

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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Abstract

Global small ruminants' sector is under scrutiny and is challenged by sustainability issues. The infections caused by gastrointestinal nematodes (GIN) are some of the main concerns that are responsible for both economic and productive losses. Natural extracts may be a useful as a part of an integrated parasite control. It has been shown that both Garlic (Garlic) and *Salix babylonica* (Salix) have anti-microbial and anti-parasitic properties. The objective of this study was to determine the effect of dietary calcium soaps of Garlic and Salix extracts on nematode loads, nutrient intake and digestibility, nitrogen balance, and rumen fermentation kinetics in dairy goats. A completely randomized design was used with 9 Saanen does, 3 in each of the three treatments over a period of 28 days. Animals were fed a diet based on alfalfa hay and a concentrate that was supplemented (65 g/kg DM) with calcium soaps of either safflower (Control), Garlic, or Salix. Intake of dry matter (DM), organic matter (OM), and neutral detergent fiber (NDF) were not affected by dietary calcium soaps. However, the highest digestibility of DM ($P = 0.02$) and OM ($P = 0.01$) was observed in Salix supplemented goats besides a tendency to increase ($P = 0.06$) NDF digestibility. *In vitro* gas kinetics and fermentation profile were not affected by diets. Results from fecal egg count indicated a reduction ($P = 0.003$) for Salix (67%) and Garlic (66%) compared to Control. Overall, calcium soaps of garlic or Salix extracts can be used to reduce gastrointestinal parasites in goats without compromising animal's productive traits and rumen function.

Introduction

Worldwide, gastrointestinal nematodes (GIN) remain the main challenge associated with small ruminant production since these infections impose a serious problem on health, welfare, productivity, and reproduction of animals (Hoste et al. 2022; Oliveira Santos et al. 2019). Goats and sheep are infected with the same GIN species, mainly *Haemonchus contortus*, *Trichostrongylus axei*, *Teladorsagia circumcincta* (abomasum), *T. colubriformis*, *Strongyloides papillosus*, *Nematodirus* spp. (small intestine), *Oesophagostomum co. lumbianum*, and *O. venulosum* (large intestine) (Bentounsi et al. 2012). Nematode infections can decrease milk production, reduce growth, provoke weight loss and diarrhea, and reproductive disorders as well as changes in carcass quality (Oliveira Santos et al. 2019). Lastly, massive infections can result in high mortality rates (Froehlich et al., 2022).

Since the mid-1960s, GIN control in livestock has heavily relied on anthelmintics (Hoste et al. 2022), however, over-dependency and misuse of these drugs has led to anthelmintic resistance (AR) that has now become a growing problem on small ruminant farms in many countries (Froehlich et al., 2022; Oliveira Santos et al. 2019). Today, not only the efficient control of GIN infections is essential to improve livestock health and production (Bentounsi et al. 2012), but also widespread resistance to synthetic anthelmintic has inspired research for alternative strategies of parasite control, including the use of plant extracts (Hoste et al. 2022; Oliveira Santos et al. 2019).

It is well documented that the natural-derived feedstuffs can contain a wide array of plant secondary metabolites (PSM) arranged in a variety of chemical classes (Hoste et al. 2022) that may provide an alternative, cost-effective treatment to gastrointestinal parasites, limiting both drug use and development of anthelmintic resistance (Froehlich et al., 2022). According to Jerónimo et al. (2010), the continuous consumption of PSM-rich plants may cause an increase in the concentration of salivary proteins. These proteins have high affinity for tannin, which can lead to reducing the effect of this active compound. It has been suggested (Hoste et al. 2022; Novobilský et al. 2011) that feeding small ruminants with tannin-rich plants [30–40 g of condensed tannins per kg dry matter (DM)] promotes anthelmintic effect. However, condensed tannins, at higher concentrations (7–8% DM), can depress feed intake, decrease nutrient digestibility and overall production (Min et al. 2003).

Garlic (*Allium sativum*) has been reported to be a parasiticide, amebicide, larvicide and immune-stimulant, and garlic essential oil can be used as alternative or supplement to the current anthelmintic therapies (Zra et al. 2019). Garlic powder has been shown to increase rumen propionate, increase N retention and absorption in cattle (Kamruzzaman et al. 2011). Garlic oil has been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria (Zra et al. 2019; Arreola et al. 2015) which have been related to several small terpenoid and phenolic compounds (Kamruzzaman et al. 2011).

Little is known about the nutritive value of *Salix babylonica* (Salix) in goat diets; however, extracts of Salix have been evaluated as feed additives in ruminant diets due to its anti-microbial effects and its ability to modulate ruminal fermentation and improve nutrient utilization (Valdes et al. 2015). In addition, Salix extract enhanced digestibility and average daily gain (ADG) of lambs (Salem et al. 2014a), and improved milk production of cows (Salem et al. 2014b). It is also well established that Salix has natural anthelmintic activity (Cedillo et al. 2015; Salem et al. 2016).

