Effects of dietary saponins on fecal bile acids and neutral sterols, and availability of vitamins A and E in the chick

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Saponins are a heterogenous group of triterpene or steroidal glycosides that are widely distributed among food plants. They have been implicated in reduced animal growth, interference with the absorption of cholesterol and bile acids, and with fat digestibility, but also have shown potential in the reduction of blood cholesterol levels. This study investigated whether these effects of saponins affect the availability of the fat-soluble vitamin A and vitamin E. Chicks fed gypsophila and quillaja (triterpenoid) saponins, at 0.9% of diet, had reduced weight gains, feed intake, digestibility of lipid, marked increases in cholesterol excretion, but no changes in excretion of coprostanol or bile acids. Blood total cholesterol and high density lipoprotein cholesterol were unaffected. Both saponins appeared to interfere with the absorption of vitamin A and vitamin E, as indicated by reduced concentrations of plasma retinol and vitamin E, and liver retinol, vitamin A palmitate, and vitamin E. Feeding a steroidal saponin (sarsaponin) had virtually no effect on any of the parameters measured. Further studies will be needed to determine if saponins have specific or general inhibitory effects on nutrient availability and how much these effects contribute to reduced animal performance. (J. Nutr. Biochem. 5:134–137, 1994.)

Keywords: saponins; availability of vitamins A and E; fecal bile acids and neutral sterols

Introduction

Saponins are a heterogenous group of triterpene or steroidal glycosides that occur in a wide range of plants that are consumed by animals and humans.[1–3] Numerous biological effects have been attributed to saponins, some with positive and others with negative implications.[2] For example, saponins have been found in some circumstances to reduce feed intake, inhibit growth of swine and poultry, and contribute to various toxicological problems associated with weeds.[2] On the other hand, there have been numerous indications that saponins may have potential as dietary additives in lowering serum cholesterol levels.[4] In this regard, orally administered saponins have reduced serum cholesterol in experimental animals by interfering with cholesterol absorption or by increasing excretion of bile acids.[2,4]

No comprehensive studies have been conducted to determine the mechanisms whereby saponins, on some occasions, retard the growth rate of animals. It has been suggested that the bitterness of saponins results in reduced palatability and intake of feeds.[4] An alternate explanation has been a limiting of the availability of essential micronutrients, either by forming unavailable complexes or by interfering with mucosal cell activity.[4] However, there have been two reports showing that saponins can increase fecal fat excretion,[5,6] which would be expected if bile acids were complexed by saponins and resulted in less effective fat solubilization, digestion, and absorption. It was considered that this higher excretion of fat might interfere with the utilization of fat-soluble vitamins, and the present experiment was conducted to test this possibility. Chicks were fed a diet for 4 weeks containing one of two triterpene saponins (gypsophila, quillaja) or a steroidal saponin (sarsaponin), at a concentration of 0.1%, 0.3%, or 0.9%. Measurements included the effect of dietary saponins on individual weight gains; group feed intakes; fecal excretion of neutral sterols, bile acids, and lipids; concentrations of vitamins A and E in blood plasma and liver; and plasma total and high density lipoprotein (HDL) cholesterol.

Methods and materials

Animals, diets, collection of samples

Two hundred 1-day-old unsexed chicks of the Centre for Food and Animal Research (Ottawa, Ontario, Canada) meat strain 31[7] were individually banded, fed a commercial starter diet for 3 days, then

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Table 1 Composition of basal diet (weight %)

Ingredients	
Glucose	66.8
Casein	20.0
Cellulose	3.0
L-arginine · HCl	1.0
Tallow	4.0
Salts premix, macro[a]	4.0
Salts premix, micro[b]	0.2
Vitamin premix[c]	1.0

[a]Macro salts mixture (g per 100 g diet): $CaHPO_4 \cdot 2H_2O$ 1.87; $CaCO_3$ 0.65; KH_2PO_4 0.69; MgO 0.08; NaCl 0.60

[b]Micro salts mixture (mg per 100 g diet): $FeSO_4 \cdot 7H_2O$ 41.4; $MnSO_4 \cdot H_2O$ 33.3; KI 0.26; $CuSO_4 \cdot 5H_2O$ 1.67; ZnO 6.0; $Na_2SeO_4 \cdot 10H_2O$ 0.047

