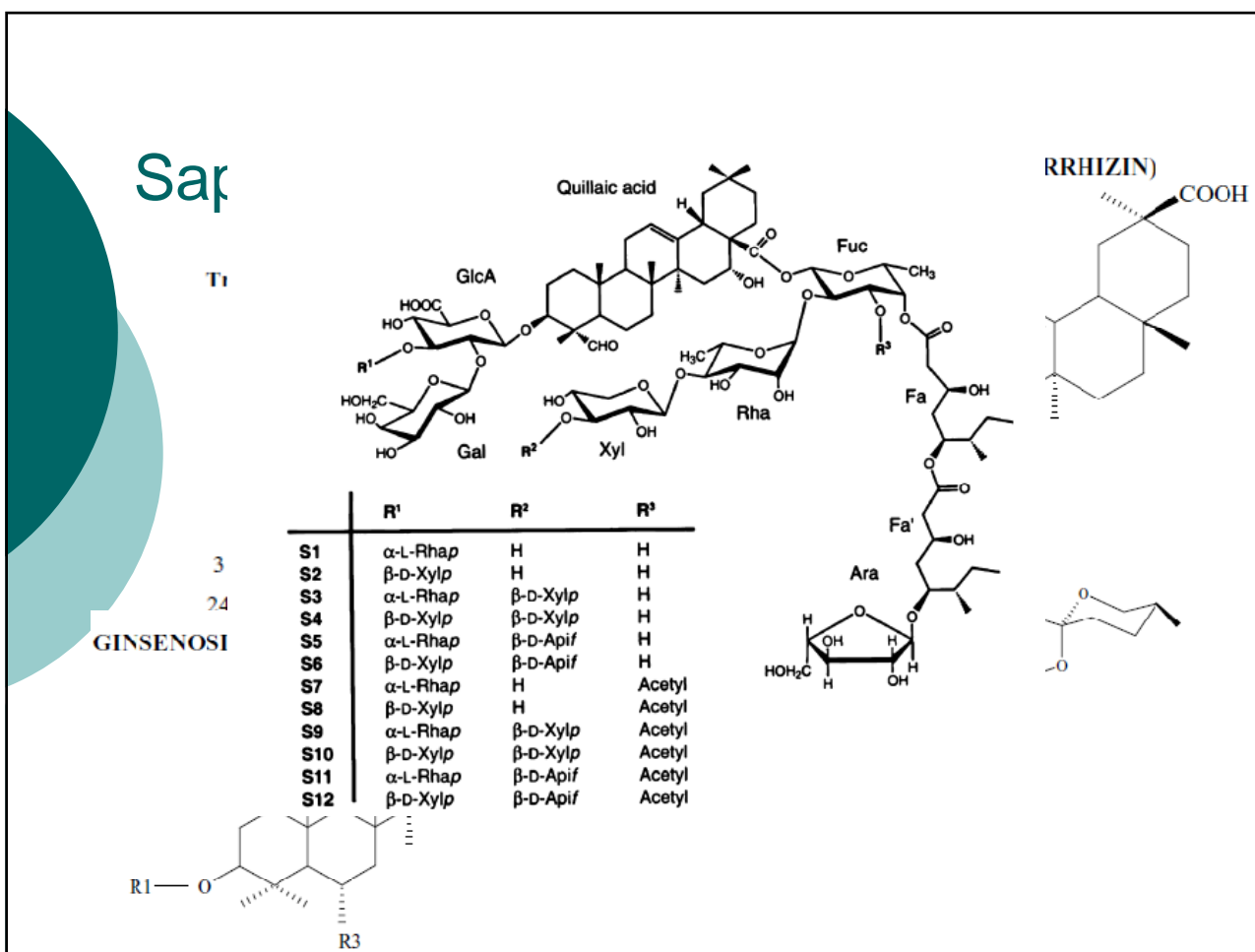


Use of saponins in livestock farming

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General

*Saponins are a **diverse** group of compounds **widely distributed** in the plant kingdom, which are characterized by their structure containing a **triterpene** or **steroid** aglycone and **one or more** sugar chains...*



General

The characteristics of saponins differ in accord with their individual structure.