Until now, the effects of using rumen bypass (as calcium soaps) plant extracts have not been analyzed in dairy goat nutrition and parasite loads. Therefore, the objective of this study was to determine the effect of dietary calcium soaps of Garlic and Salix extracts on nematode loads, nutrient intake and digestibility, nitrogen balance, and rumen fermentation kinetics in dairy goats. Results from this study will improve our knowledge on plant material alternatives to the use of conventional anthelmintic drugs.

Materials And Methods

Study site

The study was carried out in the Experimental farm from the Faculty of Veterinary Medicine of the Universidad Autónoma del Estado de México. The farm is located at 19° 24" north latitude, 99° 54" west longitude, and the average annual temperature is 27.2°C with a maximum 22°C for the summer season and a minimum of -3°C for the winter months.

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.

Animals, Experimental Design, And Diets

All animal studies were conducted according to the Animal Care and Use Committee of Veterinary and Animal Science from Universidad Autonoma del Estado de México (Toluca, Mexico) (Project ID UAEMex4335/2017).

Nine adult non-lactating Saanen does were grouped into a complete randomized block design with 3 treatments (n = 3). At the onset of the study, average body weight was 36.7 ± 6.2 kg (means $\pm$ SD) while body condition score (scored on a 5-point scale) was of 2.0 ± 0.1 (means $\pm$ SD). Prior to the beginning of the experiment, animals were grazing ryegrass (*Lolium perenne*), with daytime grazing from 0800 to 1600 h and night enclosure. For 28 days, animals were fed a diet based on alfalfa hay offered *ad libitum* and a concentrate (based on crushed rapeseed, sorghum grain, soybean meal, wheat grain, and corn stover) supplemented with calcium soaps of Garlic or Salix extract as potential anthelmintic.

Animals received 400 g of concentrate (16% CP and 2.8 Mcal ME/kg DM) every morning at 0700 h. The concentrate was supplemented with 65 g/kg DM of calcium soaps from either safflower (Control), garlic extract (Garlic), or *Salix babylonica* extract (Salix) and treatments were manually mixed. Table 1 shows ingredients and chemical composition of experimental treatments. Animals were housed in individual roofed pens with concrete flooring and straw bedding. Animals had individual drinkers and feeders. Water was offered *ad libitum*. Individual body weights were recorded on days 0, 7, 14, 21 and 28.

Table 1
Ingredients and chemical composition (g/kg DM) from control, Garlic and Salix diets

Item	Treatments ¹		
	Control	Garlic	Salix
Ingredients (g/kg DM)	600	600	600
Alfalfa hay			
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 <i>Salix babylonica</i> 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.			

Parasite Analysis

All goats were naturally infected with gastrointestinal parasites and were divided into three groups. Animals were monitored for parasite loads of *Haemonchus contortus*, *Moniezia spp.*, *Ostertagia spp.*, *Chabertia spp.*, and *Trychostrongilus spp.* Fecal samples (4 g/animal) were taken from the rectum on days 0, 14, 21 and 28. Fecal samples were removed using examination gloves with lubricant, and then samples were individually bagged, labeled, and kept refrigerated at 4°C until further analysis.

Fecal samples were analyzed on the same day of the collection, where number of eggs per gram of feces (FEC) were quantified using the modified McMaster technique (Morales and Pino, 2009) and a sugar centrifugal flotation technique with a saturated sugar solution ($\delta = 1200\text{--}1300$), and the efficiency of the treatments (%) was estimated. The fecal egg count reduction per gram of feces (FECRT) was determined according to the method described by Coles et al. (1992). The FECRT was calculated by comparing the post-treatment arithmetic mean FEC. Resistance was defined as < 95% reduction in FEC with a 95% confidence interval (CI) of less than 90%. The results were considered inconclusive if the FEC differentiated from the control group for a particular genus and was less than 25 eggs/g (Waghorn et al. 2006).

Rumen Fermentation Kinetics

After 28 d of the nutritional intervention, for 5 days, does were housed in a roofed pen with individual metabolic cages (1.20 × 0.80 m) with slatted floor and had free access to water. Each cage had individual drinker and feeders. During those days, daily records of feed intake, and amount of feces and urine were collected. Samples of feed, feces, and urine were collected at 0800 h and were frozen at -20°C until analyzed. For feces and urine, only 10% of the total sample collected was used for analysis.

Feces and urine samples were used to determine N intake and excretion. Urine N was analyzed using a standard procedure (954.01) (AOAC 1990). For feces, dry matter (DM; 930.15), organic matter (OM; 942.05) and nitrogen (N; 990.02) were determined using the AOAC (1990) methods, while neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined following Van Soest et al. (1991) methods with alpha amylase and uncorrected for ash. Nutrient digestibility (g/kg) was determined as [(nutrient intake, g/d – nutrient excreted, g/d) / (nutrient intake, g/d)] × 1000.

