

## Research Article

**Cite this article:** Vargas-Bello-Pérez E, Ghavipanjan N, Torres-Gonzalez T, Angeles-Hernandez JC, Velázquez-Ordoñez V, Castelán-Ortega OA, Robles Jimenez LE, Roskopf SD and Gonzalez-Ronquillo M (2024). Effect of calcium soaps from garlic (*Allium sativum*) and willow (*Salix babylonica*) extracts on nematode loads, nutrient intake and digestibility, nitrogen balance and rumen fermentation kinetics in dairy goats. *Journal of Dairy Research* **91**, 19–24. <https://doi.org/10.1017/S0022029924000141>

Received: 11 January 2023

Revised: 23 January 2024

Accepted: 6 February 2024

**Keywords:**

Anthelmintic; *Allium sativum*; calcium soaps; gastrointestinal parasites; *Salix babylonica*

**Corresponding author:**

Einar Vargas-Bello-Pérez;

Email: [e.vargasbelloperez@reading.ac.uk](mailto:e.vargasbelloperez@reading.ac.uk);

Manuel Gonzalez-Ronquillo;

Email: [mrg@uaemex.mx](mailto:mrg@uaemex.mx)

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

Einar Vargas-Bello-Pérez<sup>1,2</sup>, Navid Ghavipanjan<sup>3</sup>, Teresa Torres-Gonzalez<sup>4</sup>, Juan Carlos Angeles-Hernandez<sup>5</sup>, Valente Velázquez-Ordoñez<sup>6</sup>, Octavio Alonso Castelán-Ortega<sup>4</sup>, Lizbeth E. Robles Jimenez<sup>4</sup>, Sergio Daniel Roskopf<sup>7</sup> and Manuel Gonzalez-Ronquillo<sup>4</sup>

<sup>1</sup>Department of Animal Sciences, School of Agriculture, Policy and Development, University of Reading, P.O. Box 237, Earley Gate, Reading RG6 6EU, UK; <sup>2</sup>Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua, Periférico R. Aldama km 1, 31031 Chihuahua, México; <sup>3</sup>Department of Animal Science, Faculty of Agriculture, University of Birjand, Birjand 97175-331, Iran; <sup>4</sup>Department of Animal Nutrition, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Instituto Literario 100. Toluca, Estado de México, 50000, México; <sup>5</sup>Área Académica de Medicina Veterinaria y Zootecnia, Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, Av. Universidad km. 1, Tulancingo de Bravo, Hidalgo, 43600, México; <sup>6</sup>Centro de Investigación y Estudios Avanzados en Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Instituto Literario 100. Toluca, Estado de México, 50000, México and <sup>7</sup>Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Kreder 2805 (3080). Esperanza, Santa Fe, Argentina

**Abstract**

The objective of this study was to determine the effect of dietary calcium soaps from garlic (*Allium sativum*) and willow (*Salix babylonica*) extracts on nematode loads, nutrient intake and digestibility, nitrogen balance and rumen fermentation kinetics in dairy goats. Nine adult non-lactating Saanen goats were grouped into a complete randomized block design with 3 treatments ( $n = 3$ ) over a period of 28 d. Animals were fed a diet based on alfalfa hay and a concentrate that was supplemented (65 g/kg DM) with calcium soaps of safflower (control), garlic or willow. 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 and OM were observed in willow supplemented goats. In vitro gas kinetics and fermentation profile were not affected by diets. Results from fecal egg count indicated a reduction in total count, *Haemonchus* spp. and *Trichostrongylus* spp. for both garlic and willow compared to control. Our results suggest that calcium soaps of garlic or willow extracts can be used to reduce gastrointestinal parasites in goats without compromising productive traits or rumen function.