The characteristics of saponin concentrate depend upon the unique composition, which in turn is determined by the plant origin, plant tissue, physiological state, and extraction protocol.

The biological activity depends not only on the saponin structure, but also on the matrix, tissue or organ,

Just a little history - adverse effects

- Saponins were commonly recognized by animal nutritionists to be deleterious compounds (Cheeke, 1996, Sen et al. 1998).
- Dietary saponins depressed growth, feed consumption in gerbils and egg production in poultry (Sim et al. 1984; Terapunduwat & Tasaki, 1986; Potter et al. 1993; Jenkins & Atwal, 1994).

Just a little history - possible causes for the adverse effects

- reduced feed intake caused by the astringent and irritating taste of saponins (see Oleszek et al. 1994, and Patra, 2010)
- reduction in intestinal motility (Klita et al. 1996)
- reduction in protein digestibility (Shimoyamada et al. 1998)
- inhibition of nutrient transport,
- production of active metabolites by intestinal bacteria (Bae et al. 2000)
- pasture bloat (Rochfort, 2008)

Just a little history

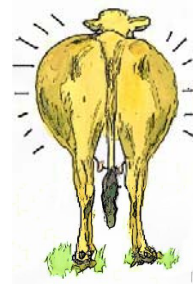


Still, the **safety** of saponins of commonly used **food and feedstuffs** such as soybeans, and alfalfa has been established by animal toxicology studies.

Ishaaya, I., Birk, Y., Bondi, A., and Tencer, Y. 1969. Soyabean saponins IX.— Studies of their effect on birds, mammals and cold-blooded organisms. *J. Sci. Food Agric.*, **20**:433–436.

Malinow, M. R., McNulty, W. P., McLaughlin, P., Stafford, C., Burns, A. K., Livingston, A. L., and Kohler, G. O. 1981. The toxicity of alfalfa saponins in rats. *Food Cosmet. Toxicol.*, **19**:443–445.

"Pasture Bloat" - are alfalfa saponins truly safe?



While improving biomass digestibility in alfalfa, its high protein content was shown to cause problems for ruminants, because it is too rapidly digested by rumen microorganisms.

This may lead, among other deleterious effects, to excess methane production in the rumen, exacerbated by foaming caused by a **combination of the high protein content** and presence of other agents such as **saponins** and leading to the condition known as **pasture bloat**.

The nature and effect of the saponin-protein interactions

- Dependent on the type of protein
- Dependent on the type of the saponin mixture
- Dependent on other components in the mixture

The nature and effect of the saponin-protein interactions

The stability of whey proteins to chymotryptic hydrolyses decreased upon addition of soybean saponins (Shimoyamada et al., 2000).

De-saponization of quinoa protein increased water hydration capacity and lowered the fat binding and buffer capacity, and total nitrogen solubility (Chauhan et al., 1999).

Back to farm animals: Reducing availability of hydrogen for methane production by ruminants

Saponins were shown to decrease protein degradation in the rumen and favor at the same time microbial protein and biomass synthesis

(Makkar and Becker, 1996).



Back to farm animals: Reducing methane production by ruminants

-
- Methane from livestock might accounted for up to 38% of the green house gases emission (recent reviews by Wina et al. 2005, Patra & Saxena, 2009, Martin et al, 2010, Patra, 2010, and Patra & Saxena, 2010).

Back to farm animals: Reducing methane production by ruminants

- It is recognized that in today's world, the production of methane during fermentation in the rumen of ruminant livestock makes a major contribution to the greenhouse effects.
- Many suggest that secondary metabolites from plants hold a promise to serve as natural, biodegradable and safe feed additives to inhibit enteric methanogenesis and replace synthetic chemicals.