The in vitro gas production technique (Theodorou et al. 1994) was used to determine the kinetics of rumen fermentation. Three rumen cannulated lactating goats were used as donors of rumen fluid (40 ± 3 kg of body weight, means ± SD) and were fed with the control diet. Equal amounts of rumen fluid were collected and filtered through four layers of cheesecloth. 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. Two additional bottles without substrate were also prepared as blanks 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. The bottles were incubated in a water bath at 39°C. The gas volume was recorded at 6, 12, 24, 48, 72, and 96 hours of incubation in three series of incubations. Gas production 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 of gas production (GP) were estimated through an iterative procedure of non-linear regression analysis (PROC NLIN, SAS Institute 2002) according to Krishnamoorthy et al. (1995), calculated as:

$$GP = B(1 - e^{-C(t-l)})$$

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.

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.

Calculations And Statistical Analysis

Efficacy was determined as follows: $\text{Efficacy} = (100[(C - T)/C])$, where C is the geometric mean between the controls treated with concentrate without natural extract and T is the geometric mean of the animals treated with Salix and Garlic.

Repeated measure analysis was performed to assess the effect of anthelmintic treatments on BW, DBW, FEC, resistance, and % of parasite eggs as dependent variables. Each data set was analyzed using Kolmogorov-Smirnov and Shapiro-Wilk to test for normality and then with Levene's test for homoscedasticity evaluation. The randomness of observations was ensured with the random allocation of experimental units to each treatment. The experimental design of repeated measures was determined to carry out multiple measures on the same experimental unit (goat), therefore, the usual assumptions about the independence and homogeneity of error variances were no longer valid, to deal with this issue mixed models were implemented, which allows modeling several covariance structures for repeated measures data, and to account within-subject correlation. Therefore, the effect of fixed effect (treatment, time, and treatment × time) and random effects (subject: goat) on dependent variables was analyzed using the following model:

$$Y_{ijt} = \mu + \alpha_i + S_{j(i)} + \Upsilon_t + (\alpha\Upsilon)_{it} + \epsilon_{ijt}.$$

Where Y_{ijt} is the dependent variable measured at time t; μ is the overall mean effect; α is the fixed effect of anthelmintic treatment; $S_{j(i)}$ is the random effect of the j^{th} goat within the i^{th} anthelmintic treatment; Υ_t is the fixed t^{th} time effect when the measurement was taken; $(\alpha\Upsilon)_{it}$ is the fixed effect of the interaction between the anthelmintic treatment and the time; and ϵ_{ijt} is the random error. Post hoc pairwise comparison was carried out using the Tukey's HSD test. Significance was declared with $P < 0.05$, and trends were declared at $P < 0.10$. Several variance-covariance matrix [first-order autoregression AR(1), Toeplitz, unstructured, compound symmetry, and first-order ante-dependence ANTE(1)] were evaluated to choose which fit better to the data set. Akaike information criteria and Bayesian information criteria were used as indicators to select the best variance-covariance matrix, which was AR(1). All analyses were implemented and calculated in the PROC MIXED package of the SAS software (PROC MIXED, SAS Institute 2002).

Intake, digestibility and N balance, and in vitro GP data were subjected to analysis of variance (ANOVA) using the General Linear Model (PROC GLM, SAS Institute 2002).

$$Y_{ijk} = \mu + A_i + T_{(j)} + E_{(ij)}$$

Where: Y_{ijk} is the response variable in animal i, treatment j; μ is the Overall mean; A_j is the effect of the animal; $T_{(j)}$ is the effect of treatment; and E_{ij} is the random error.

The Tukey test ($P \leq 0.05$) was used to interpret any significant difference between the mean values.

Results

Nutrient intake, digestibility, and weight changes

Intake of dry matter (DM, $P = 0.87$), organic matter (OM, $P = 0.87$), neutral detergent fiber (NDF, $P = 0.90$), and acid detergent fiber (ADF, $P = 0.96$) were similar between treatments. The highest digestibility of DM ($P = 0.02$) and OM ($P < 0.01$) was

observed in *Salix* supplemented goats besides a tendency to increase ($P = 0.06$) for NDF digestibility. Moreover, ADF digestibility was higher in Garlic and *Salix* fed goats ($P = 0.02$, Table 2).

Table 2
Nutrients Intake (g/LW^{0.75}/day) and digestibility (g/100g) of goats fed with calcium soaps of Garlic and *Salix* extracts.

Item	Treatments ¹			SEM	P-value
	Control	Garlic	<i>Salix</i>		
Metabolic live weight ²	14.9 ^b	15.9 ^b	19.3 ^a	3.38	0.043
Intake (g/kg LW^{0.75})					
Dry matter, DM	61.5	59.7	60.4	2.96	0.876
Organic matter, OM	53.5	51.9	52.7	2.32	0.878
Neutral detergent fiber, NDF	27.5	26.8	27.2	2.31	0.902
Acid detergent fiber, ADF	12.3	12.3	12.4	2.32	0.960
Acid detergent lignin, ADL	5.40	5.36	5.44	2.32	0.966
Digestibility, g/100g					
Dry matter, DM	66.3 ^b	69.9 ^{bc}	71.2 ^c	0.81	0.028
Organic matter, OM	67.9 ^b	71.4 ^{bc}	74.7 ^c	0.72	0.005
Neutral detergent fiber, NDF	58.9	61.5	64.8	1.31	0.064
Acid detergent fiber, ADF	46.5	47.7	50.2	2.40	0.472
Acid detergent lignin, ADL	34.3 ^b	35.3 ^c	35.5 ^c	0.62	0.028
^{a-c} Means with different superscript letters within a row are different ($P \leq 0.05$). ¹ Diets supplemented with 65 g/kg DM of calcium soaps from either safflower (Control), garlic extract (Garlic), or <i>Salix babylonica</i> extract (<i>Salix</i>), respectively. ² Metabolic live weight= (Live Weight) ^{0.75} SEM = Standard error of the mean					

