NUTRITIONAL AND PHYSIOLOGICAL IMPLICATIONS OF SAPONINS: A REVIEW[1]

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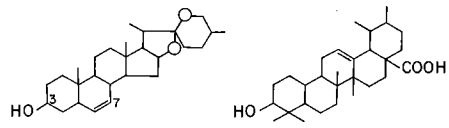
INTRODUCTION

Saponins are widely distributed in plants of agricultural importance, and in particular, in leguminous forage species such as alfalfa. They have been observed to elicit a number of responses, mostly detrimental, in animals consuming them. A review of U.S. Department of Agriculture studies on the relationship of alfalfa saponins to bloat has been published (Lindahl *et al.*, 1957), but information on other aspects of saponin influences in ruminants and monogastrics is scattered through the literature. A review of present knowledge of saponins and their effects on livestock is desirable, not only because considerable information on their biological effects has been collected but also because many gaps still exist in the understanding of their mode of action.

CHEMICAL AND PHYSICAL PROPERTIES OF SAPONINS

Saponins are glycosides; that is, they are composed of carbohydrate and non-carbohydrate, or aglycone, portions. The aglycones are often referred to as sapogenins. The sapogenin nucleus may be either of steroid or triterpenoid structure (Farnsworth, 1966; Robinson, 1963):



STEROID SAPOGENIN — TRITERPENOID SAPOGENIN

Saponins in common forage legumes are of the triterpenoid type (Lindahl *et al.*, 1957). Commercially available saponins may be steroids, derived from yucca or the triterpenoid Quillaja saponin. The latter, which has been fairly extensively used in biological research, is obtained from a South American tree, *Quillaja saponaria.* In both steroid and triterpenoid saponins, the carbohydrate side chain is usually attached at carbon-3 of the sapogenin (Robinson, 1963). Different saponins may have the same nucleus but different carbohydrate side chains (Robinson 1963). In alfalfa saponins, the predominant monosaccharides in the side chain are galactose, glucose and rhamnose, with trace amounts of arabinose and xylose (Jackson and Shaw, 1959; Lindahl *et al.*, 1957). The steroid saponin digitonin is composed of the digitogenin nucleus and a side chain of one xylose, two glucoses and two galactoses (Robinson, 1963). While the triterpenoids may be found in plants as either the saponin or the sapogenein, the steroids never occur in the free sapogenin form (Farnsworth, 1966).

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Legume saponins differ qualitatively and quantitatively both among and within species. Coulson and Davies (1962) found at least 10 and possibly 12 constituent saponins in alfalfa. Genetic differences in the types and relative amounts of saponin in several cultivars of alfalfa have been reported (Hanson *et al.*, 1963; McNairy *et al.*, 1963; Pedersen *et al.*, 1967). In the case of Ladino clover, three saponins have been identified (Walter *et al.*, 1955).

Saponins possess surface-active or detergent properties because the carbohydrate portion of the molecule is water-soluble while the sapogenin is fat-soluble. This detergent property leads to considerable foaming of aqueous saponin solutions; the characteristic honey-comb froth produced is often employed in plant-screening work as presumptive evidence of the presence of saponins (Farnsworth, 1966). The stability and strength of forage saponin foams are closely dependent on pH (Mangan, 1959), a factor which may be related to implicated roles of saponin in development of bloat in ruminants.

Isolation of saponins from plant material involves extraction with a polar solvent, followed by various purification techniques. Alfalfa saponins form insoluble addition complexes with cholesterol, while other legume saponins have been isolated by crystallization (Lindahl *et al.*, 1957). Various chromatographic procedures may then be used to further separate individual saponins from the saponin mixture (Coulson and Davies, 1962; Lindahl *et al.*, 1957; Van Atta *et al.*, 1961). Bioassays for alfalfa saponin, employing a fungus (Scardavi and Elliot, 1967) and fish (Jones and Elliott, 1969), have been used in alfalfa breeding work for identificaton of low-saponin plants.

BIOLOGICAL EFFECTS OF SAPONINS

Ingested saponins have been observed to influence animal performance and metabolism in a number of ways. Among the responses which have been attributed to saponins are:

1. Erythrocyte hemolysis.
2. Effects on blood and liver cholesterol levels.
3. Effects on growth.
4. Bloating of ruminants.
5. Inhibition of smooth muscle activity.
6. Enzyme inhibition.
7. Effects on nutrient absorption.