Back to farm animals: Reducing methane production by ruminants

-
- Using statistical tool to visit 53 publications on the role of saponins in decreasing methane production, digestibility of OM (4.3%), especially NDF (10.6%), was **reduced by saponins**,
 - digestibility of CP (-1.3%) was hardly affected (Patra, 2010).

Back to farm animals: Reducing methane production by ruminants

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- The effect of saponins on methane production varied from **stimulatory** (19%) to **inhibitory** (42%) with an average of 11% inhibition,
 - The growth of **protozoa** ranged from an **increase** by 60% to a **decrease** by 79% with an average of 28% lower in the saponin database (Patra, 2010).

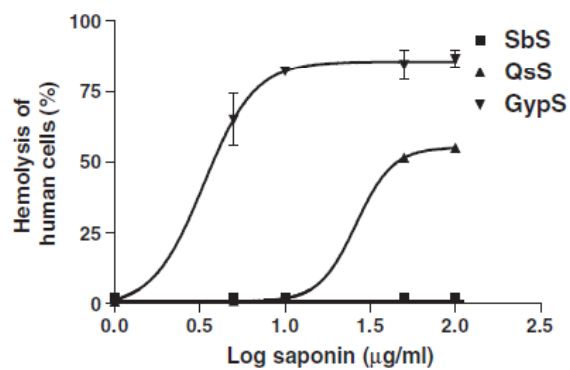
Protozoa

Protozoa cause rapid intra-rumen nitrogen cycling, and excess ammonia is excreted in the urine.

Saponins kill or damage protozoa, probably by forming complexes with sterols in the protozoal membrane surface.

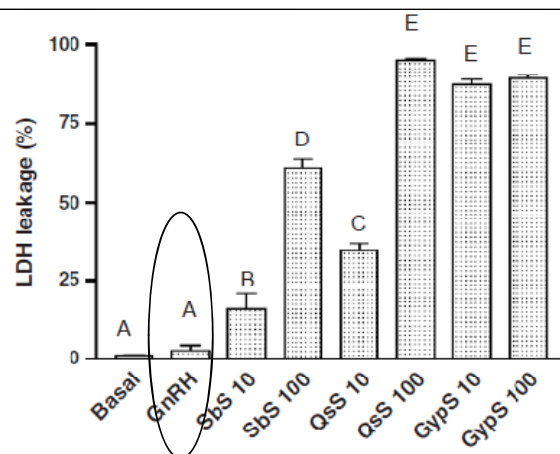
A compilation of *in vitro* and *in vivo* results on the effect of saponins or saponin containing plants on protozoa in the rumen is presented in Wina et al, 2005.

Saponins cause Hemolysis! ?



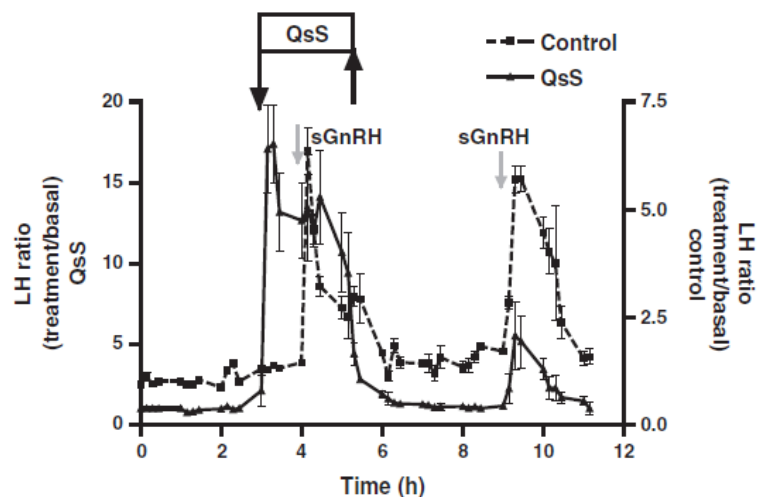
Resistance of human erythrocytes to hemolysis induced by soybean saponins (SbS), Quillaja saponaria saponin (QsS) or Gypsophila paniculata saponin (GypS) in isotonic buffer (Levavi Sivan et al, 2005)

Saponins disrupt membranes! ?