The results showed that the *Salix* fed goats had higher ($P = 0.001$) body weight compared to control and Garlic that decreased over time (time $P = 0.04$) from the beginning of the study until day 28. Daily body weight gains were similar ($P = 0.46$) between treatments. However, overall time effect was observed as daily body weight increased in day 28 as compared to day 0 (time $P = 0.001$; Table 2). The interactions between calcium soap supplements and time were not significant for both body weight (interaction $P = 0.98$) and daily body weight gains (interaction $P = 0.48$).

Nitrogen Balance

No differences were observed in N intake ($P = 0.35$), fecal N excretion ($P = 0.54$), urinary N excretion ($P = 0.78$), and N balance ($P = 0.803$) between the Control and calcium soap-supplemented goats (Table 3).

Table 3
Nitrogen balance (g N day/kg LW^{0.75}) of goats fed with calcium soaps of Garlic and Salix extracts.

Item	Treatments ¹			SEM	P-value
	Control	Garlic	Salix		
N intake	1.41	1.29	1.32	2.29	0.355
Fecal N excretion	0.44	0.41	0.41	0.032	0.544
Urinary N excretion	0.53	0.44	0.45	0.111	0.781
N balance	0.34	0.43	0.45	0.181	0.803
¹ Diets supplemented with 65 g/kg DM of calcium soaps from either safflower (Control), garlic extract (Garlic), or <i>Salix babylonica</i> extract (Salix), respectively SEM = Standard error of the mean					

Fecal egg count

Compared to control and Garlic, Salix reduced ($P = 0.05$) almost 2-fold FEC with a noticeable time effect (time $P = 0.001$) as FEC was reduced from day 0 to day 28 (Fig. 1). The fecal egg count on day 28 indicated a FECRT of 67% for Salix and 66% for Garlic compared with the control group. There were not interaction effects for any of the variables. The resistance was higher ($P = 0.001$; Table 4) for the control group compared to Salix and Garlic. Also, resistance was higher ($P = 0.002$) at day 28 compared with 14 and 21 days. The count of parasite eggs (%) for *Chabertia spp.* was higher ($P = 0.017$) for Garlic and Salix than control. *Trychostrongylus spp.* was higher in control ($P = 0.001$) compared with Garlic and Salix. No differences were found between treatments for *Haemonchus spp.*, *Moniezia spp.* and *Ostertagia spp.* The lowest concentration of *Chabertia spp.* was at day zero ($P = 0.001$), while the highest concentration (%) of *Haemonchus contortus* was at day zero ($P = 0.001$). The rest of the parasite eggs were similar among time and there were not treatment and time interactions.

Table 4

Anthelmintic effect of calcium soaps of Garlic and Salix extracts on performance and fecal parasite eggs in goats

Item	Treatment ¹			Time (days)				SEM	P-value		
	Control	Garlic	Salix	0	14	21	28		Trt	Time	Trt×Time
Body weight (kg)	34.0 ^b	36.8 ^b	46.4 ^a	43.0 ^A	39.1 ^{AB}	36.2 ^B	38.2 ^{AB}	2.70	0.001	0.048	0.980
Daily body weight gain (kg)	-0.09	-0.09	-0.22	0.0	-0.28 ^B	-0.41 ^B	0.28 ^A	0.07	0.465	0.001	0.480
Fecal egg count, FEC	416 ^a	300 ^{ab}	275 ^b	577 ^A	200 ^B	244 ^B	300 ^B	61.8	0.052	0.001	0.340
Resistance %	72.3 ^a	33.3 ^b	32.8 ^b		34.9 ^B	44.3 ^B	59.3 ^A	5.49	0.001	0.002	0.610
Parasite eggs											
<i>Chabertia</i> %	20.2 ^b	44.8 ^a	42.9 ^a	5.22 ^B	49.4 ^A	45.8 ^A	43.4 ^A	10.3	0.017	0.001	0.640
<i>Haemonchus</i> %	22.3	19.5	13.0	47.3 ^A	5.67 ^B	10.2 ^B	9.8 ^B	5.01	0.130	0.001	0.490
<i>Moniezia</i> %	4.83	11.6	4.42	12.0	5.89	4.67	5.33	5.28	0.250	0.530	0.560
<i>Ostertagia</i> %	13.5	10.4	17.9	13.1	13.6	14.2	14.7	3.94	0.100	0.970	0.780
<i>Trychostrongylus</i> %	38.9 ^a	13.6 ^b	21.7 ^b	22.2	25.3	24.7	26.5	5.21	0.001	0.970	0.130

^{a-b} Means with different superscript letters within a row are different (Treatment $P \leq 0.05$).

