Effect of calcium soaps from garlic and *Salix babylonica* extracts on nematode loads, nutrient intake and digestibility, nitrogen balance, and rumen fermentation kinetics in dairy goats

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SUPPLEMENTARY FILE

Material & methods

Preparation of the extracts

Every week, 4 L of Salix and Garlic extracts were prepared. For the preparation of Salix extract, *S. babylonica* fresh leaves were randomly collected from mature trees during the summer and autumn season (10 kg of fresh material). Leaves were chopped into 2–3 cm lengths and immediately extracted soaking leaf material with water (1 g leaf per 8 ml of water). Plant material was soaked and incubated at 25 °C for 72 h in closed jars of 5 L. After incubation, jars were heated at 39 °C for 1 h and then filtered. Filtrated material was stored at 4 °C until its use. For garlic, 10 kg of fresh material was obtained from a local supermarket. Both materials from Salix and Garlic were ground at 2mm in a blender using 500 g from each one and then 1 kg of safflower oil (*Carthamus tintorius*) was added, allowing the mixtures to stand for a period of 30 days in amber bottles for later use.

Saponification process

After a 30-day period, the process of fat saponification was performed following the double decomposition method of Jenkins and Palmquist (1984), using safflower oil as control and the Garlic and Salix extracts. Calcium soaps from safflower oil were chosen as control because they have shown to have more energy density than those from with rapeseed, corn, soybean, and sesame oils (Romero-Davila et al. 2017).

For calcium soaps preparation, in each of the three treatments, 1L of safflower oil, 250 g of calcium chloride, 50 grams of calcium carbonate, 300 ml of alcohol 96%, 1370 g sodium hydroxide at 30%, and 500 g of fresh leaves of Salix or Garlic was used. All the ingredients were mixed until obtaining a homogeneous paste; the mixture was placed in glass containers in a forced air oven at 60 °C for 48 hours to dehydrate each of the samples. This process was carried out separately for each treatment.

Rumen fermentation kinetics

In vitro gas production (GP) test was performed according to Theodorou *et al*. (1994). Briefly, 800 mg of DM of each diet was placed in 125 mL glass flasks and 90 mL of buffer solution and 10 mL of rumen fluid were added to them. The buffer solution was prepared according to the technique of Menke and Steingass (1988), in which 0.800 g DM of each ingredient and each diet mixture were incubated in glass flasks of 125 ml, to which 90 ml of buffer solution and 10 ml of rumen fluid were added, to make three bottles per sample. Three incubation runs were carried out as replicates, in each incubation run, three flasks per treatment were used, a set of appropriate blanks were included to adjust for the potential effects of other soluble materials in the extracts on overall gas production and for readings correction of substrates, including bottles from self-fermentation of rumen inoculum. Rumen fluid (300 ml per animal) was collected from the three-rumen cannulated lactating goats (40 ± 3 kg of body weight, means ± SD) into a pre-warmed thermos flask, and then filtered and flushed with CO_2. GP was measured using a pressure transducer (Delta Model HD 8804, Italy) at 6, 12, 24, 48, 72, and 96 hr and a set of blanks were included. GP was registered using a pressure transducer (Delta Model HD 8804, Italy). After incubations, samples were filtered and dried for 48 h at 65 °C for dry matter disappearance (DMd) determination. For relative gas production (RGP, mL gas/g DMd) determination, gas production at 96 h was correlated with DMd. Kinetic parameters GP were estimated through an iterative procedure of non-linear regression analysis (PROC NLIN, SAS Institute Inc., Cary, NC) according to Krishnamoorthy *et al.* (1995):

$$GP = B\,(1\text{-}e^{-C(t-1)})$$

Where GP is the volume of gas (ml gas / g DM) at time t; B is the asymptotic GP (ml/gDM); C is the fractional rate of GP (g/h), and *l* (h) is the discrete lag time prior to gas production.

After the in vitro incubation period, the samples were filtered and dried (48 h, 60 °C) to measure the dry matter disappearance (DMD96h), and gas yield production at 24 h (GY24) was determined. The volume of gas (ml gas/g DM) produced after 24 h of incubation was calculated by dividing the amount of DMD (g):

Gas production (GY24) = ml gas 24h /g DMD96h

The gas production (GP) at 96 h was correlated with DM disappeared to produce relative gas yield (RGY; ml gas/g DMd) (González Ronquillo et al. 1998).

RGY = ml gas 96h / g DMd96h

Short chain fatty acids concentration (SCFA) was calculated according to Getachew et al. (2002) as: SCFA (mmol/200 mg DM) = 0.0222 GP - 0.00425. Where: GP is the 24 h net gas production (ml/200 mg DM).

Microbial biomass production (MCP) was calculated according to Blümmel et al. (1997) as: MP (mg/g DM) = mg IVDMD - (ml gas × 2.2 mg/ml). Where 2.2 mg/ml is a stoichiometric factor, which expresses mg of C, H and O required for the SCFA gas associated with production of one ml of gas (Blümmel et al. 1997).