1. Erythrocyte hemolysis

Saponins have pronounced hemolytic properties (Sollman, 1957). Reaction of the saponin with cholesterol in the erythrocyte wall, resulting in permeability changes, may be responsible for the hemolytic activity (Glanert *et al.*, 1962), although a number of hemolytic saponins do not form cholesteroids (Jones and Elliott, 1969). Ewart (1931) for a number of species has classified susceptibility of erythrocytes to hemolysis as follows: guinea pig, horse > dog, rat, rabbit > man, pig > goat, sheep, cattle. The hemolytic effects of saponins are apparently not correlated with other properties; no relationship has been noted between foam strength (Lindahl *et al.*, 1957; Woodward and Alsberg, 1916), bloat-producing ability (Lindahl *et al.*, 1957), or acute toxicity in monogastrics (Lindahl *et al.*, 1957) and the hemolytic properties of saponins.

2. Effects on blood and tissue cholesterol levels

Since many legume saponins form insoluble addition complexes with cholesterol (Lindahl *et al.*, 1957), effects of dietary saponin on cholesterol metabolism might be anticipated. Griminger and Fisher (1958) reported that with the presence of saponin in chick diets the plasma cholesterol level was lowered. They hypothesized that cholesterol in the bile combines with saponin in the gut, and in this manner is prevented from being reabsorbed. Newman *et al.* (1958) noted that when both saponin and cholesterol were added to chick diets, the liver, but not the serum, cholesterol level was reduced, while there was no significant effect of saponin when cholesterol was omitted from the diet. Studies of the effect of ingested saponin on tissue cholesterol levels have apparently not been conducted with other monogastrics. Fonnesbeck and Symons (1969) found that horses fed alfalfa hay had lower serum cholesterol levels than those receiving grass hays, and attributed the difference to the cholesterol-binding activity of alfalfa saponin. If it is considered desirable to reduce the cholesterol content of eggs, and of swine and poultry meat, it appears that the saponin-cholesterol interaction might offer possibilities for producing animal products of greater consumer acceptability.

No direct studies on the effects of saponin on cholesterol metabolism in ruminants have been reported, but there is some evidence that the situation may be different from that in monogastrics. Binns and Pedersen (1964) found no effect of feeding a high-saponin alfalfa hay on the blood cholesterol level of calves. If saponins are degraded in the rumen, as has been demonstrated by Gutierrez *et al.* (1958) and Gutierrez and Davis (1962), then an influence on cholesterol reabsorption from the intestinal tract would be slight. The report of Jackson *et al.* (1959) that the serum cholesterol level increased when cattle were fed alfalfa as pasture, and that this increase was highly correlated with bloat severity, is difficult to interpret in terms of saponin activity.

On the basis of the limited evidence available, it appears that in monogastrics dietary saponin tends to result in a reduction of tissue cholesterol levels, while in ruminants there is little effect.

3. Effects on growth

High levels of alfalfa meal in diets for monogastrics have growth depressing effects, beyond those that may be accounted for by effects of fiber on caloric intake or endogenous nitrogen losses. Peterson (1950*a*, 1950*b*) and Kodras *et al.* (1951) noted that the growth inhibition caused by alfalfa meal in chick diets could be largely overcome by dietary supplementation with 1% cholesterol, suggesting that the saponin fraction was involved. Heywang and Bird (1954) found that alfalfa saponin at levels of 0.2% or more in the diet of chicks retarded growth and restricted feed intake and feed efficiency. Later studies (Heywang *et al.*, 1959) also showed a depressant effect of alfalfa saponin on egg production. Similarly, Anderson (1957) noted that levels of 0.1% or more alfalfa saponin in chick diets reduced growth and feed efficiency. Addition of 1% cholesterol completely overcame the growth depression produced by 0.3% saponin, the highest level used. Growth depression in chicks and rats fed alfalfa saponin was reported by Coulson (1957), who noted that higher levels (2–3%) of alfalfa saponin were required to produce growth depression in rats. This may explain why no detrimental effects were observed in another study (Lindahl *et al.*, 1957) in which