^{A-B} Means with different superscript letters within a row are different (Time $p < 0.05$)

¹ 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

FEC = eggs per gram of feces

Rumen Fermentation Kinetics

Total gas production (A, $P = 0.25$), dry matter digestibility (DMD, $P = 0.89$), gas yield at 24 h (GY24, $P = 0.36$), estimated short-chain fatty acids (SCA, $P = 0.352$), microbial crude protein (MCP, $P = 0.02$), and metabolic energy (ME, $P = 0.83$) were similar between treatments (Table 5). Also, in vitro fermentation kinetics were similar between treatments (Table 4).

Table 5

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 <i>Salix babylonica</i> extract (Salix), respectively SEM = Standard error of the mean					

Discussion

Global small ruminants' sector is under scrutiny and is challenged by sustainability issues (Hoste et al. 2022). The infections caused by GIN are some of the main concerns for goat production as they affect animal health and productivity (Froehlich et al. 2022; Novobilský et al. 2011), also due to the increasing phenomena of AR and the risk of anthelmintic residues in the environment. In particular, the small ruminant sector should adopt more sustainable practices and principles to become more resilient and competitive. The improvement of sustainability represents the center of the debate on the future of global agriculture (Hoste et al. 2022; Olivas-Salazar et al. 2018). Notably, finding alternatives to control gastrointestinal parasites in livestock is not easy (Froehlich et al. 2022), but natural compounds and plant extracts are a promising alternative in this direction. Thus, this study aimed to investigate the effect of using calcium soaps of Garlic or Salix extracts on GIN control in goats as well as their impact on nutrient intake, digestibility, and N balance. The results of these field tests, not only highlighted the importance of anthelmintic efficacy studies of ethnoveterinary remedies, but also pave the way for the use Salix and Garlic as an alternative and sustainable method to reduce the use of chemicals and to counteract the phenomena of AR.

The GIN infection in small ruminants have been largely found to decrease DMI, nutrients digestion, and weight gain of host animals (Mavrot et al. 2015; Hoste et al., 2022). Though having the above negative effects on the host animals, the main economic losses caused by GIN infection are not only from the mortality rate, but also from nutrients digestion loss (Mavrot et al. 2015). Our results revealed that ingestion of Salix at doses of 65 g/kg DM led to higher body weight besides an enhanced digestibility of DM, OM, and NDF which followed by Garlic diet. However, intake of DM, OM, NDF, and ADF were not affected by dietary calcium soaps. The effects of feeding Garlic extracts on the nutrients digestibility and livestock growth were not consistent in the literature. Some studies (Zhong et al. 2019; Hasan et al. 2015) reported that the intakes and body weight of GIN-infected goats increased with a Garlic supplementation, whereas others (Chaves et al. 2008) reported that dietary Garlic supplementation had no effects on DMI and weight gain of lambs with parasitic infection. However, the present improvement of nutrient digestibility in Salix fed goats may be due to positive impacts of plant secondary metabolites (PSM) on ruminal fermentation and nutrient digestibility (Salem et al. 2014b). Although about 59 PSM were identified in Salix, none of the individual active compounds was tested for its effect on animal nutrition. In contrast, previous publications (Dearing et al. 2000; Salem et al. 2017) have been shown that ingestion of Salix secondary metabolites increase digestibility of NDF and ADF. In agreement, Jiménez-Peralta et al. (2011) suggested that extracts of Salix could positively modify rumen gas production and fermentation activities, which may improve nutrient utilization in lambs mainly due to its rich PSM. Moreover, it is well known (Salem et al. 2016) that the PSM of Salix extract such as phenolic compounds, alkaloids, or saponins could improve synchronization between energy and N release and improve microbial protein synthesis that positively affect body weight. Indeed, saponins have antimicrobial properties on ciliate protozoa growth, peptidase-producing bacteria, and cellulolytic bacteria (Francis et al. 2002) which normally effect SCFA production (i.e., acetate, not propionate; Salem et al., 2014b) which could improve body weight gain. In line with our results, Cedillo et al. (2015), in a 72-day trial, showed that of Salix extract (at doses 20 and 40 mL/lamb/d) tended to increase body weight by about 5 and 8.2%, respectively.