[c]Vitamin premix (mg per 100 g diet): niacin 5; calcium pantothenate 2; thiamine · HCl 1; riboflavin 1; pyridoxine · HCl 0.45; folic acid 0.4; menadione 0.05; D-biotin 0.02; vitamin B_{12} 0.002; vitamin A palmitate 500 IU; vitamin D_3 38 ICU; vitamin E 0.3 IU; choline chloride 150

randomized into 20 groups of 10 chicks each, providing 10 dietary treatment groups in duplicate. Each group of 10 chicks was then randomly assigned a pen in the battery brooders. The subsequent feeding trial was for 4 weeks. Housing consisted of wire-floored battery brooders, electrically heated, with 23 hours of light provided daily. Fresh feed and water were provided daily for ad libitum consumption. Birds were individually weighed and feed consumption of each pen taken on a weekly basis. Pen excreta were collected daily and frozen for the last 7 days of the experiment with the total amount mixed and freeze-dried.

The composition of the basal diet is shown in *Table 1*. Saponins were included in the basal diet (replacing glucose) to produce 10 treatments as follows: no addition (control); 0.1% or 0.3% or 0.9% sarsaponin; 0.1% or 0.3% or 0.9% gypsophila saponin; 0.1%, or 0.3% or 0.9% quillaja saponin. Diets were prepared on a weekly basis and stored at $-5°$ C.

At the end of the experiment, blood samples were taken from each bird, the birds then killed by CO_2 asphyxiation, and the livers removed. All samples were stored at $-15°$ C.

Analyses

Analyses of feed and feces for dry matter and lipid were according to the standard methods of the Association of Official Analytical Chemists.[8]

Vitamin E in blood plasma was determined by a high performance liquid chromatography (HPLC) method described by Hidiroglou.[9] For hepatic vitamin E, 1 g of liver was homogenized with a mixture of 2 mL water and 10 mL ethanol. After centrifugation, the supernatant was extracted with 10 mL hexane, a 5 mL aliquot of the extract evaporated to dryness under nitrogen, dissolved in 2 mL hexane, and analyzed for vitamin E by HPLC, as for plasma. Retinol was also read on these liver samples using excitation 326 nm and emission 480 nm. Serum retinol and liver total vitamin A (retinol plus retinyl palmitate) were determined by the method of Davila et al.[10] Fecal neutral steroids (cholesterol, coprostanol) were analyzed by the method of Miettinen et al.[11] (separation by thin layer chromatography and quantitated by gas-liquid chromatography (GLC)) and fecal bile acids (cholic acid, deoxycholic acid, chenodeoxycholic acid, lithocholic acid) by the procedure of Grundy et al.[12] (GLC of trimethylsily ethers). Plasma HDL cholesterol was determined using Sigma Procedure 352 and plasma total cholesterol using Sigma cholesterol esterase kit 352-20 (Sigma Chemical Co., St. Louis, MO USA).[13]

Materials

Quillaja saponin was obtained from J.T. Baker (Toronto, Ontario, Canada), sarsaponin from The Distributors Processor Inc. (Porterville, CA USA) and gypsophila saponin from BDH Chemicals (Toronto, Ontario, Canada).

Statistical analyses

All data are presented as mean ± SEM. Two-way analysis of variance (ANOVA) was used to analyze the data, and the Duncan test was used to check the significance of differences ($P < 0.05$) between the means.[14]

Results

The three dietary concentrations of sarsaponin had no effect on weight gains and feed intake over the 28-day trial, or on digestibilities of dietary dry matter and lipid for the last 7 days *(Table 2)*. In contrast, both triterpenoid saponins (gypsophila and quillaja) affected these performance parameters at one or more of the intake levels. For example, gypsophila at 0.3% and 0.9%, and quillaja at 0.9% significantly reduced ($P < 0.05$) weight gains and food consumption and both saponins at 0.9% markedly reduced digestibility of dry matter and lipid.

