa, confirmed that alfalfa saponins, not just alfalfa fibre, was responsible for reducing cholesterol absorption (17,18,19,20). Other work by Carroll *et al* had shown a cholesterol-lowering effect in animals fed plant proteins relative to animal protein (21). Pathirina *et al* (22), argued there was no support for the hypocholesterolaemic effect of plant proteins being due to the presence of saponins. Although experimental- methods differed from those used by Oakenfull and Fenwick (15), they cited work by Calvert and Yeates (23), in which saponins were not responsible for bile acid binding to fibrous substances *in vitro* and stated there was little evidence for the '*saponin theory*' of hypocholesterolaemia. Subsequent work with semi-purified diets and purified proteins supported an effect of amino acids on plasma cholesterol independent of saponins (24).

Although some controversy remains, there were two theories emerging for the effect of saponins; a direct binding of cholesterol in the gut and reduction in absorption, thus affecting exogenously produced hypercholesterolemia; a binding of bile acids in the gut which, by decreasing the enterohepatic circulation of bile acids resulted in a reduction of endogeneous hypercholesterolemia. Results in human trials however are conflicting. Initial experiments using soy flour biscuits as the source of saponin, showed increased faecal excretion of bile acids and neutral sterols, but no effect on plasma cholesterol (25), which considering the low saponin intake (approx. 500 mg/day) and the normolipoaemic nature of the healthy subjects may not be surprising. A follow up trial (approx. 500 mg/day) by Calvert *et al* (26), using hypercholesterolemic subjects did not produce any effect on plasma cholesterol or excretion, although Oakenfull and Topping (27) comment that the diet and out patient subjects of the second trial, were not as closely monitored as in the previous trial, and compliance may have been a problem (25). None the less, several animal experiments using a variety of saponins (Table 1), have supported an effect on faecal bile acids and neutral sterol excretion (7,28,29).

Overall, the work done in relation to the hypocholesterolaemic effect of saponins confirm antihypercholesterolaemic activity at least in animals but do not exclude other factors. Comparisons are difficult between trials, due to the use of different types and food sources of saponins (Table 1). These can have a profound effect on the results produced.

Saponin Variation in Common Food Plants

Quillaja, soya (6,30) and alfalfa saponins (6) have a range of binding capacities with cholesterol and this may contribute to the differing results. In addition, the quantity present may also contribute to this variation with alfalfa sprouts > soyabean > chick-pea > navy bean > runner beans > lentils, in saponin content (1,7). Variation between cultivars (31) can also affect the quantity of saponin present, with alfalfa being reported as containing 1.4 - 17.1g/kg (32) depending on the cultivar. These differences have resulted from selective breeding over the years, because of the implications for ruminant bloat (31). *In vitro*, alfalfa plant and sprouts bind cholesterol (33), however bile acid binding by alfalfa plant material was not reduced by the extraction of saponins (7,33), suggesting other components may contribute. None the less, animal studies (Table 1), with chick pea, navy beans (34,35), ginseng (36,37), quillaja (38) and fenugreek (39), have demonstrated a hypocholesterolaemic effect in animals. Sauvaire *et al* (40), suggests that fenugreek saponins and diosgenin produced by gastrointestinal hydrolysis, may contribute to this effect. Whilst Amigo *et al* (41), confirmed the starch fraction of beans (*Phalaseolus vulgaris*) was responsible, Stark & Madar (42), demonstrated a similar result using an ethanol extract from defatted fenugreek seeds, which

suggests the hypocholesterolaemic component, is saponins. It is worth noting that some of the sugars attached to the aglycone in saponins are β-1-4 linked and therefore would be considered soluble fibre (43). The effects of phytates and saponins on digestion and metabolism have been linked with fibre (44) which could also contribute to a cholesterol lowering effect of saponins (45). Thus these and other food components found in association with saponins may have a synergistic effect.