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 (Oliveira Santos *et al.*, 2019; Hoste *et al.*, 2022; Nolinda *et al.*, 2024). 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 lumbianum* and *O. venulosum* (large intestine: Dehuri *et al.*, 2021; Hoste *et al.*, 2022). Since the mid-1960s, GIN control in livestock has relied heavily on anthelmintics (Froehlich *et al.*, 2022; Hoste *et al.*, 2022), however, over-dependency and misuse of these drugs has led to anthelmintic resistance that has now become a serious problem on small ruminant farms in many countries (Oliveira Santos *et al.*, 2019; Froehlich *et al.*, 2022; Nolinda *et al.*, 2024). Today, not only the efficient control of GIN infections is essential to improve livestock health and production (Dehuri *et al.*, 2021; Hoste *et al.*, 2022), but also widespread resistance to synthetic anthelmintic has inspired research for alternative strategies of parasite control, including the use of plant extracts (Oliveira Santos *et al.*, 2019; Nolinda *et al.*, 2024).

It is well documented that natural-derived feedstuffs can contain a wide array of plant secondary metabolites (PSM) arranged in a variety of chemical classes (Castagna *et al.*, 2021) that may provide an alternative, cost-effective treatment to gastrointestinal parasites, limiting both drug use and development of anthelmintic resistance (Dehuri *et al.*, 2021; Froehlich *et al.*, 2022). It has been suggested (Oliveira Santos *et al.*, 2019; Hoste *et al.*, 2022) that feeding

© The Author(s), 2024. Published by Cambridge University Press on behalf of Hannah Dairy Research Foundation. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.



small ruminants with tannin-rich plants (30–40 g of condensed tannins per kg DM) promotes anthelmintic effect. However, condensed tannins, at higher concentrations (7–8% DM), can depress feed intake, decrease nutrient digestibility and overall production (Salem *et al.*, 2016; Castagna *et al.*, 2021).

Garlic (*Allium sativum*) has been reported to be a parasiticide, amebicide, larvicide and immune-stimulant, so garlic essential oil can be used as an alternative or supplement to the current anthelmintic therapies (Kamruzzaman *et al.*, 2011; Zhong *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 (Zhong *et al.*, 2019) which have been related to several small terpenoid and phenolic compounds (Kamruzzaman *et al.*, 2011).

Little is known about the nutritive value of willow (*Salix babylonica*; Salix) in goat diets, however, extracts of Salix have been evaluated as feed additives in ruminant diets due to their antimicrobial effects and ability to modulate ruminal fermentation and improve nutrient utilization (Salem *et al.*, 2016). In addition, Salix extract enhanced digestibility and average daily gain 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; Dehuri *et al.*, 2021).

Until now, the effects of using rumen bypass (for example, calcium soaps) plant extracts have not been analyzed in dairy goat nutrition and parasite loads. Therefore, the aim of this study was to determine the effect of using dietary calcium soaps with garlic and Salix extracts on nutrient intake and digestibility, nitrogen balance and rumen fermentation kinetics in dairy goats, as well as studying their effect on gastrointestinal nematode load. The hypothesis of this study is that calcium soaps of plant extracts will not affect intake. Results from this study will improve our knowledge on plant material alternatives to the use of conventional anthelmintic drugs.

## Materials and methods

### Goats, experimental design, and dietary treatments

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, all goats were adapted to the diets for 7 d, and then were weighed. Individual fecal samples were taken, and the fecal egg count and parasite identification were determined. At the onset of the study, average body weight was  $36.7 \pm 6.2$  kg (mean  $\pm$  SD) while body condition score (scored on a 5-point scale) was  $2.0 \pm 0.1$ . For 28 d, animals were fed ad libitum a basal diet consisting of 60% forage (alfalfa hay) and 40% concentrate (containing crushed rapeseed, sorghum grain, soybean meal, wheat grain, and corn stover) supplemented with calcium soaps of safflower, garlic, or Salix extract as potential anthelmintic (Tables S1 and S2), with free access to water. Garlic and Salix extracts were prepared weekly, and the process of fat saponification was performed following the double decomposition method of Jenkins and Palmquist (1984), using safflower oil as control. The concentrate

was supplemented with 65 g/kg DM of calcium soaps from safflower (control), garlic, or Salix extract and treatments were manually mixed. Animals were housed in individual roofed pens with concrete flooring and straw bedding. Individual body weights were recorded on days 0, 7, 14, 21 and 28.