Table 2 Food consumption, body weight changes, and dietary dry matter and lipid digestibilities for chicks fed various diets

Treatment	Weight gains g/28 days	Food consumption g/28 days	Digestibility (21–28 days)	
			Dry matter (%)	Lipid (%)
Control	480 ± 9[a]	727 ± 17[a]	90.1 ± 1.2[a]	88.0 ± 2.3[a]
Sarsaponin, 0.1%	479 ± 9[a]	737 ± 23[a]	90.6 ± 2.1[a]	85.6 ± 2.1[a]
Sarsaponin, 0.3%	512 ± 13[a]	768 ± 18[a]	87.4 ± 2.1[ab]	86.8 ± 2.8[a]
Sarsaponin, 0.9%	501 ± 12[a]	730 ± 32[a]	88.2 ± 2.9[ab]	85.1 ± 2.9[ab]
Gypsophila, 0.1%	476 ± 9[a]	722 ± 21[a]	88.5 ± 1.6[a]	88.5 ± 2.0[a]
Gypsophila, 0.3%	387 ± 8[b]	620 ± 44[bc]	85.7 ± 2.6[abc]	88.3 ± 1.6[ab]
Gypsophila, 0.9%	144 ± 5[d]	348 ± 18[d]	78.9 ± 2.8[c]	75.1 ± 2.2[c]
Quillaja, 0.1%	478 ± 11[a]	736 ± 26[a]	88.2 ± 2.1[a]	86.9 ± 1.5[a]
Quillaja, 0.3%	475 ± 13[a]	698 ± 29[ab]	86.5 ± 2.0[ab]	86.1 ± 1.8[a]
Quillaja, 0.9%	312 ± 8[c]	570 ± 30[c]	81.7 ± 2.2[bc]	79.4 ± 2.0[bc]

The results are expressed as means ± SEM for 20 animals/group (duplicate pens of 10 animals). Values in the same columns without a common superscript are significantly different based on ANOVA and the Duncan test ($P < 0.05$).

The effects of type and dietary concentration of saponin on concentrations of neutral sterols and bile acids are shown in *Table 3*. As with the growth and food intake and digestibility data, dietary sarsaponin had no effect on excretion of neutral sterols or bile acids. The gypsophila and quillaja treatments, however, caused very large increases ($P < 0.05$) in cholesterol excretion, with greater effects for the highest saponin intakes. Concentrations of coprostanol and the bile acids was generally unaffected; there were relatively small increases in coprostanol, cholic acid, and chenodeoxycholic acid for the highest level (0.9%) of gypsophila.

The effects of the saponin treatments on concentrations of vitamins A and E in blood plasma and liver and cholesterol in plasma, are shown in *Table 4*. None of the saponin treatments affected the total and HDL cholesterol concentrations. Conversely, vitamin E concentrations in plasma were depressed ($P < 0.05$) by both the 0.9% gypsophila and 0.9% quillaja treatments, and liver vitamin E levels were decreased ($P < 0.05$) by five of the six dietary treatments involving these saponins. Similarly, plasma retinol, liver retinol concentration, and vitamin A palmitate concentration were reduced ($P < 0.05$) by the highest dietary concentrations of gypsophila and quillaja. Sarsaponin at 0.9% also reduced liver vitamin A palmitate levels.

Discussion

Although the concentration of saponins in the diets of humans and farm animals varies considerably according to the content of contributing materials, it is evident from published data on the saponin content of materials that the saponin intakes of both humans and livestock could exceed the 0.3% and 0.9% levels used in the present study.[4,15–17] Foods that could provide an appreciable dietary intake of saponins are soybeans, asparagus, peanuts, spinach, tomato, lentils, garden peas, and soft drink beverages; foodstuffs include alfalfa, clovers, oats, forages, lupines, and sunflower.[4,15–17] Chick peas are an appreciable source of saponins in the Middle East, India, and Pakistan, soybeans and yams in much of Southeast Asia, and peanuts in Central Africa.[17]

Our results showed that gypsophila saponins reduced chick growth and feed intake starting at the 0.3% dietary level, whereas a similar effect for quillaja saponins required the 0.9% intake. Sarsaponin had no effect on these parameters at

Table 3 Effect of dietary saponins on concentrations of neutral sterols and bile acids in fecal dry matter