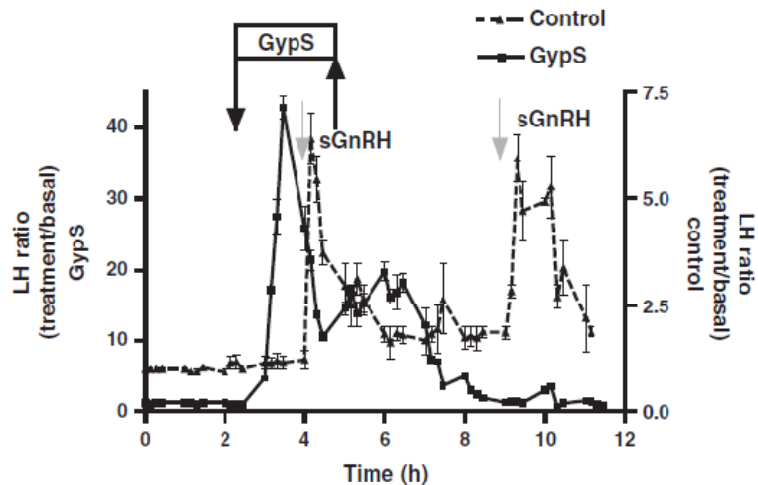
Effect of various concentrations (10 and 100 $\mu\text{g}/\text{mL}$) and different saponins on cell membrane permeability as measured by lactate dehydrogenase (LDH) leakage. (Levavi Sivan et al, 2005)

Saponins induce hormone secretion from fish tissues



Luteinizing hormone (LH) secretion from perifused tilapia pituitary fragments in response to *Quillaja saponaria* saponins (QsS; 2 h; 10 Ag/mL; black arrows) in the presence or absence of salmon GnRH (sGnRH; 5 min; 10 nM; grey arrows), (mean \pm FSEM). (Levavi Sivan et al, 2005)

Saponins induce hormone secretion from fish tissues - not all operate the same



Luteinizing hormone (LH) secretion from perifused tilapia pituitary fragments in response to *Gypsophilla paniculata* saponins (GypS; 2 h; 10 Ag/mL; black arrows; left axis) in the presence or absence of salmon GnRH (Levavi Sivan et al, 2005)

Disruption of tight junction integrity, and induced mammary involution in lactating goats by saponins - more about membrane integrity

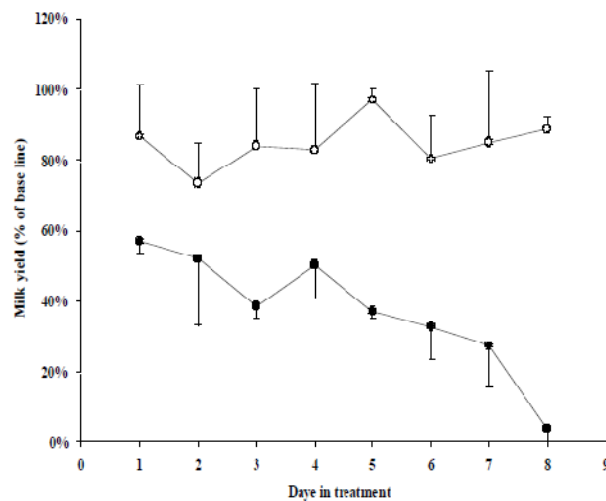
Mammary gland involution lead to cessation of milking, apoptosis of epithelial cells and tissue remodeling.

Unilateral cessation of milking in goats and teat-sealing in mice induced involution in the treated gland only.