### Experimental measurements, samplings, and analyses

All goats were naturally infected with gastrointestinal parasites. The animals were grazing a pasture where they are naturally infested with parasites and these animals had not been dewormed in the last 6 months prior to the study. Animals 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 the number of eggs per gram of feces (FEC) as fresh matter were quantified using the modified McMaster technique (Boareki *et al.*, 2021). Briefly, fecal samples of 4 g were mixed well with 56 mL of flotation solution. The mixture was then strained and put in the two-chamber McMaster slide and allowed to sit for 5 min before counting the eggs under microscope. The fecal egg count reduction per gram of feces was determined according to the method described by Coles *et al.* (1992).

After 28 d of the nutritional intervention, for 5 d, all animals were housed in a roofed pen with individual metabolic cages (1.20  $\times$  0.80 m) with slatted floor and had free access to water. Each cage had individual drinkers and feeders and the goats were fed with their individual diet. 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  $-20^{\circ}\text{C}$  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. Nutrient digestibility was determined as  $[(\text{nutrient intake, g/d} - \text{nutrient excreted, g/d}) / (\text{nutrient intake, g/d})] \times 1000$ .

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. Three incubation runs were carried out as replicates. In each incubation run, three flasks per treatment were used and a set of appropriate blanks were included. Rumen fluid (300 ml per animal) was collected from the three-rumen cannulated lactating goats into a pre-warmed thermos flask, and then filtered and flushed with  $\text{CO}_2$ . GP was measured using a pressure transducer (Delta Model HD 8804, Italy) at 6, 12, 24, 48, 72, and 96 h and a set of blanks were included.

Detailed information on in vitro experimental procedures and calculation as well as preparation of the extracts and calcium soaps are given in the online Supplementary File.

### Statistical analysis

A MIXED model of repeated measurements was implemented to analyze the effect of garlic and Salix administration in goat on in vivo variables using R statistical program. The model included the fixed effects of treatment, time of measurement, the random effect

of goat nested within treatment and the residual error. Interactions between treatments and sampling times were included in the model if they were significant. The results are presented as least squares means (LSM). The differences in LSM were tested using the Tukey–Kramer procedure. For all data, significance was declared at  $P \leq 0.05$ . Each variable analyzed was subjected to three covariance structures including autoregressive order, ante-dependence and unstructured and the one resulting in the lowest Akaike information criterion was chosen. For the dependent variables body weight (BW), metabolic BW and BW change, BW at baseline was used as a covariate.

The data were tested for normality using the Shapiro–Wilk test and since most of the FEC variables were not normally distributed they were transformed prior to analysis. Fecal egg counts were transformed as  $\ln(\text{FEC} + 100)$  prior to analysis to improve normality. The resulting LSM were presented on either of the transformed scales and after back-transformation of LSM as  $(e^{\text{LSM}} - 25)$  and standard error (SE) of back-transformed means were estimated by multiplying them by SE of LSM. The percentages of FEC for each parasite genus to be derived from the frequency of counts were subjected to an angular or arcsine transformation. However, as the arcsine transformation only works on values between 0 and 1, we first converted each value using the following formula:  $y = x/\max(x)$  where  $y$  is the transformed value,  $x$  is the original value and  $\max$  is the highest value in the data set.

## Results

Intake of dry matter, organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were similar between treatments ( $P > 0.05$ , Table 1). The highest digestibility of DM ( $P = 0.02$ ) and OM ( $P < 0.01$ ) was observed in Salix supplemented goats. No significant differences were observed in N intake, fecal N excretion, urinary N excretion, and N balance between diets (Table 1).