young rats were fed 1% saponin. No growth inhibition was observed in young guinea pigs and rabbits receiving a dietary level of 2% alfalfa saponin (Lindahl *et al.*, 1957). From these reports it is evident that there are species differences in response by monogastric animals to dietary saponin, poultry being much more sensitive than other monogastrics. Cheeke (unpublished data) has observed that mice are much less tolerant of alfalfa saponin than are rats; at a dietary level of 2% saponin, no response was noted with rats, while with mice feed intake was markedly depressed and weight loss occurred.

The importance of saponin-induced growth inhibition of monogastrics under practical circumstances will vary. In poultry, there is ample evidence that levels of around 20% alfalfa meal in chick rations result in growth depression that is almost entirely due to the saponin content (Heywang and Bird, 1954; Peterson, 1950*a*, 1950*b*). Levels of 20% alfalfa have been incorporated into swine grower rations with no adverse effects on growth (Cheeke, unpublished data); Bohman *et al.* (1953) have established the feasibility of using 30–40% alfalfa in swine rations when economic conditions permit. Under some circumstances, high quality alfalfa meal can be used to meet a significant portion of the protein needs of growing swine. The presence of saponins would probably be an important factor limiting the level of alfalfa that could be used.

Using isocaloric diets, Bell (1960) found that the response of growing mice to fibrous materials varied with the fiber source used. While a number of physical characteristics such as bulk density, tendencies to swell when wet, and others, may account for some of the differences, the effects of organic constituents such as saponins may also account for some of the differential response to fiber source.

The effect of saponins on the growth of ruminants has received little attention; Binns and Pedersen (1964) found no detrimental effects on growth when alfalfa hay containing 2.62% and 1.72% saponin was fed to calves. Hale *et al.* (1961) reported that a steroidal saponin, smilagenin, at a level of 17.6 mg/kg of feed, improved the growth rate of fattening lambs. They suggested that the mechanism of the growth stimulating effect was due to a structural similarity of the saponin to diethylstilbestrol.

The biochemical mechanisms accounting for the growth depressing effects of saponins are not identified. Apparently, the entire saponin molecule is involved, since purified alfalfa and soybean saponins inhibit chick growth while the sapogenins do not (Potter and Kummerow, 1954). Cheeke and Oldfield (1970) demonstrated *in vitro* inhibition by alfalfa saponin of enzymes involved in intermediary metabolism, and suggested that saponin-induced growth depression may be a result of inhibition of cellular enzymes. Another possibility is inhibition of digestive enzymes, which would lower the digestibility of certain nutrients. For example, inhibition of chymotrypsin and trypsin activity by soybean saponin has been demonstrated (Ishaaya and Birk, 1965).

Part of the growth inhibition caused by saponins is probably due to anorexic effects. Quillaja saponin has, in fact, been employed as an anorexic agent in studies of amino acid nutrition (Netke *et al.*, 1969).

It has been suggested that tannins may counteract the inhibitory effects of saponins (Ewart, 1931). However, since tannins have been shown to be growth inhibiting in rats (Glick and Joslyn, 1970), chicks (Rayudu *et al.*, 1970) and rabbits (Dollahite *et al.*, 1962), their use as supplements to overcome saponin-

induced growth inhibition is unlikely to be beneficial, unless a tannin-saponin complex is formed which eliminates the toxicity of both. This aspect warrants investigation.

It is interesting to note that saponins have been reported to have effects on plant growth. Helmkamp and Bonner (1953) observed that saponin stimulated the growth of wheat and pea embryos. It has been suggested that saponins have a role in the regulation of seed germination (Nord and Van Atta, 1960).

4. Bloat in ruminants

Mangan (1959) and McArthur, Miltimore and co-workers (1964, 1966, 1969*a, b,* 1970) have investigated factors which are important in the etiology of bloat. These can be summarized from their work as follows:

1. Presence of foaming agents in the ingested forage.
2. Vigorous gas production in rumen.
3. Acidic rumen pH favoring stable foam formation.
4. Low levels of natural anti-foaming agents.
5. Presence of certain cations involved in foam formation.