Tight junction (TJ) in the epithelial cells of the mammary gland forms a barrier between the systemic (basolateral) and the milk (apical' sides) and prevents paracellular transport.

Milk stasis causes the accumulation of local signals, which makes the TJ leaky.

Disruption of tight junction integrity, and induced mammary involution in lactating goats by saponins



Milk yield of udder halves of goats subjected to intramammary injection of GpS (full circles) or control solutions (open circles; Mabjeesh et al, 2007)

Saponins that disrupt membranes do work, but do they get to their target?

Yet another common saying is that saponins are not absorbed:

For killing protozoa - OK. This happens in the GI tract.

For transient drying of mammary gland - OK. This follows injection to the relevant tissue.

For hormonal action - ? Saponins must be absorbed? If they do - might there be other effects?

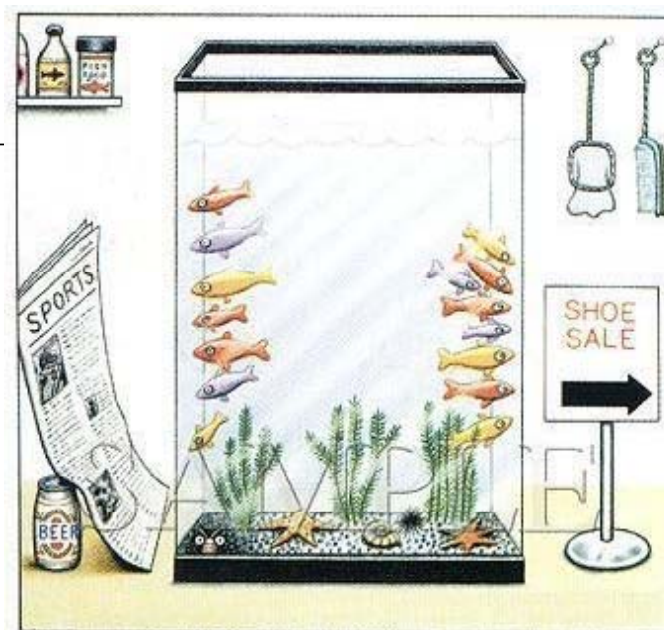
Saponins that disrupt membranes do work, but do they get to their target?

Before one answers this, why do they act as hormones, and is there an interest in their hormonal action?



Determination of sex in fish?

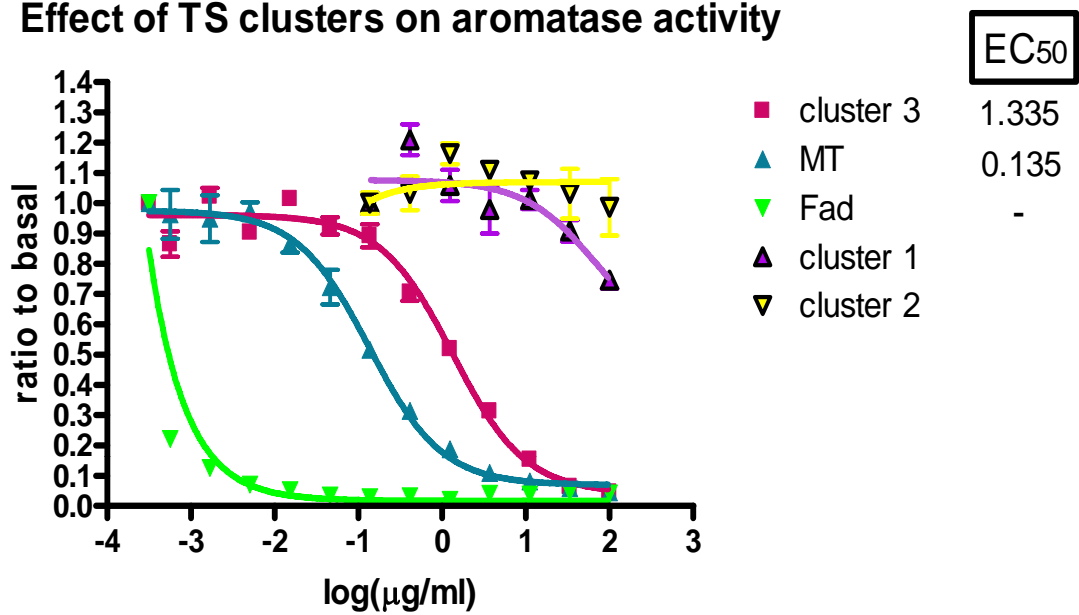
• sex inversion by non-metabolizable androgens. are hazardous to the ecological systems.