Total FEC reduced ( $P = 0.001$ ) with ingestion of both calcium soaps. The goats fed garlic and Salix had approximately 6.9 and 5.6% lower total FEC compared to the control group (Table 2). There were no interaction effects for any of the variables. The resistance was higher ( $P < 0.001$ ; Table 2) for the control group compared to salix and Garlic groups. The count of fecal parasite eggs (%) of *Haemonchus* spp. ( $P < 0.05$ ) and *Trychostrongylus* spp. ( $P < 0.001$ ) was lower for garlic and Salix than control (Fig. S1). No significant differences were found between treatments for *Chabertia* spp., *Moniezia* spp. and *Ostertagia* spp. The highest and lowest concentration of *Haemonchus* spp. was at day zero and day 28, respectively and the effect of time was significant ( $P = 0.001$ ). The rest of the parasite eggs were similar in time and there were no treatment and time interactions.

Total GP, DM, gas yield at 24 h, estimated short-chain fatty acids, microbial crude protein and metabolic energy were similar between treatments (Table S3). Also, in vitro fermentation kinetics were not affected (Table S3 and Fig. S2).

## Discussion

Finding alternatives to control GINs in livestock is not easy (Dehuri *et al.*, 2021; Froehlich *et al.*, 2022), but natural compounds and plant extracts are a promising move 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 potential alternative and sustainable methods to reduce the use of chemicals.

GIN infection in small ruminants has been found to decrease DMI, nutrient digestion and weight gain of host animals (Froehlich *et al.*, 2022; Hoste *et al.*, 2022). Our results revealed that ingestion of calcium soaps of Salix at doses of 65 g/kg DM led to higher body weight besides an enhanced digestibility of DM and OM, and we observed similar but lesser effects with garlic. However, intake of DM, OM, NDF, and ADF were not affected by dietary calcium soaps. The effects of feeding garlic extracts on nutrient digestibility and livestock growth are not consistent in the literature. Some studies (Kamruzzaman *et al.*, 2011; Zhong *et al.*, 2019) reported that the intakes and body weight of GIN-infected goats increased with garlic supplementation, whereas others (Zhong *et al.*, 2019) 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). It has been reported (Salem *et al.*, 2016) that 59 of these PSM (including tritetracontane, hexadecanoic acidmethyl ester, phytol and aliphatic hydrocarbons) were identified in Salix, however, none of the individual active compounds was tested for its effect on animal nutrition. In contrast, previous publications (Salem *et al.*, 2016) have shown that ingestion of Salix secondary metabolites increases 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 content of 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 affects body weight. Indeed, saponins have antimicrobial properties on ciliate protozoal growth, peptidase-producing bacteria and cellulolytic bacteria (Froehlich *et al.*, 2022) which normally effect short-chain fatty acid 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 Salix extract (at doses 20 and 40 ml/lamb/d) increased BW of lambs 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 and OM based on a stimulating effect on fermentation. This will increase microbial protein production, which will positively affect 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 (Jenkins and Palmquist, 1984). N intake and N balance were not affected by dietary garlic and Salix supplementation. However, numerically (but non-significant), higher N balance in both garlic and Salix diets was found and this was probably due to bioactive components being 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 (Salem *et al.*, 2016), since its PSM prevented microbial degradation of plant proteins, causing an increased amino acid

**Table 1.** Nutrient intake, digestibility and nitrogen balance of goats fed with calcium soaps of garlic and *Salix* extracts

Item	Treatments <sup>1</sup>			SEM	P-value
	Control	Garlic	Salix		
Intake (g/kg LW <sup>0.75</sup> )					
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 coefficients					
Dry matter, DM	0.663 <sup>b</sup>	0.699 <sup>ab</sup>	0.712 <sup>a</sup>	0.0081	0.0285
Organic matter, OM	0.679 <sup>b</sup>	0.714 <sup>ab</sup>	0.747 <sup>a</sup>	0.0072	0.0053
Neutral detergent fiber, NDF	0.589	0.615	0.648	0.0131	0.0645
Acid detergent fiber, ADF	0.465	0.477	0.502	0.0240	0.4726
Acid detergent lignin, ADL	0.343 <sup>b</sup>	0.353 <sup>a</sup>	0.355 <sup>a</sup>	0.62	0.9758
Nitrogen balance, g N day/kg LW <sup>0.75</sup>					
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
Fecal N excretion (%)	31.5	32.2	30.9	1.05	0.76
Urine N excretion (%)	38.3	34.1	35.0	4.15	0.05
N retention (%)	25.5	33.6	34.0	4.81	0.05

SEM, Standard error of the mean.