Of the foaming agents present in forages, saponins (Lindahl *et al.,* 1957), pectins (Conrad *et al.,* 1958), and certain proteins (Jones and Lyttleton, 1969; Mangan, 1959; McArthur *et al.,* 1964; McArthur and Miltimore, 1966) have been studied in relation to bloat. Mangan (1959) investigated the foaming properties of various legume saponins, a "cytoplasmic protein" from red clover, and rumen liquor taken from bloated cattle. The "cytoplasmic protein" and the rumen liquor showed very similar *in vitro* relationships of foam strength to pH, leading Mangan (1959) to conclude that the "cytoplasmic protein" fraction was the primary foaming agent in bloating forages. McArthur, Miltimore and co-workers have further examined the soluble proteins of alfalfa, and have isolated 18S or Fraction 1 protein which they believe is the foaming agent responsible for bloat (McArthur *et al.,* 1964; McArthur and Miltimore, 1966, 1969*a,* 1969*b*; Miltimore *et al.,* 1970). Some of the observations supporting the Fraction 1 protein theory might also be considered to support the possible involvement of other foaming agents, such as saponins, either acting directly in foam production, or acting in a synergistic capacity with the Fraction 1 protein. Legumes are implicated more often than grasses in bloat; legumes are higher in both Fraction 1 protein (McArthur and Miltimore, 1969*b*) and saponins (Lindahl *et al.,* 1957) than are grasses. Birdsfoot trefoil is a non-bloating legume (McArthur and Miltimore, 1969*b*); it is low in both Fraction 1 protein (McArthur and Miltimore, 1969*b*) and in saponin (Walter, 1961). The rumen pH of bloated animals is acidic (McArthur and Miltimore, 1969*a*); the maximum foam strengths of both saponins and Fraction 1 protein occur under acidic conditions (Mangan, 1959). A correlation between calcium content of alfalfa and bloating incidence has been observed (Miltimore *et al.,* 1970); calcium is a constituent of saponin foam (Mangan, 1959) while Fraction 1 protein molecules are bound together by cations such as calcium (Mangan, 1959; McArthur and Miltimore, 1969*a*) which increases foam strength (Mangan, 1959). A correlation between forage zinc and bloat incidence was also observed (Miltimore *et al.,* 1970); it is of interest that a relationship between soil zinc and forage saponin levels has been suggested (Henrici, 1952). Finally, support for a possible saponin involvement in bloat comes from the fact that administration of legume saponins to

ruminants does in fact cause bloating (Lindahl *et al.*, 1957), although the levels employed were high compared with normal intakes. However, Jackson *et al.* (1962) were unable to correlate a bloat severity index with the level of total saponins in Ladino clover forage.

Additional evidence against a role of saponin in bloat production includes the observation that the maximum foam strengths in red clover and alfalfa saponins occur at a pH lower than is most usually found in bloat (Mangan, 1959). However, these measurements were not made under conditions simulating an intraruminal environment. It would be more valid if conclusions were based on the foaming properties of saponins in rumen fluid, rather than in buffer solutions. It is not inconceivable that the foam strength-pH curve might be shifted, especially in view of Mangan's (1959) observation that the foam strength maximum of protoplasmic protein was shifted considerably by changes in the nitrogen and salt concentrations of the medium. Before concluding that saponins are not important in stable foam formation in the rumen, it is necessary to establish the foam strength-pH relationship under the conditions that exist in the rumen. With regard to the acceptance of the Fraction 1 protein theory, Clarke and Reid (1970) state: "This contention probably leans rather too heavily on comparisons between the pH of rumen contents during bloat, and the pH optima for *in vitro* foam-strengths of leaf protein and of possible alternative foaming agents, saponins, and pectins". As Dougherty (1970) aptly points out, there are dangers entailed in extrapolation from *in vitro* foaming to *in vivo* foaming. Clarke and Reid (1970) also note that the Fraction 1 content of stems is low, but that New Zealand work has shown that bloat has occurred in cows fed clover stems from which all leaves were removed.