• We are currently in search for natural bioactive products. that will be used in both Israeli & Palestinian aquaculture

Using isolated saponins from *Trigonella foenum-graecum* to inhibit aromatase

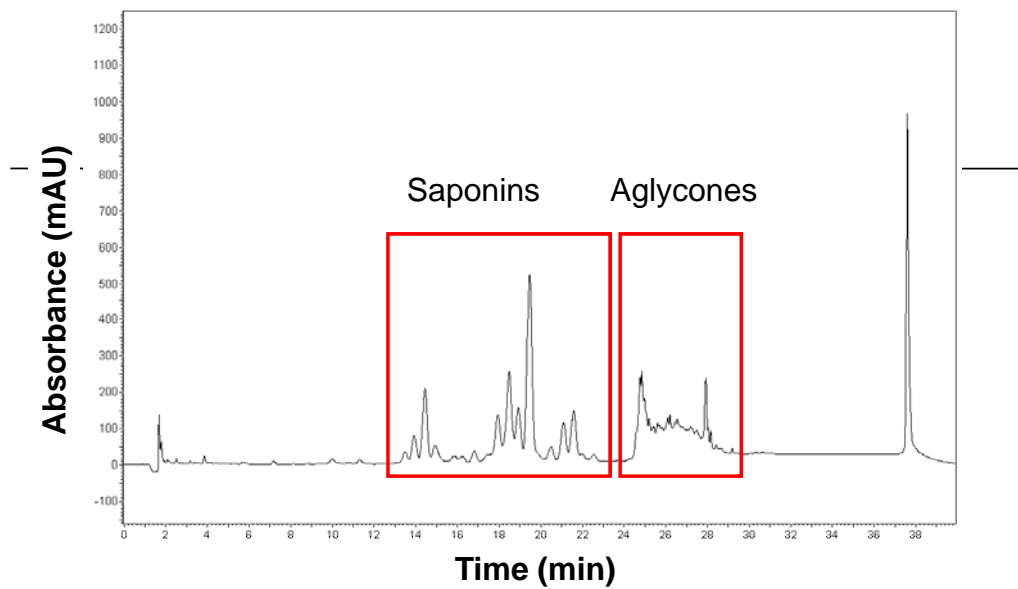
Effect of TS clusters on aromatase activity



Saponins... but do they get to their target?