Potential Negative effects of Saponin Intake

The bitter nature of saponins has been shown to reduce feed intake in pigs and rats (46) when fed cultivars of alfalfa, high in saponins. Direct evidence of toxicity on blood or tissue parameters (Table 1), has not been found for alfalfa top saponins in rats (47) or monkeys (48,49), or for alfalfa seeds in rats (18) or in short term feeding (3 weeks) in humans (18). However, in the longer term (6 weeks) 160g/day of alfalfa seeds in one human experiment produced mild anaemia and pancytopenia which reverted to normal after cessation of intake (50). Prolonged feeding (9 months) of alfalfa seeds to monkeys resulted in 3/5 developing systemic lupus erythematosus (SLE) type symptoms (51). Autoclaving or heat treating the seed prevented the development of these symptoms whilst retaining the hypocholesterolaemic effect in monkeys (52). L-canavanine (Fig 3) a structural analogue of arginine is present in high concentration in alfalfa seeds and is destroyed by heating. No toxic effects were noted in humans fed heat treated seeds (at 40g/day for 8 weeks) (53). L-canavanine acts as an allelochemical in alfalfa seedlings controlling the growth of radicles (54) and providing protection from insect attack (55).

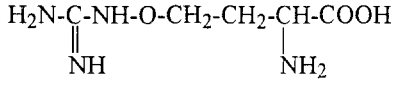

FIG. 3 L-canavanine

The effect of saponins on the gut mucosa has also been studied in view of their potent hemolytic action. They have been used as oral adjuvants (rabies vaccine) to enhance the uptake of antigenically active material from the gut (56). The presence of saponins increase the permeability of intestinal mucosal cells in vitro (57), via the formation of saponin-membrane cholesterol complexes (34). This process has been used to develop an assay for saponins, involving growth inhibition of the micro-organism *Trichoderma virde* (58). Both alfalfa plant and sprout saponins produce villi and colonic mucosal damage in rat intestinal cells (33). The resultant increased rate of exfoliation has been proposed as one mechanism by which saponins are hypocholesterolaemic (57,59); increased gut cell turnover producing increased loss of cell membrane cholesterol (Table 1). Whether increased cell turnover occurs in humans remains to be determined.

Saponins can also form complexes with minerals which at high dose could have a negative effect. Growth depression produced by alfalfa root saponins (3,60) may involve the binding of trace minerals rendering them unavailable for absorption. *In vitro*, saponins from alfalfa root bind zinc and iron (61) and rats fed demineralised soya flour developed zinc

deficiency (62) with concomitant increased bile acid synthesis and excretion. Alfalfa meal decreased plasma zinc and calcium in pigs (63) and iron absorption decreased in rats fed gypsophila saponins (64). Soya, gypsophila and alfalfa saponins have all been shown to affect iron status in rats (65). Relative deficiencies of minerals can be hypocholesterolaemic (66,67). These mineral interactions with saponins may also contribute to the observed hypocholesterolaemic effect, at least in animals (Table 1).

In humans, there is a potential for some negative effects of excessive intake of saponins, including local effects on gut mucosa and secondary effects produced by mineral interactions decreasing availability for absorption. Exactly what constitutes excessive intake remains to be determined. Vegetarians may consume in excess of 100 mg/day, an amount which in the long term may compromise iron status in individuals marginal for intake (65). This has not been demonstrated in any studies so far and remains speculative. However the accuracy of the data for saponin intake from the food supply is poor while individual saponins remain uncharacterised (1,7).

CONCLUSIONS

In summary, the mechanisms by which saponins may reduce cholesterol levels are:-

1. The formation of insoluble addition complexes with β-hydroxysteroids (1,10,20), decreasing intestinal absorption of cholesterol, producing an increase in faecal sterol excretion.

2. Adsorption of bile acids to dietary fibre is enhanced in the presence of saponins (15,17,20), forming large molecular weight micelles, which exclude the bile acids from reabsorption. The ensuing loss of bile acids, is offset by the enhanced conversion of cholesterol to bile acid in the liver (16,68), resulting in hypocholesterolemia (Table 1).

3. Interaction with intestinal mucosal cells leading to an increase in cell permeability and subsequent rapid loss of normal cellular function (57), increasing exfoliation and promoting proliferation. The increased loss of gut cells contribute to an additional increase in faecal cholesterol excretion (Table 1).

4. Their content of β-1-4 linked sugars increasing soluble fibre intake and promoting volatile fatty acid (VFA) production in the large bowel resulting in decreased hepatic cholesterol output.