<sup>a-c</sup>Means with different superscript letters within a row are different ( $P \leq 0.05$ ).<sup>1</sup>Diets supplemented with 65 g/kg DM of calcium soaps from either safflower (control), garlic extract (garlic), or *Salix babylonica* extract (salix), respectively.

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 enhance the N balance (Salem *et al.*, 2016). However, the effect of PSM on N balance is still not clear and there are inconsistencies (Kamruzzaman *et al.*, 2011; Hoste *et al.*, 2022). Future studies should consider the use of *Salix* and garlic extracts focusing on rumen function and N retention. Likewise, the *in vitro* ruminal gas kinetics and fermentation profile of goats fed calcium soaps of garlic and *Salix* extracts were not affected. It is well known (Theodorou *et al.*, 1994) 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 a stimulating effect on nutrient digestibility and microbial protein production (Salem *et al.*, 2014b). It has also been reported that moderate doses of *Salix* extract (0.6 and 1.2 ml/g DM) but not high (1.8 ml/g DM) had positive impact on ruminal GP and fermentation parameters. This could be explained by the ability of rumen microorganisms to degrade low and moderate levels of PSM in plant extracts (Salem *et al.*, 2014b; Dehuri *et al.*, 2021). Previous studies have shown (Jiménez-Peralta *et al.*, 2011; Castagna *et al.*, 2021) that the effect of PSM and plant extracts on ruminal fermentation parameters are often influenced by the physicochemical composition of the 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 or none of these factors affect GP or some of these factors neutralized the other effects.

Our calcium soaps of *Salix* extract showed promising results for controlling *Trychostrongylus* spp. and *Haemonchus*, spp. probably due to the presence of tannins (Cedillo *et al.*, 2015). Consistently, *in vitro* studies have shown that *Salix* extracts have significant activity against ruminant nematodes (Jiménez-Peralta *et al.*, 2011). The anthelmintic efficacy of *Salix* extract was confirmed by Cedillo *et al.* (2015). In addition, 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 condensed tannins have shown anthelmintic activity against gastrointestinal nematodes of sheep and goats (Oliveira Santos *et al.*, 2019). A recent paper (Nolinda *et al.*, 2024) has demonstrated that the anthelmintic effects of PSM are associated with the deactivation of several key enzymes within the parasite. 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

**Table 2.** Anthelmintic effect of calcium soaps of garlic and salix extracts on performance and fecal parasite eggs in goats