An additional factor to consider when evaluating the foam strength-pH curves of Mangan (1959) is that Buckingham (1970) has reported a very well-defined effect of temperature on foam strength. He suggests that small changes in intraruminal temperature could have marked effects on cytoplasmic protein foam strength; possibly a similar effect could be seen with saponins.

Sufficient evidence, obtained under physiological conditions, has not been obtained to evaluate conclusively the significance of saponins in legume bloat. It is likely that under appropriate circumstances both soluble proteins and saponins may be involved. The foam strength curves of Mangan (1959) would indicate that the contribution of saponins to foam formation might be of greatest significance under the more acid rumen conditions. It would be highly desirable to redetermine the relationship of saponin foam strength to pH, but under physiological conditions.

In order for the causative factors of bloat to be fully elucidated, it would be desirable for investigators in the area to evaluate as many factors as possible simultaneously, including Fraction 1 protein, saponins and pectins. Mangan (1959) has suggested that such compounds as saponins and salivary mucoprotein might interact synergistically with cytoplasmic protein to increase foam strength. Another possibility includes studying the interactions of the various foaming agents with poloxalene, a compound that very effectively prevents bloating (Foote *et al.*, 1968).

Kendall (1966) reported that non-bloating legumes contain high levels of tannin (about 10% of the dry weight) whereas those associated with bloat have low levels (about 2%). This is interesting in view of the suggestion of Ewart (1931) that tannins may overcome the detrimental effects of saponins on biological systems; Segelman *et al.* (1969) also reported that very low levels of tannins would

render red blood cells immune to hemolysis by saponins. If, as might be inferred from these observations, tannins complex with saponins, a possible explanation for the low bloat potential of high-tannin legumes is that the saponin has been tied up in a tannin-saponin complex. In addition, tannins have been postulated to precipitate the soluble leaf proteins of forages and render them unavailable for foam production (Jones *et al.*, 1970).

5. Saponin inhibition of smooth muscle activity

In an extensive series of studies, Lindahl *et al.* (1957) examined the effects of alfalfa saponin on the motility of smooth muscle. Intraruminal administration of saponin to sheep resulted in a pronounced reduction in rumen motility. Intravenous administration of saponin also resulted in reduced rumen motility. In these experiments, the site of action was not identified; not all the inhibition could be explained by direct effects on the ruminal musculature. Alfalfa saponin was also found to inhibit eructation, with evidence that direct effects on the central nervous system were involved. The significance of these observations lies in their relationship to the bloat situation; besides a possible role in stable foam formation, saponins may interfere with gas loss from the rumen by inhibiting eructation.

6. Inhibition of enzymes

Shaw and Jackson (1957) found that Ladino clover extracts inhibited the respiration of isolated rat diaphragm tissue. Further work with alfalfa implicated saponins as the cause of respiratory inhibition (Jackson and Shaw, 1959; Shaw and Jackson, 1959). A possible explanation for this observation is that saponin may inhibit respiratory enzyme activity. This aspect has been examined by Cheeke and Oldfield (1970), who found that alfalfa saponin inhibits the *in vitro* oxidation of succinate, an important Kreb's cycle intermediate, by rat liver enzymes. Ethanolic extracts of alfalfa also inhibit *in vitro* succinoxidase activity (Cheeke and Oldfield, 1969, 1970; Roughan, 1965); other factors besides saponin are evidently involved (Cheeke and Oldfield, 1970). *In vivo* inhibition of enzymes involved in cellular metabolism by saponins offers a tenable explanation for their detrimental effects on growth. Besides apparent effects on cellular metabolism, saponins might influence digestive enzyme activity. Ishaaya and Birk (1965) reported that soybean saponin inhibits cholinesterase, chymotrypsin and trypsin activity. Further examination of the effects of saponin on enzyme function is indicated.

7. Saponin effects on nutrient absorption

Some effects of ingested saponins on nutrient absorption have been suggested. Sollman (1957) and Ewart (1931) cite a suggestion by Kofler (1927, Die Sapoine; Springer, Vienna) that saponins favor the absorption of sugars. Apparently, no definitive studies have been undertaken to verify this.