The beneficial effects on plasma cholesterol may be somewhat speculative in humans. Intakes up to 500 mg/day have not had particularly marked effects on plasma cholesterol (69,70), although alteration to the enterohepatic circulation of bile acids and neutral sterol excretion may be of benefit. Part of the reasons for these minimal effects may be the lack of accurate characterisation of "*saponins*" from various sources (1,7). The potential hazards related to mineral complexing (Table 1), is an under-researched area and with the recent development of a synthetic saponin for use as an oral hypocholesterolaemic agent (71,72) the risk/benefits need to be carefully analysed. Research needs to be undertaken to characterise dietary sources so that their potential usefulness can be better determined.

TABLE 1.

Chronological Overview of Saponin Research

YEAR	Ref No	ANIMAL	SAPONIN/TYPE	DIET	TERM	EFFECTS/FINDINGS
1960	4	rat experiments x 12	Quillaja Saponin white (Gypsophila)	basal + various supplements. saponin 2g/100g diet.	4-6wk	Both saponins depressed weight gain. Quillaja saponin reduction was reversed by addition of cholesterol. The essential fatty acid, Linoleic, when esterified or protected by antioxidant, increased rate of gain, whereas free linoleic acid had no effect.
1967	11	rabbit Exp 1. 3x9 Exp 2. 2x9	Alfalfa	calf meal pellets only CMP+0.6g chol alfalfa pellet+0.6g chol alfalfa diet+0.6g chol	12 to 18mth	Rabbits fed alfalfa diet were unaffected by administration of large doses of daily cholesterol. No evidence of experimental atherosclerosis. Alfalfa feeding had no effect upon established lesions, but probably prevented further lesions. Suggests that alfalfa prevents absorption of cholesterol in the gut.
1968	73	mice rat chick	Soybean saponin extracted flour	+20% soy flour	10day	Neither soy saponin or sapogenins were found in blood. Ingested soy saponins were hydrolysed into sapogenins/sugars by caecal micro flora, enzymes from micro flora showed low degree of specificity. In vitro hemolytic activity of soy saponins of red blood cells were fully inhibited in presence of plasma or its constituents.

1968	12	rabbit 4x5	Alfalfa	diet+0.3g chol diet+25%alfalfa+0.4g chol diet+50%alfalfa+0.5g chol diet+100%alfalfa+0.6g chol	8wk 16wk	Ingestion of alfalfa (300g/wk per rabbit) very efficient in preventing hypercholesterolemia. Suggests that an active component of alfalfa exerts its effects by blocking the cholesterol absorptive mechanisms of the intestinal mucosa in the gut.
1969	6	chick 18x10	Soybean saponin extract	varied +lucerne saponin +cholesterol +vitamin	12day	Saponins did not impair growth in chicks & mice, even when increased x5 but caused slight growth retention in larvae. Showed detrimental effect on tadpoles.
		mice 4x3 tadpole larvae	Lucerne saponin		12day	Lucerne saponin growth impairment counteracted by addition of cholesterol, due to complex formation. Soy saponin does not form a complex and are hydrolysed by caecal micro flora in rat & chick. Suggested cholesterol binding difference between lucerne (increased) & soy, due to structural differences.
1971	13	rabbit 3x12 Ileal bypass (x 2 groups)	Alfalfa	diet diet+12.5% alfalfa diet+25% alfalfa diet+50% alfalfa diet+100% alfalfa varied chol /period	40wk	Rabbits with shortened small intestine (ileal bypass) required less alfalfa to prevent serum cholesterol elevation than those with normal gut length. Could be due to decreased ability to absorb cholesterol from lumen. Supports hypothesis that alfalfa prevents hyper-cholesterolemia by forming unabsorbable complexes with cholesterol, in the lumen.

