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ALTERNATIVES FOR MINIMIZING THE USE OF ANTHELMINTICS IN FARM ANIMALS. A REVIEW

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Abstract

Pasture livestock systems have an essential role in promoting sustainable farming practices in Europe, but they have disadvantages, especially in terms of animal health, as grazing animals are highly susceptible to parasitic infections. They can apply a significant economic weight to the production process, so the most frequently used control method is the administration of anthelmintics. This review summarizes the research on the use of alternative anthelmintics on global scale, reprising the policy for helminth control with envisage of more sustainable solutions including safety, quality and risk assessment. This is in line with the objectives of the national and international plans on resistance to medications. The competitiveness in the livestock sector should stimulate us to look for more efficient and profitable alternatives for their farming. Helminths can cause chronic and sometimes fatal diseases that infect an estimated two billion people worldwide, but the misuse and overuse of antiparasitic drugs can cause serious global drug resistance problems as well. This necessitates the isolation and identification of new anthelmintic drugs for veterinary and human medicine.

***Key words**: anthelmintics, drug resistance, livestock.*

INTRODUCTION

Livestock sector is the fastest-developing subsector in agriculture on global scale today (Perry & Dijkman, 2021), and grazing is included to assure better production quality. In extensive farming, animal diseases can pose serious challenges, particularly gastrointestinal nematodes (GINs) such as *Haemonchus contortus*, *Ostertagia ostertagi, Teladorsagia circumcincta, Cooperia oncophora,* and *Trichostrongylus colubriformis*, along with other severe infections that can be acquired from pasture (Charlier et al., 2020a; Adduci et al., 2022). These issues can lead to chronic conditions characterized by symptoms like diarrhea and anemia, or in some cases, they can even be fatal (Craig, 2018). The impact of GIN infections has resulted in substantial economic losses in productivity, threatening animal health on a global scale (Charlier et al., 2020b; Sargison, 2020).

Three primary classes of broad-spectrum anthelmintics are currently utilized for managing GIN infections: benzimidazoles, imidothiazoles/tetrahydro-pyrimidines, and macrocyclic lactones (Hassan & Ghazy, 2022). Nevertheless, not only did such control result in the presence of drug residues in the foods of animal origin but it also led to drug resistance (Vineer et al., 2020; Charlier et al., 2022). Therefore, there is an urgent need for new effective methods to cope with GINs so to obtain sustainability of livestock production system.

This is a study reviewing the research on the use of alternative anthelmintics, with an attempt to reprise the strategies for helminth control and to envisage new solutions with regard to sustainability including quality, safety and risk assessment.

MATERIALS AND METHODS

An in-depth comprehensive search of scientific literature was conducted to study the problem in focus. The present review used information from articles obtained from Google Scholar, Research Gate, PubMed, Scopus and Web of Science.

RESULTS AND DISCUSSIONS

Around the world, intestinal parasitic nematodes affect approximately two billion individuals. With no vaccines available for these infections, our efforts to control them heavily depend on chemotherapy. However, the rise of drug resistance is becoming a significant challenge. This underscores the urgent need to discover and develop new anthelmintic medications, particularly those that operate through innovative mechanisms. Great expectations are placed on medicinal plants for veterinary applications, involving anthelmintic treatment. They have been traditionally used in the past with their positive effects, or if they have toxic side effects they are well known. However, active compounds against nematodes have not yet been recognized in most medicinal plants. Against human intestinal nematodes are used four main anthelmintic drugs: albendazole, pyrantel pamoate, mebendazole and levamisole. For application in human medicine, they had been developed and marketed as veterinary drugs in the first place. Albendazole and mebendazole are the drugs of choice for mass drug administration programs and are most effective in ascariasis and hookworm infections. The pharmaceutical progress in the development of anthelmintic drugs has been retarded in the recent 40 years some of them showing side effects (Liu et al., 2020).

Pathogens various helminth species, including gastrointestinal nematodes (GINs), lungworms and liver flukes found in cattle, sheep and goats cause severe pathological conditions with adverse effect on productivity in all grazing ruminants (Charlier et al., 2022).