Saponin reduces the absorption of cholesterol, as has been already discussed, by forming an insoluble addition product. It might be anticipated that saponin would combine with other sterols of similar structure such as those with vitamin D activity and interfere with their absorption. However, Coulson and Evans (1960) found no influence of Quillaja saponin on the absorption of ergocalciferol or on its curative effect upon rickets. Pudelkiewicz and Matterson (1959) reported evidence of a fraction in alfalfa, that they suggested might be saponin, which interfered with the absorption of vitamin E. Later work showed that the inhibitory

fraction was not saponin (Olson *et al.*, 1966). Ewart (1931) states that saponins favor the absorption of salts of calcium and magnesium, but does not cite the source of this information. He also states that saponins enhance the absorption of various alkaloids, so that the lethal dose is lowered in the presence of saponins.

METABOLISM OF SAPONINS

No direct studies of the metabolism of forage saponins have been reported, to the author's knowledge, but a few general observations can be made. Saponins are non-dialyzable (Lindahl *et al.*, 1957), so their absorption would be expected to be low. A low level of absorption can also be deduced from toxicity studies; Sollman (1957) states that saponins are from 10 to 1000 times more toxic when administered intravenously than when given orally. Alfalfa saponin was found to be about 50 times more toxic to sheep when given intravenously than intraruminally (Lindahl *et al.*, 1957). Gestetner *et al.* (1968) were not able to find either saponin or sapogenin in the blood of chicks, rats and mice following oral administration of soybean saponin. However, their studies also indicated that soybean saponins do not produce growth inhibition in these species, indicating that potency may be less than that of other legume saponins. The lack of substantial biological effects might just be a result of their not being absorbed. Hence, these studies cannot be considered as conclusive evidence that saponins are not absorbed. While absorption of saponins across the rumen wall has not been observed, the passage of saponins from the blood to the rumen has been indirectly demonstrated. Lindahl *et al.* (1957) found that intravenously administered saponin resulted in bloat in sheep. Although this suggests a passage of saponin from the blood to the rumen, an effect on the central nervous system causing impaired eructation cannot be completely discounted.

Destruction of saponins in the digestive tract of both ruminants and monogastrics has been observed. Gutierrez *et al.* (1958) and Gutierrez and Davis (1962) reported breakdown of legume saponins by rumen bacteria, with the production of volatile fatty acids, carbon dioxide and a slime. Gestetner *et al.* (1968) studied the metabolism of soybean saponins in rats, mice and chicks. Ingested soybean saponins were hydrolyzed into sapogenins and sugars by the cecal microflora of all three species. A saponin-hydrolyzing enzyme of low specificity was isolated from the cecal microflora of rats; it is interesting that it also hydrolyzed alfalfa saponin. Since saponin in the cecum is past the major sites of absorption, the significance of cecal saponin dissimilation in monogastrics is uncertain.

CONCLUSIONS

Dietary saponin inhibits the growth of chicks, and is a factor limiting the amount of alfalfa that may be incorporated into poultry rations. Other monogastric species have received less attention, but there is evidence of species specificity in growth response to ingested saponins. No direct studies of effects of saponin on swine performance have been reported. Since the use of alfalfa to meet a portion of the protein needs of swine may be economical under some conditions, the significance of alfalfa saponin to swine performance should be evaluated. Such information would also be of value to plant geneticists, since selection for low-saponin alfalfa seems feasible (Jones and Elliott, 1969). Evaluation of the effects of saponins in such monogastric laboratory animals as the guinea pig and rabbit,

which are often fed high-alfalfa rations, would also be appropriate. Saponins may have useful applications in some instances, since in monogastrics they form complexes with cholesterol and may help to lower tissue cholesterol levels by reducing its reabsorption from the intestinal tract. In ruminants, neither growth depression nor reduced tissue cholesterol levels due to saponin ingestion have been demonstrated, possibly because of bacterial dissimilation of the saponin in the rumen. Saponins may be significant factors in the development of bloat in ruminants. Although in recent years interest in saponins as causative factors of bloat has lagged, their properties suggest that they could be involved in stable foam formation in the rumen.

This review has summarized the limited knowledge of saponin metabolism and indicated some areas where further investigation is warranted. Since saponins are a part of the diet of any animal consuming alfalfa or other forage legumes, it is desirable to be aware of what effects, if any, these compounds may have on metabolic processes.

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