	Neutral sterols		Bile acids			
Treatment	Coprostanol nmol/g	Cholesterol nmol/g	Cholic acid nmol/g	Chenodeoxycholic acid nmol/g	Deoxycholic acid nmol/g	Lithocholic acid nmol/g
Control	134 ± 14^{bc}	2772 ± 140^{g}	470 ± 40^{bc}	26 ± 2.0^{b}	167 ± 17^{a}	125 ± 20^{ab}
Sarsaponin, 0.1%	160 ± 18^{b}	2868 ± 188^{g}	506 ± 52^{bc}	33 ± 2.3^{ab}	140 ± 16^{a}	104 ± 17^{ab}
Sarsaponin, 0.3%	168 ± 26^{b}	2870 ± 124^{g}	418 ± 30^{bc}	29 ± 2.6^{ab}	155 ± 33^{a}	132 ± 15^{ab}
Sarsaponin, 0.9%	125 ± 12^{bc}	2584 ± 172^{g}	523 ± 46^{b}	26 ± 2.2^{b}	175 ± 25^{a}	91 ± 13^{b}
Gypsophila, 0.1%	143 ± 14^{b}	4459 ± 202^{e}	434 ± 36^{bc}	30 ± 3.3^{ab}	127 ± 21^{a}	122 ± 13^{ab}
Gypsophila, 0.3%	124 ± 26^{bc}	9051 ± 302^{c}	473 ± 42^{bc}	33 ± 3.1^{ab}	141 ± 18^{a}	127 ± 14^{ab}
Gypsophila, 0.9%	428 ± 42^{a}	14214 ± 760^{a}	701 ± 69^{a}	36 ± 3.5^{a}	123 ± 14^{a}	156 ± 19^{a}
Quillaja, 0.1%	112 ± 16^{bc}	3377 ± 156^{f}	394 ± 33^{c}	34 ± 2.6^{ab}	145 ± 18^{a}	97 ± 17^{b}
Quillaja, 0.3%	98 ± 12^{c}	5944 ± 288^{d}	521 ± 55^{bc}	24 ± 1.9^{b}	129 ± 17^{a}	100 ± 15^{b}
Quillaja, 0.9%	93 ± 18^{c}	9955 ± 340^{b}	406 ± 46^{bc}	27 ± 2.3^{b}	150 ± 15^{a}	142 ± 21^{ab}

The results are expressed as means ± SEM for 20 animals/group (duplicate pens of 10 animals). Values in the same columns without a common superscript are significantly different based on ANOVA and the Duncan test ($P < 0.05$).

Table 4 Effect of dietary saponins on concentrations of vitamins A and E in blood plasma and liver, and cholesterol in plasma

	Liver (dry matter)			Blood plasma			
Treatment	Vitamin E nmol/g	Retinol nmol/g	Vitamin A palmitate nmol/g	Total cholesterol mmol/L	HDL cholesterol mmol/L	Vitamin E μmol/L	Retinol μmol/L
Control	6.7 ± 0.51^{a}	9.4 ± 0.72^{a}	53.3 ± 3.1^{a}	4.1 ± 0.18^{a}	3.1 ± 0.17^{a}	4.9 ± 0.37^{ab}	2.3 ± 0.13^{ab}
Sarsaponin, 0.1%	6.5 ± 0.72^{a}	8.5 ± 0.70^{a}	49.4 ± 3.1^{abc}	3.9 ± 0.26^{a}	3.0 ± 0.22^{a}	5.3 ± 0.46^{ab}	2.4 ± 0.17^{a}
Sarsaponin, 0.3%	5.8 ± 0.44^{ab}	10.5 ± 0.86^{a}	54.3 ± 2.9^{a}	4.2 ± 0.20^{a}	3.3 ± 0.19^{a}	4.9 ± 0.29^{ab}	2.1 ± 0.16^{abc}
Sarsaponin, 0.9%	7.2 ± 0.63^{a}	8.8 ± 0.75^{a}	44.1 ± 2.0^{bcd}	3.9 ± 0.24^{a}	3.0 ± 0.15^{a}	5.1 ± 0.35^{ab}	2.3 ± 0.14^{ab}
Gypsophila, 0.1%	5.6 ± 0.67^{abc}	9.9 ± 0.92^{a}	56.6 ± 4.2^{a}	4.1 ± 0.32^{a}	3.2 ± 0.23^{a}	5.6 ± 0.52^{a}	2.2 ± 0.09^{ab}
Gypsophila, 0.3%	4.2 ± 0.38^{cd}	9.2 ± 0.88^{a}	50.7 ± 3.1^{ab}	4.0 ± 0.23^{a}	3.2 ± 0.20^{a}	4.2 ± 0.37^{bc}	2.3 ± 0.12^{ab}
Gypsophila, 0.9%	4.4 ± 0.46^{bcd}	6.0 ± 0.47^{b}	41.4 ± 2.7^{cd}	3.8 ± 0.28^{a}	3.0 ± 0.18^{a}	3.7 ± 0.28^{cd}	1.8 ± 0.15^{c}
Quillaja, 0.1%	4.6 ± 0.53^{bcd}	8.4 ± 0.63^{a}	51.7 ± 3.6^{ab}	4.3 ± 0.21^{a}	3.1 ± 0.21^{a}	4.6 ± 0.38^{ab}	2.3 ± 0.13^{ab}
Quillaja, 0.3%	3.7 ± 0.49^{d}	9.5 ± 0.80^{a}	52.6 ± 3.3^{a}	4.0 ± 0.20^{a}	3.0 ± 0.16^{a}	4.6 ± 0.33^{ab}	2.5 ± 0.16^{a}
Quillaja, 0.9%	3.5 ± 0.34^{d}	6.3 ± 0.55^{b}	38.2 ± 2.0^{d}	3.8 ± 0.26^{a}	3.1 ± 0.18^{a}	2.8 ± 0.24^{d}	1.8 ± 0.14^{c}