Current global trends demonstrate that Anthelmintic resistance (AR) is becoming a serious issue for the efficent control of parasites in ruminants. Combating anthelmintic resistance (AR) will require a concerted effort in research to innovate diagnostic tests for detecting helminth infections, develop sustainable treatment strategies, and create complementary control methods, including vaccination and plant-based approaches (Charlier et al., 2022). Accurately gauging the prevalence of AR can be difficult, as farmers typically report resistance only after observing clinical signs of parasitic gastroenteritis in their livestock. These initial reports usually pertain to just one class of anthelmintics and one type of parasite. Multispecies resistance, which involves multiple parasite species, is being reported more frequently (Bartley et al., 2021; Sargison et al., 2001). To effectively implement integrated parasite control programs in ruminant livestock, there needs to be a greater emphasis on the adoption of anthelmintic efficacy and resistance tests. Unfortunately, the diagnosis of anthelmintic resistance (AR) in farm animals has been overlooked for years, and there has been a lack of investment in developing and enhancing diagnostic tools. This neglect undermines the effectiveness of anthelmintic control programs (Charlier et al., 2020c).

Anthelmintic resistance inevitably appears after persistent administration of anthelmintic drugs due to the selection pressure they impose on the populations of parasites. The control GINs in ruminants should be focused on solutions for the different situations based on the characteristics of the host and the GIN species, as well as on farming systems and local epidemiological factors that play effect on GIN biological functions. This calls for alternatives in helminth control, like pasture management, genetic selection, medicinal plants, nematophagous fungi (Charlier et al., 2022).

Throughout history, plants have played a vital role in combating parasites. Research indicates that various plant-derived compounds - like terpenes, glycosides, saponins, flavonoids, tannins, and alkaloids - exhibit properties effective against worms. Despite this potential, there are currently no commercially available plant-based remedies for helminth control. Instead, reliance on a few powerful medications known as anthelmintics has increased, leading to significant concerns over drug resistance on a global scale. This necessitates an urgent need to isolate and identify new anthelmintic drugs for animals and humans. Parasitic nematodes are of two types: intestinal nematodes and tissue or blood nematodes. Helminths live in the gastrointestinal tract of the host and feed on nutrients from the host causing infections/diseases. They also cause damage to the immune system. Secondary metabolites such as alkaloids, terpenes, flavonoids, resins, and phenolic compounds demonstrate promising anthelmintic properties. Terpenes, composed of multiple isoprene units (C_5H_8), have been shown to effectively damage the intestinal walls of parasites. Additionally, glycosides exhibit strong efficacy against a range of helminths. Cardenolides disrupt the transport of sodium and potassium ions in helminths and cause death. Saponins contain triterpene or steroid-aglycone with sugar chains, show their anthelmintic activity by inhibiting acetylcholinesterase and thus lead to their paralysis and death. Flavonoid-rich plants play a significant role in disrupting the phosphorylation process, which inhibits energy production in parasitic worms, ultimately leading to their demise. For instance, quercetin interferes with the ability of worms to absorb essential nutrients from their host cells. When larvae consume condensed tannins, these compounds bind to their intestinal mucosa, resulting in autolysis. Additionally, alkaloids exhibit anthelmintic properties by targeting acetylcholine receptors and blocking glucose uptake, causing the worms to starve (Liu et al., 2020).

In a first survey for Slovakia, Babják et al. (2018) studied the incidence of AR in 30 goat farms in the grazing season conducting larval development test (LDT) in three species of larvae and used resistant benzimidazole (BZ) concentrations observed after treatment. The Fecal Egg Count Reduction Test showed percentage reductions of 69 to 86% for single dose and 36-45% for double dose. AR is observed to be more common in goats compared to sheep. The authors found that BZ resistance is rather widespread in the studied farms.