Table 1. (Continued)

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YEAR	Ref No	ANIMAL	SAPONIN/TYPE	DIET	TERM	EFFECTS/FINDINGS
1971	5	chick 3x12	Digitonin Gypsophilla	diet diet + saponin 0.25%	3wk	Digitonin & saponins from Gypsophilla depressed growth, which was reversed by adding dietary cholesterol.
1977	17	monkey 2x5 rat 2 x 6	Alfalfa root	diet diet +1% Alfalfa root saponin 20mg Alfalfa root saponins	2wk	Alfalfa root saponins prevented expected increase in plasma cholesterol associated with ingestion of high butter/cholesterol diet. Rat experiment indicated alfalfa root saponins, reduced cholesterol absorption.
1977	14	15 rat gps	Alfalfa root & tops	10-20mg intragastically	hrs	Alfalfa top saponin, non-hydrolysed, reduced absorption of cholesterol, but acid hydrolysis enhanced ability to inhibit cholesterol absorption.
1978	74	monkey 2x4	Digitonin	chow chow +0.4% saponin	3wk X over study	Rise in plasma cholesterol prevented by saponin/diet. Both Digitonin and mildly hydrolysed Digitonin, precipitated cholesterol and decreased intestinal absorption of cholesterol.
1978	75	human x66 x156 arthritis pts	Yucca saponin extract	tablets Placebo trial/ blind study	4mth-16mth	Reduction in blood cholesterol, blood pressure, blood triglycerides.

1978	61	in vitro	Alfalfa root Alfalfa leaf Digitonin Quillaja Glycyrrhizin	in vitro		Alfalfa root & glycyrrhizin, complexed zinc & iron but not magnesium. Therefore proposes a possible mechanism for suppression of growth.
1978	46	rat x10 Exp 1	Soybean Low saponin alfalfa Unselected alfalfa	Soybean meal Cotton seed meal Low Sap Alfalfa Unselected alfalfa	28day	No difference in growth response with low saponin & unselected alfalfa meal, but was very much less for soybean & cottonseed meals.
		Exp 2		as above	5day	No difference in digestibility from alfalfa leaf meals with different saponin contents.
		Exp 3		as above	5day	Suggest saponins do not exert their negative effects by influencing protein or fat digestibility.
		Exp 4	alfalfa	alfalfa saponin & quinine sulfate	24hr	As concentration of quinine increased the rats reduced voluntary feed intake indicating sensitivity to bitter tastes.
		swine 5x5	alfalfa	low sap high sap unselected sap control	35day	Low saponin alfalfa has an advantage over unselected or high saponin alfalfa, so reducing saponin content of alfalfa improves its feeding value for swine, but other factors may also be responsible for low nutrient value of alfalfa.

Table 1. (Continued)

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YEAR	Ref No	ANIMAL	SAPONIN/TYPE	DIET	TERM	EFFECTS/FINDINGS
1978	76	rat 4x5	Purified saponin	control diet control diet + 1% saponin control diet + 1% chol control diet +saponin +chol	3wk	Saponin diet caused an increase in faecal bile acids and neutral sterols, supporting hypothesis that saponins bind bile acids and prevent reabsorption.
1978	81	monkeys 1x8 4x18	Alfalfa	monkey chow semipurified diet + chol semipurified diet + chol + alfalfa meal	6mth induct-ion 18 mth regress-ion	Decrease in plasma cholesterol and phospholipids & reduction in the extent of aortic & coronary atherosclerosis were observed in cholesterol fed monkeys consuming a semipurified diet plus alfalfa meal.
1979	77	rat 4x8	Saponaria officinalis	casein casein+saponin soy protein soy protein +saponin	45day	Saponin decreased aorta & liver cholesterol concentration, without modifying serum cholesterol, which could be attributed to increased secretion of neutral sterols and bile acids and to formation of insoluble complexes in digestive tract. Effect of saponins was reduced in presence of soy protein in the diet.
1979	28	rat 4x5	Saponin white	control diet+1% saponin diet+1% cholesterol diet+1% cholesterol +1% saponin	3wk	Dietary saponins increased the excretion of bile acids and neutral sterols in the faeces and they induced a further increase in the rate of bile acid secretion when this was stimulated by a high - cholesterol diet.
1979	19	rat x24	Alfalfa	Alfalfa tops dehydrated alfalfa 264mg/100g	10day	Alfalfa saponins not alfalfa fibre, were responsible for effect of alfalfa meal, in reducing cholesterol absorption.