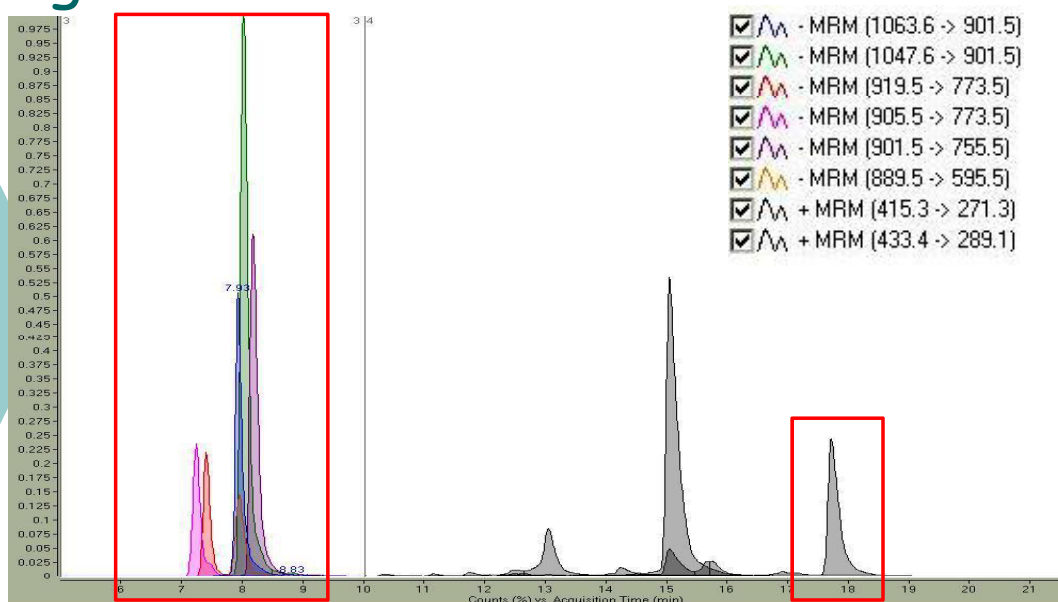
-
- In some cases hormonal action is sought for.
 - Only small fraction of a complete set of saponins in a plant may be responsible for a desired biological activity.
 - Will it get to its target? In this case - the gonads?

Saponins... but do they get to their target?



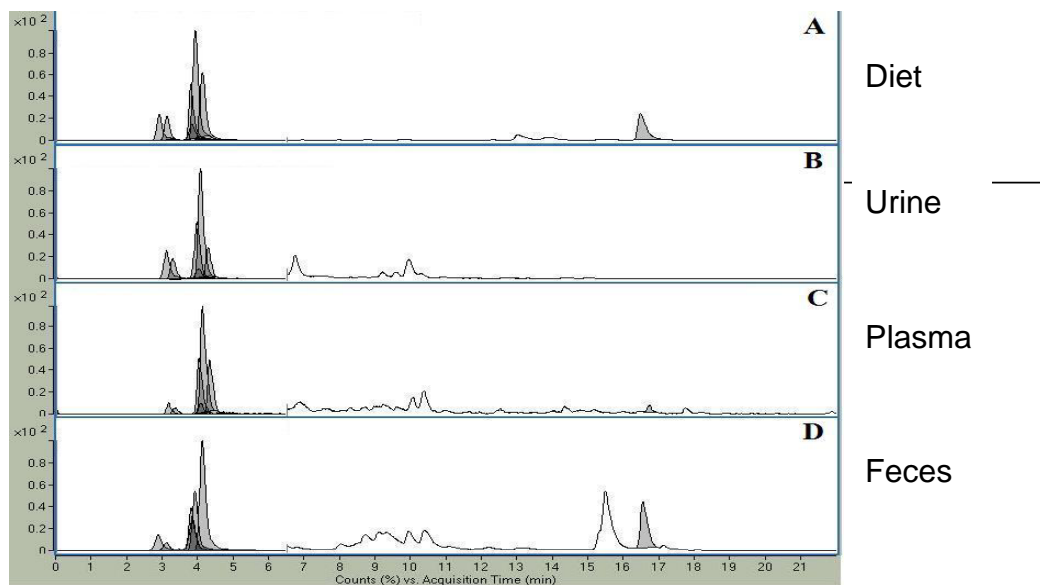
Using HPLC to fractionate saponins from *Trigonella*

Saponins... but do they get to their target?



Using LC-MS, and MRM to identify the various saponins from *Trigonella*

Saponins... but do they get to their target?