Overall, our results demonstrated that the calcium soap of Salix is expected to be beneficial to digestibility of DM, OM, and NDF based on their stimulating effect on fermentation, and increased nutrient degradability, as well as by increasing microbial protein production, which also positively altered body weight changes (Jiménez-Peralta et al. 2011; Cedillo et al. 2015). Nitrogen balance is considered as an important index of protein status in ruminants. Neither N intake nor N balance were affected by dietary Garlic and Salix supplementation. However, numerically, higher N balance in both Garlic and Salix diets was found and this was probably due to plenty of bioactive components available in those extracts. It has been shown (Kamruzzaman et al. 2011) that Garlic bioactive components might have a positive impact on N balance by influencing microbial proteolytic activities of rumen fluid in sheep fed with garlic silage diet. Previously, the favorable impact of Salix on N balance has been reported (Valdes et al. 2015), since its PSM preventing microbial degradation of plant proteins, causing an increased amino acid flow to the duodenum from the rumen (Salem et al. 2014a). Besides having a protective effect on protein in the rumen, Salix extract can promote duodenal absorption of amino acids and enhancing the N balance (Valdes et al. 2015). However, the effect of PSM on N balance is still not clear and have some inconsistencies (Hoste et al. 2022; Kamruzzaman et al. 2011), thus for greater scientific reach, further studies should consider the use of Salix and Garlic extracts focusing on rumen function and N retention.

Nither *in vitro* rumen gas kinetics nor fermentation profile of goats fed with calcium soaps from Garlic and Salix extracts were affected. It is well known (Theodorou et al. 1994; Menke and Steingass, 1988) that gas production is generally an indicator of nutrient digestibility and fermentability, as well as rumen microbial protein production. Salix extract was expected to be beneficial to rumen function based on their stimulating effect on nutrients digestibility, as well as by increasing microbial protein production (Salem et al. 2014b). Also, it has been reported that the moderate doses of Salix extract (0.6 and 1.2 ml/ g DM) had positive impact on ruminal GP and fermentation parameters, but not with high dose (1.8 ml/g DM). this could be explained by ability of rumen microorganisms to degrade low and moderate levels of PSM in plant extracts (Theodorou et al. 1994; Salem et al. 2014b). Previous studies have shown (Jiménez-Peralta et al. 2019; Patra et al. 2006) that the effect PSM and plant extract on ruminal fermentation parameters often influenced by the physicochemical composition of diet, and the structure of the forage as well as the level of PSM use. In our study, since it is hard to separate the effects of all these factors, possibly either none of these factors affect GP or some of these factors neutralized the other effects.

It is well known that most commercial drugs used for parasitic infections lose their effectiveness after some years of use because gastrointestinal parasites develop resistance (Froehlich et al. 2022). The excessive use of synthetic nematocides generates a great monetary cost worldwide. Goats and sheep have been reported to have resistance to drugs aiming to control *Haemonchus contortus* (Zra et al. 2019; Oliveira Santos et al. 2019). The irrational use of these drugs has generated anthelmintic resistance; in addition to the presence of chemical residues in meat and milk of treated animals (Hoste et al. 2022), so the use of natural products as anthelmintic controls has been explored (Froehlich et al. 2022).

In temperate regions, parasitic problems are controlled through the seasonal use of anthelmintic and pasture management; this is not the case for tropical and subtropical regions since the problem persists (Salem et al. 2016; Oliveira Santos et al. 2019). More specifically, *Haemonchus contortus* is responsible for economic losses and represents enormous animal welfare problems for small ruminants (Novobilský et al. 2011). Previously, Salem et al. (2016) reported the anthelmintic activity of Salix in small ruminants, and now, in the present study, calcium salts of Salix extract showed promising results for controlling *Haemonchus contortus*, probably due to the presence of tannins (Mejía-Hernández et al. 2014; Cedillo et al. 2015). Consistently, *in vitro* studies have been shown that Salix extract have significant activity against ruminant nematodes (Jiménez-Peralta et al., 2019). The anthelmintic efficacy of Salix extract was confirmed by Mejía-Hernández et al. (2014). In addition, Mupeyo et al. (2011) reported that the Salix ingestion in infected sheep resulted in a lowered *Haemonchus contortus* and female *Teladorsagia circumcincta* worm loads in the abomasum. Similarly, Castagna et al. (2021) indicated that the weekly administration of an aqueous extract of Salix (at a dose of 20 mL/ animal) reduced the fecal egg and oocyst counts of many parasitic species in both sheep and goats. Hence, it would be reasonable to suggest that the observed anthelmintic activity of the Salix extracts against different nematode species could be attributed to the presence of PSM. It is well established that the condensed tannins have shown anthelmintic activity against gastrointestinal nematodes of sheep and goats (Novobilský et al. 2011). In addition, condensed tannins that can be found in Salix, can impair parasite feeding because tannins bind free proteins for larvae nutrition causing larval starvation and death, and tannins can disrupt the integrity of parasite cuticles (Salem et al. 2017). Moreover, Salix's tannins can affect parasites in the gastrointestinal tract directly through the inhibition of oxidative phosphorylation, causing larval death (Salem et al. 2016). Guarrera (1999) has been reported that Salix extract contains considerable amounts of PSM (namely saponins, alkaloids, tannins, other polyphenols, nonprotein amino acids, lignin, and glycoside) all of which demonstrated antiparasitic effects. Salem et al. (2016) pointed out that not only tannins are responsible for the anthelmintic activity of Salix extract, but it is likely that alkaloids in the plant extract may also contribute to the paralysis and consequent worm death. In confirmation, extracts of several tannins and alkaloids have been shown to cause a delay or total inhibition of the exsheathment process of *H. contortus* and *T. colubriformis* larvae (Froehlich et al. 2022; Novobilský et al. 2011). In this study, tannins were not quantified and neither larval exsheathment, however those factors should be further explored. Previously, Arreola et al. (2015) have reported garlic parasitocidal properties against *Ostertagia spp.* and *Trychostrongylus spp.* However, the present study used calcium soaps of garlic extract as a nematode control strategy as nematodes have become resistant to commercial drugs have (Hoste et al., 2022). The presence of drug-resistant helminths represents an important restriction for the sustainability of the current helminth control strategies in sheep and goats. For example, Madsen et al. (1990) analyzed fecal residues of Ivermectin, and their effects on beetles and other organisms such as springtails and mites that spend part of their biological cycle scavenging feces. These types of anti-helminthic chemicals have negative impact on agroecosystems (Herd et al. 1993; Hoste et al., 2022).