The results are expressed as means ± SEM for 20 animals/group (duplicate pens of 10 animals). Values in the same columns without a common superscript are significantly different based on ANOVA and the Duncan test ($P < 0.05$).

all intakes. These observations concur with results published by Morgan et al.[18] and Newman et al.,[19] who found that chick growth was reduced by dietary gypsophila at 0.25%, and quillaja saponin at 0.9%, respectively. It has been well established by numerous investigators that sarsaponin has no growth-inhibiting effects in poultry, and in fact has been fed to birds by poultry producers to control fecal ammonia levels.[2]

Our study shows for the first time that feeding gypsophila or quillaja saponins to chicks, at the 0.9% level, depressed fat utilization, although a similar effect on fat utilization has been reported for other saponins and animal species. For example, Reshef et al.[5] fed mice a diet containing 0.5% alfalfa saponins and found lower lipid digestibility and increased fecal cholesterol and neutral sterols. Similarly, rats fed alfalfa saponins (0.6%) had elevated fecal concentrations of lipids, cholesterol, and bile acids.[6]

There have been numerous studies showing that certain saponins can interact with sterols in the gastrointestinal (GI) tract of animals, causing an increased fecal excretion of neutral sterols (cholesterol, coprostanol, plant sterols), and bile acids, which in some instances has been accompanied by a reduction of serum cholesterol concentration.[2,4,17] The kind of sterol affected in the GI tract has been characteristic of the type of saponin fed and the animal species, but there has been a common observation in virtually all studies, i.e., a reduction of serum cholesterol never occurred from saponin feeding unless a hypercholesterolemic diet had been fed.[17,20] This also occurred in our present study with chicks, where no cholesterol was added to the diets. None of the saponin treatments we used had any effect on serum cholesterol concentrations. Although the gypsophila and quillaja treatments markedly increased endogenous cholesterol excretion, this loss of cholesterol apparently was replaced by increased liver synthesis. It would appear that dietary saponins are effective in reducing blood cholesterol concentrations when the levels are high due to a high dietary intake of cholesterol, and this reduction is caused by an interference with the absorption of cholesterol and bile acids.

This study provides support to the proposition that a reduction in weight gains experienced by some animals fed saponins may be due, in part, to a decrease in the availability of certain essential nutrients. It was shown that saponins can affect the availability of dietary vitamin E and vitamin A in the chick depending on the kind and concentration of the saponin fed. It was unlikely that this effect was a major cause of the marked reductions in weight gains seen here for some of the treatments, as much of the vitamin A and vitamin E was still being absorbed. However, there is the possibility that the availability of other nutrients also was affected and contributed to the poor gains. As ingested saponins are not absorbed into the blood stream to any extent,[4] it is considered likely that the most physiological effects of saponins are caused either by interactions with materials in the gut contents or by alterations in the permeability of the gut membrane.[4]

The main reductions in availability of vitamin E and vitamin A were with the 0.9% gypsophila and 0.9% quillaja intakes. Both treatments appeared to inhibit absorption of the vitamins, as indicated by reduced concentrations of plasma retinol and vitamin E, liver retinol, vitamin A palmitate, and vitamin E. Direct measurements of the vitamins in feces were avoided due to the difficulties with extensive losses during the collecting and storing of fecal samples. The mechanism(s) involved in the reduced availabilities of vitamins A and E are not provided by this study but may have been related in part to an increase in fecal fat or a reduction in feed intake. Subsequent studies will investigate these possibilities using paired feeding techniques, and increasing fat intakes.

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