Item	Treatment <sup>1</sup>				Time (days)					P-value		
	Control	Garlic	Salix	SEM	0	14	21	28	SEM	Trt	Time	Trt × Time
Body weight (kg)	37.7	37.7	38.1	1.24	39.6	39.1	36.2	38.2	0.61	0.51	0.81	0.54
Metabolic body weight <sup>1</sup>	15.2	15.2	15.3	0.41	15.2	15.6	14.7	15.3	0.22	0.55	0.81	0.58
Body weight changes (kg)	-0.19	-0.14	-0.07	0.19	0.0	-0.28	-0.41	0.28	0.28	0.78	0.21	0.42
Arcsine transformed percentage of fecal egg count and resistance												
<i>Chabertia</i> %	0.49	0.78	0.78	0.17	0.24	0.88	0.84	0.78	0.29	0.09	0.41	0.81
<i>Haemonchus</i> %	0.69 <sup>a</sup>	0.51 <sup>ab</sup>	0.35 <sup>b</sup>	0.12	1.24	0.22	0.31	0.31	0.99	0.03	0.86	0.25
<i>Moniezia</i> %	0.33	0.32	0.32	0.003	0.33	0.32	0.32	0.32	0.003	0.95	0.42	0.55
<i>Ostertagia</i> %	0.72	0.61	0.90	0.09	0.71	0.74	0.75	0.77	0.11	0.13	0.99	0.50
<i>Trychostrongylus</i> %	0.88 <sup>a</sup>	0.44 <sup>b</sup>	0.59 <sup>b</sup>	0.06	0.60	0.66	0.63	0.66	0.08	0.0001	0.94	0.20
Resistance %	1.08 <sup>a</sup>	0.61 <sup>b</sup>	0.61 <sup>b</sup>	0.102		0.62	0.74	0.94	0.16	0.0001	0.36	0.55
Log transformed fecal egg count												
Total FEC	6.22 <sup>a</sup>	5.79 <sup>b</sup>	5.87 <sup>b</sup>	0.26	6.48	5.64	5.77	5.95	0.49	0.001	0.65	0.18
<i>Chabertia</i>	5.17	5.13	5.19	0.28	4.86	5.2	5.26	5.35	0.55	0.88	0.93	0.96
<i>Haemonchus</i>	5.23 <sup>a</sup>	5.01 <sup>b</sup>	4.96 <sup>b</sup>	0.06	5.89 <sup>A</sup>	4.72 <sup>B</sup>	4.81 <sup>B</sup>	4.84 <sup>B</sup>	0.09	0.004	0.001	0.09
<i>Moniezia</i>	4.78	4.8	4.78	0.27	5.12	4.7	4.65	4.68	0.54	0.96	0.91	0.55
<i>Ostertagia</i>	5.02	4.88	4.99	0.06	5.14	4.86	4.90	4.94	0.06	0.23	0.06	0.32
<i>Trychostrongylus</i>	5.51 <sup>a</sup>	4.92 <sup>b</sup>	5.07 <sup>b</sup>	0.05	5.4	5.0	5.09	5.17 <sup>B</sup>	0.06	0.0001	0.07	0.005

<sup>a,b,A,B</sup>Means with different superscript letters within a row are different to treatment (lower case) and time (upper case), respectively ( $P \leq 0.05$ ). <sup>1</sup>Metabolic live weight = (Live Weight)<sup>0.75</sup>.

(Salem *et al.*, 2016). 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). Cedillo *et al.* (2015) 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) suggested that tannins are not solely 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 ensheathment process of *H. contortus* and *T. colubriformis* larvae (Froehlich *et al.*, 2022; Nolinda *et al.*, 2024). In this study, tannins were not quantified, and neither was larval ensheathment, however, those factors should be explored further. Previously, Zhong *et al.* (2019) have reported garlic parasiticidal 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 (Dehuri *et al.*, 2021; 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. These types of anti-helminthic chemicals have negative impact on agroecosystems (Hoste *et al.*, 2022). Our results showed 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, in some cases, such as a reduction in feed intake and performance (Salem *et al.*, 2014a). 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 (Salem *et al.*, 2016; Hoste *et al.*, 2022).

In conclusion, we investigated the effects of adding 65 g/kg DM of calcium soaps from garlic or *Salix* extracts in goat diets, and the findings revealed that both treatments not only enhanced digestibility of DM and OM but also reduced total FEC and fecal egg resistance, as well as reducing fecal parasite eggs of *Haemonchus* spp. and *Trychostrongylus* spp. Results from this study suggest that calcium soaps from garlic or *Salix* extracts can be used for nematode control without deleterious effects on performance, N balance and rumen fermentation kinetics of goats.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0022029924000141>

**Acknowledgments.** This study was funded by the Autonomous University of the State of Mexico, with project UAEM 4335/2017. During the study, Dr Einar Vargas-Bello-Pérez was a visiting scholar also supported by project number 4974/2020CIB from Universidad Autónoma del Estado de Mexico.

## References

Boareki MN, Schenkel FS, Willoughby O, Suarez-Vega A, Kennedy D and Cánovas A (2021) Comparison between methods for measuring faecal egg count and estimating genetic parameters for gastrointestinal parasite resistance traits in sheep. *Journal of Animal Science* **99**, skab341.