Parasitic gastroenteritis (PGE), caused by GIN infections, is a sheep disease causing enormous loss estimated to €157-477 million per year to European sheep meat producers (Charlier et al., 2020c). Without specific vaccines to be applied, anthelmintic treatment of GIN infections is practiced - usually by oral drenches or injections. However, AR prevails the flocks due to too frequent treatment, wrong dosage as per bodyweight and repeated use of one and the same anthelmintic class instead of alternative drug groups (Williams et al., 2021). Something more, keeping sheep on clean pastures after treatment is a reason for AR development because resistant worms are allowed to re-infect sheep (Papadopoulos, 2008). There is AR against 4 out of 5 known groups of GIN anthelmintics, including monepantel which is available in the recent decade (Raza et al., 2016). A recent thorough literature review of Williams et al. (2021) found no studies on strategies applied by sheep farmers for specific control on GIN in sheep on global scale. Gastrointestinal helminth infection is the major factor threatening sustainability of pasture sheep husbantry (Charlier et al., 2020a). Their impact is generally subclinical with adverse effect on productivity and health and also on veterinary costs. This subclinical effect is the reason for its underestimation in the presence of other more visible pathological conditions. In Italy, helminth infections add roughly €30 million (distributed evenly between production and treatment), including €22.5 million in dairy sheep sector, €6 million in meat sheep and 2.5 million € in dairy goats (Charlier et al., 2020c).

Chemical anthelmintics supplied by pharmaceutical companies have been the primary method of controlling these parasitic diseases for a number of decades. AR-related treatment failure is a significant problem for GIN management in small ruminants. Drug-resistant populations have emerged largely due to the careless or inappropriate use of anthelmintic medications for treating gastrointestinal nematode (GIN) infections (Charlier et al., 2020). There's been a global uptick in reports highlighting the severity of this issue, including the increase of multi-resistant strains and the accelerated development of antibiotic resistance in GIN populations (Vineer et al., 2020). Although chemical anthelmintics are still the most widely used method by Italian farmers for controlling helminths in livestock, there are various complementary and alternative strategies that can be employed. These include biological, immunological, and nutritional interventions such as vaccination, genetic selection, grazing management, nutritional supplementation, and biological control methods (Charlier et al., 2022). Because of its anti-parasitic qualities, using natural bioactive chemicals is seen as a legitimate alternative among these strategies (Hoste et al., 2015). Plant extracts and plant-derived substances are examples of natural bioactive compounds that can be utilized as herbal remedies, dietary supplements, or as models for the production of novel pharmaceuticals (Hoste et al., 2022). Strong and well-established ethnoveterinary knowledge exists, and numerous herbs are specifically utilized in ethnoveterinary medication to manage parasites in small ruminants (Castagna et al., 2021). These items were chosen on the basis of their anthelmintic efficacy against other nematode species as shown in earlier research, as well as their content in secondary metabolites that may be active against GINs. Saponin combinations from *Medicago* spp. plants inhibited GIN eggs in a concentration-dependent manner in the study by Maestrini et al. (2021). At higher doses, the majority of them demonstrated activity comparable to the reference medication (thiabendazole, TBZ). Saponins' unique interactions with cell membranes, which alter cell permeability, are typically ascribed to their biological effects (Cavalcanti et al., 2016). A second study explored the in vitro effects of glycyrrhetinic acid and an aqueous extract from *Glycyrrhiza glabra* roots, which contained 10% glycyrrhizic acid, on gastrointestinal nematodes (GINs) in sheep (Maestrini et al., 2021). Both products showed considerable effectiveness against eggs, but glycyrrhetinic acid exhibited significantly higher anthelmintic efficacy against larvae compared to *G. glabra*. At the highest dosage, its performance was comparable to that of thiabendazole. Additionally, recent research has indicated that *G. glabra* root supplementation can enhance the chemical and physical properties of cow's milk and cheese (Bennato et al., 2019). Furthermore, glycyrrhizic acid has been found to positively influence the ruminal concentrations of total volatile fatty acids, such as acetate, propionate, and butyrate in sheep, by modifying the rumen bacterial ecology (Guo et al., 2019).