1980	29	pig x4	Saponin white	low cholesterol diet diet+saponin 20g/day +0.33% solution in water ration Double change over design	3wk x2 4wk x1 2wk x1	Saponins resulted in an increased concentration of faecal bile acids and neutral sterols and an increase in primary bile acids to excretion. Pigs were not fed cholesterol. Neither concentration of total LDL or HDL cholesterol were affected. Both cholestyramine and saponins, show preference for absorption of di-hydroxy bile acids.
1980	78	rat x30	saponin white	soya soya+saponin 1% casein casein+saponin 1%	56day	No difference in serum cholesterol observed between soya or casein fed rats. Dietary saponins did not influence serum cholesterol. Variation between saponin sources must not be excluded, as non-cholesterol fed rats showed decreased serum cholesterol in presence of alfalfa saponins.
1980	18	rat 4x6	Alfalfa	diet +alfalfa seeds (5% & 15% & 30%)	3wk	Alfalfa seeds decreased concentration of plasma cholesterol in rats and increased excretion of bile acids & neutral sterols.
		human x3 males		alfalfa seeds 160g/day	3wk	No signs of toxicity. Suggest intestinal effects may be related to the inhibition of cholesterol absorption by alfalfa saponins and to absorption of bile acids to alfalfa fibre, with a consequent increase in their excretion.

Table 1. (Continued)

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YEAR	Ref No	ANIMAL	SAPONIN/TYPE	DIET	TERM	EFFECTS/FINDINGS
1980	30	rat	Quillaja soya saponin	pure saponin extract 1% of dict	3wk	Soya saponins resulted in increased bile acids and neutral sterol excretion in rats on a high cholesterol diet. Quillaja saponins primarily increased neutral sterol excretion. The higher cholesterol excretion was due to Quillaja saponins forming complex with dietary cholesterol. Soya and Saponin White did not.
1980	20	rabbit 3x18	Alfalfa	diet diet+1% alfalfa saponin diet+40% alfalfa seeds all diets+27mg/100g chol	4mth	Alfalfa saponin & seeds reduced aortic sudanophilia & concentration of cholesterol in aortic intima-plus-media & in the liver. Reduced cholesterol induced hyper-cholesterolemia.
1980	25	human x9	defatted soy flour	biscuits(soya flour 50g/day) biscuits + extracted saponin soya flour Double-blind X over study	4wk	No effect on plasma cholesterol. Intake of saponins was much less than in the animal experiments. Volunteers all had normal plasma cholesterol levels. An increase in faecal bile acid excretion was recorded.
1981	26	human x10 hyperchol- esterolaemic males	soybean saponin	soy flour biscuits (soya flour 50g/day containing 4g or 22g saponins) Double-blind X over study	2 x 4wk	Neither diet had any effect on plasma cholesterol or faecal bile acids or neutral sterol excretion.

1981	47	rat 3x8	Alfalfa	semipurified diet diet+1% alfalfa top saponin diet+2% alfalfa top saponin all diets + 1% cholesterol	 6mth 2mth	Long term feeding of alfalfa top saponins had no effect on growth/survival. Serum cholesterol & triglycerides decreased 1% in 6mth group. Saponins showed no evidence of toxicity.
1981	48	monkey 2x4	Alfalfa	diet +cholesterol diet +cholesterol +0.6% alfalfa top saponin.	4wk	Total cholesterol to HDL cholesterol ratio, was reduced. Saponins decreased intestinal absorption of cholesterol, increased faecal excretion of endogenous and exogenous bile acids and neutral sterols. In monkeys, no sign of toxicity was seen.
1981	22	rabbit 4x6	Saponin white	cow milk protein cow milk protein+saponin 10g/kg soya protein soya protein+saponin	28day	Soy based diets showed significantly lower serum triglycerides & cholesterol (β-lipoproteins), & increase in excretion of bile acids & neutral sterols. No additional effect of saponins. Does not support hypothesis, that the hypocholesterolaemic association with plant proteins, is due to the presence of saponins.
1981	23	in vitro	Soya Alfalfa	soya bean flour wheatbran lucerne *Pinus insignis* sawdust		Removal of saponin from soy flour did not affect bile salt adsorption. Bile salts were adsorbed onto lignin & wheatbran (deficient in saponin).