Chemical analysis for diets, feces, and urine

To determine the DM content of feed**,** refusals and feces**,** samples were dried in a forced air oven (60°C, 48 h), and subsequently ground in a Wiley mill 3mm Ø (Arthur H. Thomas, Philadelphia). Organic matter, ether extract, and crude protein (N × 6.25) contents were determined using standard procedures (AOAC 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the ANKOM technique (Van Soest et al. 1991) with alpha amylase and uncorrected for ash.

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Table S1. Chemical composition (g/kg DM) of ingredients used in experimental diets.

Item	Safflower calcium soap	Garlic calcium soap	Salix calcium soap	Alfalfa hay	Rapeseed	Sorghum grain	SBM	Wheat bran	Corn stover
Dry matter	886	874	860	930	920	898	880	874	917
Organic matter	607	628	463	900	930	980	930	955	945
Crude protein	2	9	4	170	360	100	460	160	50
RDP	2	4	2	119	252	70	322	112	35
Fat	701	615	533	30	32	35	20	40	20
NDF	14	15	18	449	305	430	134	487	722
ADF	8	10	12	254	152	58	92	122	410
ADL	3	2	7	128	98	44.5	35	68	90
ME, Mj/Kg DM	25.0	23.0	21.0	10.0	12.1	13.5	13.0	11.0	7.8

SBM, soybean meal; RDP, Rumen degradable protein; NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber; ADL, AcidDetergent. Lignin; ME, Metabolizable energy (MJ/kg DM).

Table S2. Ingredients and chemical composition (g/kg DM) from control, Garlic and Salix diets

Item	Treatments[1]		
	Control	Garlic	Salix
Ingredients (g/kg DM)			
Alfalfa hay	600	600	600
Corn stover	60	60	60
Sorghum grain	121	121	121
Soybean meal	40	40	40
Rapeseed	40	40	40
Wheat bran	40	40	40
Calcium soaps	65	65	65
Vitamin and mineral premix[2]	33	33	33
Chemical composition			
Dry matter, DM	939	938	937
Organic matter, OM	878	867	884
Crude protein, CP	143	136	136
Neutral detergent fiber	426	412	440
Acid detergent fiber, ADF	224	216	228
Acid detergent lignin, ADL	97	72	62.5
Ether extract, EE	120	118	128

[1] Diets supplemented with 65 g/kg DM of calcium soaps from either safflower (Control), garlic extract (Garlic), or *Salix babylonica* extract (Salix), respectively.

[2]Containing in 1.0 kg DM the following: 25 mg of antioxidant, 4.5 g of calcium carbonate, 6 g of salt, 30 g of ionophore, 50 g of zinc oxide, 6 g of sodium bicarbonate, 6 g of copper sulphate, 20 g of ferrous sulphate, 125 g of sodium sulphate, 18,000 IU of vitamin E, 3,000,000 UI of vitamin A, 3,750,000 IU of vitamin D, 140 g of potassium chloride, 0.500 g of EDD. I ethylene-dynamine, 0.090 g of cobalt carbonate, 500 mg of magnesium oxide, 36 g of manganese oxide and 0.090 g of selenium.

Table S3. *In vitro* rumen gas kinetics (ml gas/g DM) and fermentation profile of goats fed with calcium soaps of Garlic and Salix extracts.

Parameters[1]	Treatments[2]			SEM	P-value
	Control	Garlic	Salix		
A	179	191	183	9.43	0.250
B	0.04	0.04	0.42	0.003	0.333
Lag time	2.39	2.23	2.00	0.29	0.237
Gas production, ml gas/g DM					
6 h	18.9	21.3	24.2	3.21	0.120
12 h	67.9	67.4	72.3	6.47	0.521
24 h	104	109	112	6.28	0.257
48 h	146	155	152	7.74	0.314
96 h	177	187	183	8.55	0.256
DMD96	585	587	582	15.0	0.897
RGP	302	319	315	15.9	0.340
GY24	178	186	192	13.1	0.361
SCA	0.30	0.30	0.30	0.002	0.356
MCP	555	557	550	16.8	0.835
ME, MJ/kg DM	14.0	13.6	13.9	0.34	0.224

[a-c] Means with different superscript letters within a row are different ($P \leq 0.05$).

[1] A, total gas production (ml gas/g DM incubated); B, fermentation rate (h-1); C, fermentation rate (h-1/2); L, the initial delay before gas production begins (h); DMD96, DM degraded substrate (mg/g DM); RGP, relative gas production (mL gas/ g DMD96); SCFAs, short chain fatty acids (mmol/g DM); GY24, gas yield at 24 h (mL gas/g DMD); MCP, microbial CP production (mg/g DM).