In a recent *in vitro* study conducted by Ragusa et al. (2022), hydroalcoholic extracts from the leaves and flowers of *Isatis tinctoria* were found to be highly effective in inhibiting the hatching of GIN eggs in sheep. The researchers suggest that these extracts could be utilized for treating infected sheep, or even consider using whole parts of *I. tinctoria* as a feed or dietary supplement to aid in GIN control within affected populations. Conversely, a study by D'Ambola et al. (2018) demonstrated only a weak ovicidal effect of *Hypoestes forskaolii* extracts on GIN eggs.

Hydroalcoholic extracts derived from the leaves and flowers of *Isatis tinctoria* have been shown to effectively prevent the hatching of gastrointestinal nematode (GIN) eggs in sheep according to an in vitro study conducted by Ragusa et al. (2022). The authors explore the potential of utilizing the entire plant of *I. tinctoria* as either a feed or dietary supplement for infected sheep as a means of GIN management, or alternatively, using the hydroalcoholic extracts for treatment purposes. In contrast, a study by D'Ambola et al. (2018) indicated that extracts from *Hypoestes forskaolii* exhibited only modest ovicidal effects against GIN eggs.

Recent in vitro research has revealed that essential oils extracted from *Origanum vulgare, Foeniculum vulgare, Satureja montana, Satureja hortensis*, and two varieties of *Thymus vulgaris* display significant efficacy against GIN eggs in sheep (Štrbac et al., 2022b). The investigators recommend further exploration of these essential oils for their potential practical applications. Fichi et al. (2017) and Giovanelli et al. (2018) studied the in vitro anthelmintic activity of plant-derived mangiferin, rutin, quercetin, β-sitosterol, aloin, and aloe-emodin on sheep GIN eggs and larvae, among other pure active compounds. None of the substances that were examined demonstrated an efficacy on eggs that was on par with the reference medication (thiabendazole, TBZ). At the highest concentrations tested, only rutin, mangiferin, and aloin proved effective as the reference drug in killing third-stage larvae (L3). In contrast, all compounds except quercetin effectively halted the development of GIN larvae, showing efficacy similar to that of thiabendazole.

Maestrini and colleagues (2022) explored the anthelmintic properties of two sea buckthorn berry juices - SBT and SBF - which are available for human consumption. Their research focused on the effects of these juices on sheep gastrointestinal nematode (GIN) eggs and larvae. While SBF showed somewhat lower efficacy, SBT exhibited significant activity against eggs, comparable to the results from TBZ-treated controls at the highest dose.

Interestingly, both juices proved effective on larvae. The study identified glycosylated isorhamnetin as the most abundant polyphenol in both *H. rhamnoides* juices, with quercetin present in smaller quantities.

Sheep GIN eggs were employed to assess the in vitro activity of gallic acid, the methanolic fraction, and the insoluble residue derived from an aqueous macerate of *Punica granatum* (Castagna et al., 2020). Among these, the methanolic fraction exhibited the highest efficacy, followed by the insoluble residue and gallic acid. All fractions showed a considerable GIN egg hatch inhibition (>82%) at all tested doses.

Despite evidence of anthelmintic capabilities, it is important to emphasize that chemicals must be assessed for safe and efficient farm application because in vitro results are not always transferable to in vivo settings. In Italy, the in vivo usage of some chemicals has previously been the subject of numerous investigations.

Castagna et al. (2021) reported significant anthelmintic effects from the macerate of *Punica granatum*, showcasing strong *in vivo* activity against GINs. In contrast, *Artemisia campestris* displayed only low activity, while *Salix caprea* proved entirely ineffective. The primary constituents identified in the macerate of *P. granatum* included alkaloids, tannins, flavonoids, glycosides, and phenolic compounds, notably gallic and ellagic acids. Research indicates that the anthelmintic properties of these substances could explain the effectiveness observed in this extract (Dkhil, 2013; Ahmed et al., 2020). A subsequent study (Castagna et al., 2022) evaluated the effects of a single oral dose of 50 mL of this plant extract on sheep naturally infected with gastrointestinal nematodes (GINs) across two farms, confirming the in vivo anthelmintic activity of pomegranate (*P. granatum*) macerate. Results showed that between days 7 and 21 after treatment, the pomegranate macerate exhibited nearly 50% efficacy in both farms. These findings suggest that this aqueous pomegranate macerate could serve as a promising alternative to synthetic anthelmintics, potentially reducing the development of anthelmintic resistance and mitigating the environmental impact of conventional medications (Castagna et al., 2022).