Taken together, it may be concluded that both Garlic and Salix calcium soaps possess anthelmintic activities due to their rich PSM profile. However, it is very important to point out that higher concentrations of the active compounds may cause antinutritional effects, such as a reduction in feed intake and performance (Salem et al. 2014). It is suggested to carry out further assays to assess the isolation, development, and validation of the effects of these herbal extracts to provide evidence that might support their wider acceptance (Hoste et al. 2022; Salem et al. 2016).

Conclusion

Overall, inclusion of 65 g/kg DM of calcium soaps of Garlic or Salix extracts in goat diets based on alfalfa hay not only enhanced digestibility of DM, OM, and NDF but also reduced egg presence of *Haemonchus Contortus* and *Moniezia spp.*

While dietary calcium soaps of Garlic can reduce eggs from *Ostertagia spp.* and *Trychostrongylus spp.* Results from this study suggest that calcium soaps from Garlic or Salix extracts can be used for nematodes control without deleterious effects on performance, N balance, and rumen fermentation kinetics of goats.

Declarations

Ethics approval: This study was approved by the Ethics Committee on Animal Experimentation of the School of Veterinary Medicine and Animal Science of the Universidad Autonoma del Estado de Mexico (Project ID UAEMex4335/2017).

Consent to participate: Authors have permission to participate.

Consent for publication: Authors have permission for publication.

Conflicts of interest/Competing interests: The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Author contributions

Manuel Gonzalez-Ronquillo and Lizbeth E. Robles Jimenez to the study conception and design. Material preparation, data collection and analysis were performed by Einar Vargas-Bello-Pérez, Navid Ghavipanje, Teresa Torres-Gonzalez, Juan Carlos Angeles Hernandez, Valente Velazquez-Ordoñez, Octavio Alonso Castelan Ortega, Lizbeth E. Robles Jimenez, Sergio Roskof, Manuel Gonzalez-Ronquillo. The first draft of the manuscript was written by Teresa Torres-Gonzalez, Teresa Torres-Gonzalez, Sergio Roskof, and Manuel Gonzalez-Ronquillo. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