[2] Diets supplemented with 65 g/kg DM of calcium soaps from either safflower (Control), garlic extract (Garlic), or *Salix babylonica* extract (Salix), respectively

SEM = Standard error of the mean

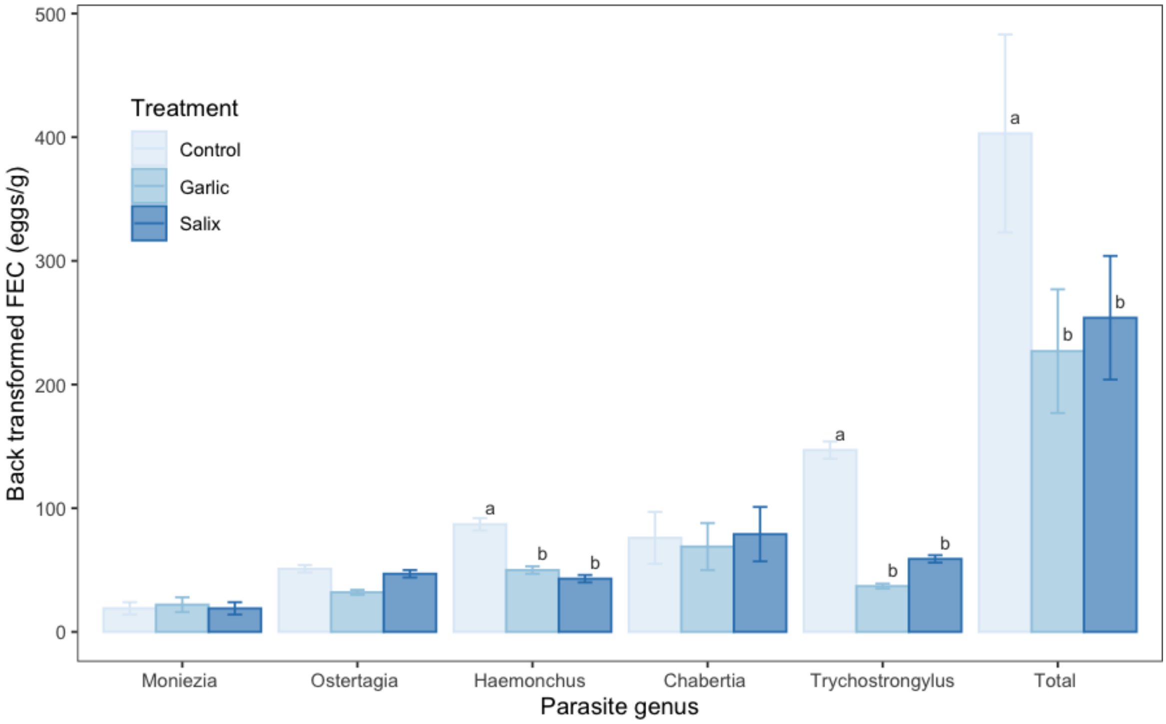

Figure S1. Back transformed FEC (egg/g) of according with parasite genus.

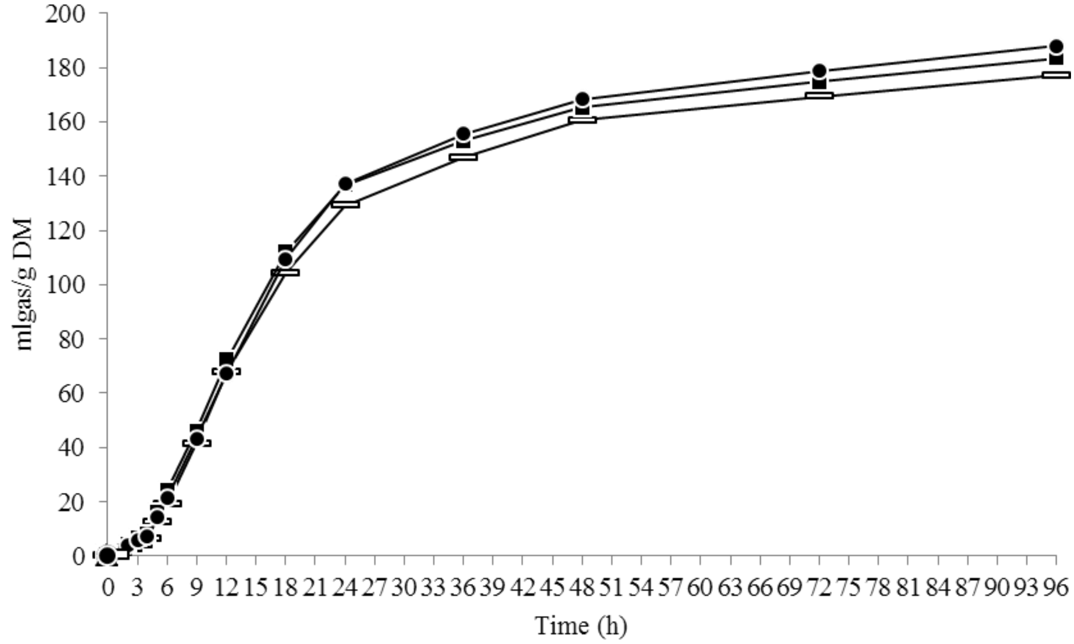

Figure S2. Cumulative gas production at 96 h (ml gas / g DM) of diets supplemented with calcium soaps of Safllower (☐), Garlic (●), and Salix (■) extracts.