- Castagna F, Piras C, Palma E, Musolino V, Lupia C, Bosco A and Britti D (2021) Green veterinary pharmacology applied to parasite control: evaluation of *Punica granatum*, *Artemisia campestris*, *Salix caprea* aqueous mace-rates against gastrointestinal nematodes of sheep. *Veterinary Sciences* **8**, 237.
- Cedillo J, Kholif AE, Salem AZM, Elghandour MMY, Vazquez JF, Alonso MU, Barbabosa A, Chagoyan JCV and Reyna AG (2015) Oral administration of Sauce lloron extract to growing lambs to control gastrointestinal nematodes and *Moniezia* spp. *Asian Pacific Journal of Tropical Medicine* **8**, 520–525.
- Coles GC, Bauer C, Borsteede FHM, Geerts S, Klei TR, Taylor MA and Waller PJ (1992) World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P). Methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology* **44**, 35–44.
- Dehuri M, Palai S, Mohanty B and Malangmei L (2021) Anti-helminthic activity of plant extracts against gastrointestinal nematodes in small ruminants – a review. *Pharmacognosy Reviews* **15**, 117–127.
- Froehlich KA, McNulty R and Greer A (2022) Loline alkaloid effects on gastrointestinal nematodes. *Animals* **12**, 996. <https://doi.org/10.3390/ani12080996>
- Hoste H, Meza-OCampos G, Marchand S, Sotiraki S, Sarasti K, Blomstrand BM and Morgan ER (2022) Use of agro-industrial by-products containing tannins in the integrated control of gastrointestinal nematodes in ruminants. *Parasite* **29**, 1–16.
- Jenkins TC and Palmquist DL (1984) Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. *Journal of Dairy Science* **67**, 978–986.
- Jiménez-Peralta FS, Salem AZM, Mejia-Hernández P, González-Ronquillo M, Albarrán-Portillo B, Rojo-Rubio R and Tinoco-Jaramillo JL (2011) Influence of individual and mixed extracts of two tree species on in vitro gas production kinetics of high-concentrate diet fed to growing lambs. *Livestock Science* **136**, 192–200.
- Kamruzzaman M, Torita A, Sako Y, Al-Mamun M and Sano H (2011) Effects of feeding garlic stem and leaf silage on rates of plasma leucine turnover, whole body protein synthesis and degradation in sheep. *Small Ruminant Research* **99**, 37–43.
- Nolinda N, Ikusika OO, Akinmoladun OF and Mpendulo CT (2024) Impact of nematode infestation in livestock production and the role of natural feed additives – a review. *Open Agriculture* **9**, 20220234.
- Oliveira Santos F, Morais-Cerqueira AP, Branco A, Moreira Batatinha MJ and Borhes Botura M (2019) Anthelmintic activity of plants against gastrointestinal nematodes of goats: a review. *Parasitology* **146**, 1233–1246.
- Salem AZM, Kholif AE, Olivares M, Elghandour MMY, Mellado M and Arece J (2014a) Influence of *S. babylonica* extract on feed intake, growth performance and diet in vitro gas production profile in young lambs. *Tropical Animal Health and Production* **46**, 213–219.
- Salem AZM, Kholif AE, Elghandour MMY, Buendía G, Mariezcurrera MD, Hernandez SR and Camacho LM (2014b) Influence of oral administration of *Salix babylonica* extract on milk production and composition in dairy cows. *Italian Journal of Animal Science* **13**, 2978.
- Salem AZM, Elghandour MMY, Kholif AE, López S, Pliego AB, Cipriano-Salazar M, Chagoyán JCV, de Oca-Jiménez RM and Alonso MU (2016) Tree leaves of *Salix babylonica* extract as a natural anthelmintic for small-ruminant farms in a semiarid region in Mexico. *Agroforestry Systems* **91**, 111–122.
- Theodorou MK, Williams BA, Dhanoa MS, McAllan AB and France J (1994) A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology* **48**, 185–197.
- Zhong R, Xiang H, Cheng L, Zhao C, Wang F, Zhao X and Fang Y (2019) Effects of feeding garlic powder on growth performance, rumen fermentation, and the health status of lambs infected by gastrointestinal nematodes. *Animals* **9**, 102.