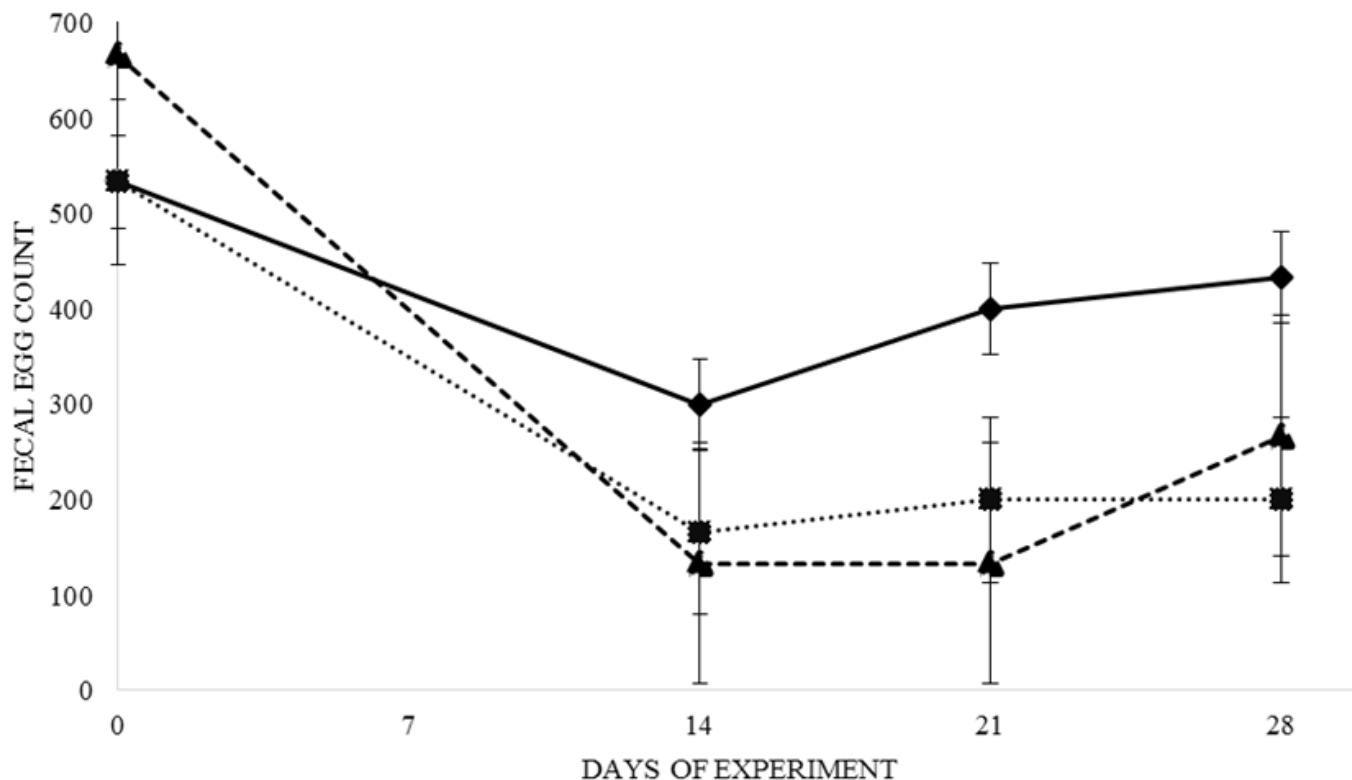


Figure 1

Fecal egg count (FEC) of goats fed with calcium soaps of Safflower (♦), Garlic (■) and Salix (▲) extracts.

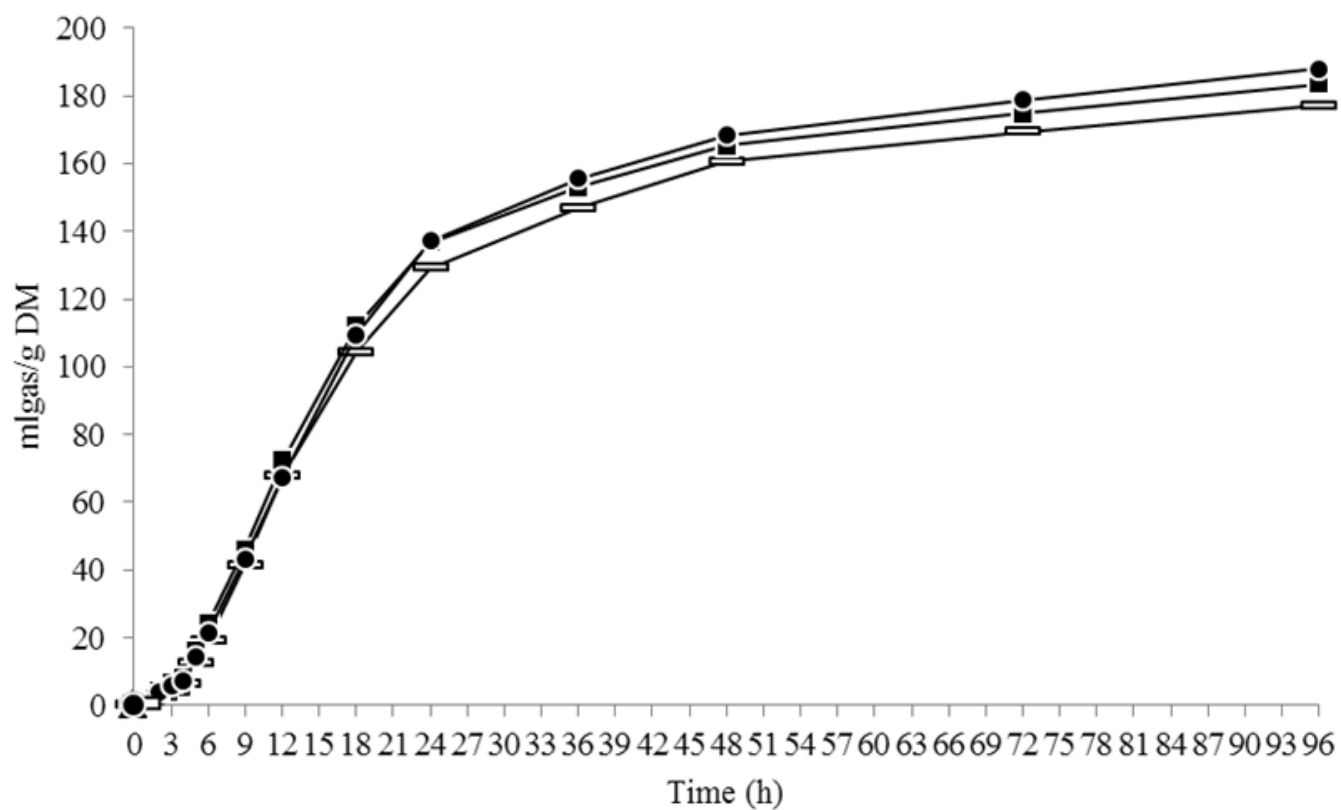


Figure 2

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

Supplementary Files

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