Table 1. (Continued)

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YEAR	Ref No	ANIMAL	SAPONIN/TYPE	DIET	TERM	EFFECTS/FINDINGS
1981	50	human x1	Alfalfa	alfalfa seeds 80-160g/day	8 x 6wk	Plasma cholesterol fell to 130-160mg/dl from 218mg/dl. A routine blood test revealed mild anaemia and pancytopenia. When seed ingestion ceased, the spleen decreased to normal. Canavanine, an arginine analogue is in high concentration in alfalfa seeds. Suggested that pancytopenia occurred when relatively large amounts of alfalfa seeds were ingested.
1982	79	rat 2x5 hamsters x5	Soybean protein/ saponin	diet diet +saponin diet + fibre diet + saponin + fibre		Experiments failed to provide evidence that saponins and fibre interact to reduce serum cholesterol.
1982	49	monkey males 5 groups	alfalfa	semi-purified diet + chol. semi-purified diet + chol + 1.2% alfalfa top saponins. chow diet. chow diet+chol +1% alfalfa top saponins. chow diet + 1% alfalfa top saponins.	18mth	Long term use of alfalfa top saponins were not associated with toxicity. Plasma cholesterol fell without changing HDL cholesterol concentration. Decreased intestinal absorption of cholesterol, increased excretion of bile acids & neutral sterols and induced regression of atherosclerosis, occurred. Suggested, they may be useful in treating patients with atherosclerosis.
1982	51	monkeys 2x5	alfalfa	semi-purified diet diet + 45% alfalfa seeds	9mth	Three of the five animals fed alfalfa saponins, developed signs of a systemic lupus erythematosus (SLE) like illness, characterised by AG-positive anaemia.

1983	80	in vitro	Saponin white			Formed large mixed micelles with bile salts in vitro, reducing the rate of dialysis of sodium cholate by 73%.
1983	36	rat 8x12	Ginseng (ginsenoside saponin) fraction 3 fraction 4	chow chow+1mg/100g body weight of saponin, orally after tumour transplant	5 to 12day	Plasma cholesterol, triglycerides & non-esterified fatty acid levels were reduced with oral administration. Incorporation of ^{14}C-acetate into total lipids, free & esterified cholesterol, triglycerides & phospholipid in liver was enhanced by fraction 3, in both normal & tumour bearing rats.
1983	37	rat 2x8	Ginseng (ginsenoside saponin)fraction 4	Chow chow +10mg/100g body weight of saponin intramuscular injected	daily/ 2wk	Elevation of plasma cholesterol and triglycerides was reduced. Elimination of ^{14}C-cholesterol from plasma was accelerated as was faecal excretion of ^{14}C bile acids and ^{14}C sterols, by administration of fraction 4 saponins.
1984	34	rat	chick pea navy beans	control chick pea saponin extracted chick pea saponin whole chick pea navy bean	3wk	Chick pea saponins & navy beans lowered plasma cholesterol, extracted chick pea saponins lower plasma cholesterol, but whole chick peas did not. Saponins increased faecal excretion of bile acids.

Table 1. (Continued)

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YEAR	Ref No	ANIMAL	SAPONIN/TYPE	DIET	TERM	EFFECTS/FINDINGS
1984	33	rat x30	alfalfa	alfalfa plant 147g/100g alfalfa saponin 147g/100g	4wk	Alfalfa plant saponin bound significant quantities of cholesterol. Alfalfa sprout saponins interacted with cholesterol & inhibited growth *Thrichodermi virde* (measure saponin/cholesterol interaction). Liver cholesterol accumulation in cholesterol fed rats was not reduced. Serum cholesterol reduced, alfalfa sprouts greater than alfalfa plant material. Increased faecal sterol excretion.
1984	63	swine 40x 2	alfalfa	basal diet. basal, less corn & soy meal +20% alfalfa meal. basal, less corn & soy meal + 40% alfalfa meal. basal, less corn +20% alfalfa meal. basal, less corn +40% alfalfa meal.	13 to 16wk	Weight gain reduced by alfalfa meal. Lower plasma calcium and zinc concentrations with pigs fed alfalfa meal. Suggests alfalfa meal reduced absorption of calcium and zinc from gastrointestinal tract. No clinical evidence of mineral deficiency in treatment groups.
1984	52	monkey x 5gps	alfalfa	semi-purified diet diet +alfalfa seeds	up to 1yr	Monkeys fed autoclaved alfalfa seeds showed no sign of systemic lupus erythematosus. Suggests L-canavanine is destroyed during autoclaving. Anti- hypercholesterolaemic effect retained after autoclaving seeds.

