

## Effects of *in vitro* and *in vivo* dietary supplementation with saponins on rumen fermentation with particular reference to volatile fatty acids, ammonia and methane

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**Introduction** Saponins occur in plants as secondary metabolites. These compounds are able to shift the rumen fermentations resulting in decreased ammonia (NH<sub>3</sub>) concentration in the rumen fluid and mitigation in methane (CH<sub>4</sub>) production (Wina *et al.*, 2005). *In vivo* results are not always consistent with data obtained *in vitro*. The aim of this study was (i) to evaluate *in vitro* the effects of a product based on saponins (Yuquina<sup>®</sup> M extract, Nor-Feed Sud, France) on VFA, CH<sub>4</sub> and NH<sub>3</sub>, and (ii) to compare the *in vivo* results to data obtained from *in vitro* experiments on goats using similar diets and amount of Yuquina<sup>®</sup> M extract/mL of rumen fluid.

**Material and methods** The total saponin content in Yuquina<sup>®</sup> M extract (YME) was estimated by gravimetric method according to Yao *et al.* (2010). The saponin content was calculated as the mass of dried butanolic extract. A substrate based on grass silage/maize silage/concentrate (30/30/40) was incubated without or added with any of 12 doses of YME (from 0.04 to 40 g/L DM, with results of 0.1, 0.2 and 1.0 g/L DM shown in Table 1) for *in vitro* rumen fermentations, conducted as described by Castro-Montoya *et al.* (2012). After 24 h at 39 °C, flasks were sampled for gas, NH<sub>3</sub> and VFA determination. A 17 days *in vivo* trial was conducted with 24 dry Murciano-Granadina goats split into 1 control group and 3 treated groups. Animals were fed twice a day a diet similar to the substrate used *in vitro*. Concentration in YME for treated animals was 0.525, 1.050 and 5.250 g/goat/day DM. Methane measurement was done at the end of the experiment in respiratory chambers during two consecutive days. Afterwards rumen content was sampled for VFA and NH<sub>3</sub> analysis. Feed intake was recorded and urine was collected for purine derivative analysis. Means were compared by two-way unbalanced variance analysis (ANOVA) with subsequent post-hoc multiple comparison test of Duncan using XLSTAT (version 2011.2.04, Addinsoft, USA).

**Results** Total saponin content was estimated at 341 mg/g in YME by gravimetric method. Similar effects were observed *in vitro* and *in vivo*. Compared to the control, methane production decreased significantly from 0.2 g YME/L DM *in vitro* and from 0.525 g YME/goat/day DM *in vivo* (p<0.05). Total VFA production (*in vitro*) and concentration (*in vivo*) increased significantly at the highest dosages (p<0.05). Ammonia, isobutyrate and isovalerate decreased from the lowest dosages *in vitro* and *in vivo*. On the contrary, the acetate/propionate ratio decreased *in vitro* only. YME significantly decreased feed intake (-8%, p<0.05) at the highest dose in goats. The concentration of total urinary purine derivatives was numerically higher (+25%) from 0.525 g/goat/day DM.

**Table 1** Rumen fluid parameters

Concentration	<i>In vitro</i> (g YME/L DM)					<i>In vivo</i> (g YME/goat/day DM)				
	0.00	0.1	0.2	1.0	s.e.d	0.000	0.525	1.050	5.250	s.e.d
Total VFA (mmol/L)	40.6 <sup>a</sup>	39.2 <sup>a</sup>	41.4 <sup>a</sup>	46.7 <sup>b</sup>	1.0	36.5 <sup>a</sup>	41.8 <sup>ab</sup>	40.7 <sup>a</sup>	58.9 <sup>b</sup>	3.7
Molar proportion (%)										
Acetate	63.2 <sup>a</sup>	63.7 <sup>a</sup>	63.7 <sup>a</sup>	61.0 <sup>b</sup>	0.3	60.1	63.4	63.2	62.8	0.8
Propionate	21.3 <sup>a</sup>	21.6 <sup>a</sup>	22.3 <sup>ab</sup>	26.2 <sup>c</sup>	0.6	15.9	15.7	17.2	16.8	0.5
Butyrate	11.9 <sup>a</sup>	11.6 <sup>ab</sup>	11.3 <sup>b</sup>	10.2 <sup>c</sup>	0.2	12.5	12.0	10.4	13.1	0.5
Isobutyrate	0.8 <sup>a</sup>	0.6 <sup>b</sup>	0.5 <sup>b</sup>	0.4 <sup>b</sup>	0.1	4.7 <sup>a</sup>	3.5 <sup>ab</sup>	3.8 <sup>ab</sup>	3.0 <sup>b</sup>	0.2
Valerate	1.2	1.2	1.2	1.1	0.3	1.9	1.5	1.6	1.5	0.1
Isovalerate	1.2 <sup>a</sup>	1.1 <sup>b</sup>	0.9 <sup>c</sup>	0.8 <sup>d</sup>	0.1	5.0 <sup>a</sup>	3.9 <sup>b</sup>	3.8 <sup>b</sup>	3.0 <sup>b</sup>	0.3
Acetate/propionate	2.97 <sup>a</sup>	2.95 <sup>a</sup>	2.86 <sup>b</sup>	2.33 <sup>c</sup>	0.1	3.85	4.06	3.78	3.79	0.1
NH <sub>3</sub> (mg/100mL)	14.5 <sup>a</sup>	11.3 <sup>ab</sup>	10.1 <sup>b</sup>	10.8 <sup>b</sup>	0.7	21.7 <sup>a</sup>	18.6 <sup>ab</sup>	17.8 <sup>ab</sup>	15.1 <sup>b</sup>	1.2
CH <sub>4</sub> (mmol/day)	0.337 <sup>a</sup>	0.332 <sup>a</sup>	0.322 <sup>b</sup>	0.298 <sup>c</sup>	0.005	798.5 <sup>a</sup>	582.8 <sup>b</sup>	666.8 <sup>ab</sup>	495.8 <sup>b</sup>	42.8
Relative CH <sub>4</sub> <sup>*</sup>	332.9 <sup>a</sup>	340.5 <sup>a</sup>	310.0 <sup>a</sup>	255.9 <sup>b</sup>	10.7	27.7	22.1	25.3	31.8	1.9

\* CH<sub>4</sub>/VFA (mmol/mol) *in vitro* and CH<sub>4</sub>/feed intake (L/kg) *in vivo*

<sup>a, b, c</sup>: Means in the same row with different superscripts differ (P < 0.05 for *in vitro* and P < 0.10 for *in vivo* trial)

**Conclusions** These results show that the concentration in YME to reduce rumen NH<sub>3</sub> is lower than the concentration to decrease CH<sub>4</sub> production *in vitro*. The lowest dose was enough to observe both NH<sub>3</sub> and CH<sub>4</sub> inhibition *in vivo*. Consequently, dose effect was observed only *in vitro* and it could be interesting to evaluate the lowest efficient dose of YME *in vivo*.

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### References

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