Demonstrating that saponins from *Trigonella* are absorbed, using LC-MS, and MRM

Accurate masses and MS/MS data of some saponins in extract from *Trigonella*

Name	RT, min	Calculated Atomic Composition	Measured Mass of [M-H]	Theoretical Mass	MS/MS Data
Trigoneoside Ia *	7.75	C ₄₄ H ₇₃ O ₁₉	905.47483	905.47515	905.4-(C ₃ H ₈ O ₄)→773.4-(C ₆ H ₁₀ O ₅)→611.4-(C ₆ H ₁₀ O ₅)→449.3 (C ₂₇ H ₄₅ O ₅)
Trigoneosides IIa or IIb*	8.56	C ₄₄ H ₇₃ O ₁₈	889.48083	889.48024	889.5-(C ₃ H ₈ O ₄)→757.4-(C ₆ H ₁₀ O ₅)→595.4-(C ₆ H ₁₀ O ₅)→433.3 (C ₂₇ H ₄₅ O ₄)
Trigofoenoside A or glycoside D or trigoneosides XIIIa or b***	8.23, 8.58, 8.75	C ₄₅ H ₇₃ O ₁₈	901.47980	901.48024	901.4-(C ₆ H ₁₀ O ₄)→755.4-(C ₆ H ₁₀ O ₅)→593.3; 755.4-(C ₆ H ₁₂ O ₆)→575.3; 593.3/575.3-(C ₆ H ₁₀ O ₅)→431.3/413.3 (C ₂₇ H ₄₁ O ₅ /C ₂₇ H ₄₃ O ₄)
Trigoneosides Xa or Xb***	7.90, 8.31	C ₄₅ H ₇₅ O ₁₉	919.49034	919.49080	919.4-(C ₆ H ₁₀ O ₄)→773.4-(C ₆ H ₁₀ O ₅)→611.4-(C ₆ H ₁₀ O ₅)→449.3 (C ₂₇ H ₄₅ O ₅)
Trigonelloside C or compound C***	8.60	C ₅₁ H ₈₃ O ₂₂	1047.53881	1047.53815	1047.5-(C ₆ H ₁₀ O ₄)→901.5-(C ₆ H ₁₀ O ₅)→755.4-(C ₆ H ₁₀ O ₅ , H ₂ O)→575.3-(C ₆ H ₈ O ₄)→431.3 (C ₂₇ H ₄₃ O ₄)
Trigoneosides Va and Vb**	8.21	C ₆₈ H ₁₁₁ O ₃₇	1519.68066	1519.68097	1519.7-(C ₆ H ₁₀ O ₅)→1357.6; 1519.7-(C ₃ H ₈ O ₄)→1387.6-(C ₆ H ₁₀ O ₅)→1225.6-(C ₆ H ₁₀ O ₅)→1063.5-(C ₆ H ₁₀ O ₅)→901.5-(C ₆ H ₁₀ O ₄)→755.4-(C ₆ H ₁₀ O ₅)→593.4

Summary

- Saponins show many biological activities, some of which may become highly important in producing farm animals
- Saponins have the great advantage of being biodegradable
- The effects of saponins in a selected bio-system vary greatly due to the great variability and complexity of their structures
- Reported results thus represent disagreements in many cases

Summary

- Some activities were not discussed here, such as immunostimulation and use for vaccines, antiviral actions, growth enhancement (i.e, not only in ruminants but also in fish!), lowering lipid absorption in monogastrics, determination of GI fauna, and interaction with subscribed drugs.
- To claim for an activity, it is obligatory to establish the mechanism of action, effective and toxic doses.



Thank you

Colleagues:

Prof K. Becker
Prof B. Levavi Sivan
Prof Y. Vodovotz
Prof S. Schwartz
Lab tech. Mrs P.
Weinberg

Students:

Adi Nudel. Haya Kazaz, Tal
Luzzato, Yizhar
Tugendhaft, Luciana
Nathan, Loai Bashir, Mor
Wilk, Mohamed Majdob,
Guy Harlev, Eti Ziv, Sivan
Cohen, Doron Hershkovitz
Uriel Ben-Haim, and Dror
Simon