1986	38	rat	Quillaja saponin	semi-purified diet diet + 0.75% saponin	8 or 24wk	No adverse effect on food consumption & body weight and no gross changes observed at autopsy. A significant increase in weight of small intestine of saponin fed rats was reported. No difference in serum lipids at 8 weeks. but after 24 weeks serum triglycerides and cholesterol levels were significantly decreased on saponin diet.
1986	56	mice 5x5	Quillaja saponin	10mg saponin in 0.4ml 4hr prior to administration of vaccine	6 doses	Quillaja saponin given by the oral route, potentiated the immune response to inactivated rabies vaccine. Saponins enhanced passage of antigenically active material from gastrointestinal tract into bloodstream (adjuvant activity) and various mechanisms were suggested. Saponins when delivered orally are of low toxicity.
1986	35	rat 5x5	navy bean	control navy bean saponin solvent extracted navy beans Gallaroy variety saponin Kerman variety saponin	3wk	Control & solvent extracted navy beans showed no cholesterol lowering. Navy bean exhibited cholesterol lowering due to saponins.

Table 1. (Continued)

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YEAR	Ref No	ANIMAL	SAPONIN/TYPE	DIET	TERM	EFFECTS/FINDINGS
1986	57	rat intestine	Gypsophila saponin Saponaria Soya saponin Tomatine	in vitro		Gypsophila inhibited carrier mediated galactose transport, but l-galactose passive transport increased. Saponaria & tomatine decreased transmural potential difference (PD). Soy saponin less effective, reduced permeability barrier to sodium at the brush border, thus discharging the electrochemical gradient & removed the driving force for sugar transport.
1986	39	rat 8x10 1x20	Fenugreek seed	8 x diets 4.8% saponin	8wk	Saponins lowered serum cholesterol as well as preventing elevation when fed with hypercholesterolemic inducing diet. Crude saponins showed hypocholesterolaemic activity. Triglyceride levels were not affected.
1987	53	human x 15	Alfalfa	Alfalfa seed 40g/day	8wk x3	Reduced total plasma cholesterol & LDL cholesterol. Apolipoprotein B reduced.
1988	59	rat 2x10	Gypsophila saponin	diet diet+saponin 1.5%	7day	Serum cholesterol reduced in saponin fed rats & cholesterol content of caecal contents increased. Loss of cholesterol via exfoliated mucosal cells may contribute to hypocholesterolaemic effect. Changes in villus morphology were observed. No evidence of inflammatory or functional damage to jejunal mucosa.

1988	64	rat 6x10	Gypsophila saponin	basal basal + saponin 0.02% low iron diet low iron diet + saponin low zinc diet low zinc diet + saponin	2day	Zn status/femur concentration not adversely affected. Significant reduction in blood cholesterol in both low iron groups. Significant reduction in iron status in basal + saponin & low iron+saponin groups, suggest saponin-mineral interaction complex.
1988	65	rats	Alfalfa	Lucerne saponin 16mg/meal	7day	Lucerne saponin reduced iron absorption, (saponin : iron ratio of 8).
1991	40	dog 4x4 Alloxan-diabetic	Fenugreek saponins subfractions	diet diet+subfraction B diet+subfraction S diet+subfraction P	21day	Faeces of alloxan diabetic dogs fed fenugreek subfractions (rich in steroid saponins) were analysed. Ingested fenugreek saponins are partially hydrolysed to diosgenin in gastrointestinal tract. Saponins may be implicated alone or together with diosgenin in the observed hypocholesterolaemic effect of fenugreek seeds in diabetic dogs.
1992	41	rat 5x5	Bean saponin fractions	Control Fraction I Fraction II Fraction III Diosgenin	5 to 6day	The effect of beans on serum, biliary cholesterol & phospholipid outputs & on hepatic cholesterol ester concentration are dependent on the starch fraction of beans, which contain sapogenin content.
1993	42	rat 3x10	Fenugreek-ethanol extract	diet diet+cholesterol diet+cholesterol + ethanol extract	4wk	Addition of ethanol extract from fenugreek seeds affects cholesterol metabolism in rats. Lower liver and plasma cholesterol concentrations may have been caused by increased excretion of faecal bile acids and a subsequent increased conversion of cholesterol to bile acids.

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