Strbac et al. (2022a) conducted a study on two sheep farms where mixed GIN infections were present. They evaluated the effectiveness of *T. vulgaris* essential oil and a blend of linalool and estragole in combating these infections. The results showed that by day 7, the average reduction in eggs per gram of feces (EPG) was 39.30% for the groups treated with *T. vulgaris* and the linalool-estragole mixture. However, by day 14, the reduction dropped to below 10%.

Castagna et al. (2019) evaluated a commercially available natural plant mixture, comprised of essential oils and extracts from various plant families including *Compositae, Cesalpinaceae, Liliaceae, Bromeliaceae*, and *Labiatae*, for its effectiveness as a dietary supplement against GIN infections in sheep. The study, conducted at the manufacturer's suggested dosages, found that the natural supplement did not have any impact on the GIN infections in the sheep.

A number of isoquinoline alkaloids were tested on the larvae of the dog roundworm *Toxocara canis* by Satou et al. (2002). The strongest anthelmintic activity was exhibited by sanguinarine, 6-methoxydihydrosanguinarine, and chelerythrine. On HL60 cell lines, however, these three substances exhibited substantial cytotoxicity, with selectivity indices (the ratio of worm IC50 to mammalian cell CC50) below 0.02.

Originating from *Mentha cordifolia*, β-Sitosterol showed similar capacity against *Ascaris suum* in vitro as pyrantel pamoate and mebendazole (Villaseñor et al., 2002). Against different model organisms, β-Sitosterol was established to be effective anthelmintic compound in a number of studies on medicinal plants (Giovanelli et al., 2018; Vijaya, 2016; Bano, 2019).

Condensed tannins were found to be a primary anthelmintic component of *Onobrychis viciifolia* - a leguminous forage that exhibited. A *Haemonchus contortus* larval migration assay revealed that rutin, nicotiflorin, and narcissin were identified through a bioassay-guided fractionation process targeting molecules with a molecular weight under 2000 Da. The migration of L3 worms showed a significant reduction at a concentration of 1200 μg/mL due to the effects of each of these compounds (Barrau et al., 2005).

Parts of *Cissampelos capensis* were fractionated using bioassay to isolate two aporphine alkaloids, namely (S)-dicentrine and (S)-neolitsine. In a test for the larval development of *Haemonchus contortus*, they demonstrated high anthelmintic action (EC90 = 6.3 and 6.4 μg/mL, respectively). The *in vivo* activity of (S)-Dicentrine was assessed in a mouse model involving *Heligmosomoides polygyrus* infection. At an oral dosage of 25 mg/kg, it showed a 67% decrease in worm counts, as opposed to 99% for the positive control ivermectin (Ayers et al., 2007).

A new anthelmintic compound has been identified from the stem bark of *Acacia oxyphylla*, a plant traditionally utilized for its anti-parasitic properties in India. The compound's structure has been detailed as 12-amino-7,17-dioxo-2-oxa-8,16-diazatricylo tetraicosa-1,3,5,18,21,23-hexaene-12carboxylic acid. At a concentration of 1000 μg/mL, it was observed to kill *Ascaridia galli* worms after 15 hours (Roy et al., 2012).

In Caribbean tradition, *Eryngium foetidum* is used to flavor food and cure intestinal worms. Using a *Strongyloides stercoralis* testing paradigm and bioassay-guided isolation, eryngial (trans-2-dodecenal) was found to be the primary anthelmintic chemical. In a 24-hour larval mortality test, its LD50 (461 μM) is less than that of the positive control ivermectin - LD50 = 2.25 mM (Forbes et al 2016).

Williams et al. (2015) found that the bark extract of *Cinnamomum verum* has anthelmintic effects against *Ascaris suum*. Their phytochemical analysis identified trans-cinnamaldehyde as the main bioactive compound responsible for this effect. To assess its effectiveness, the research tested two methods in a pig model: incorporating 1000 mg daily into the diet and administering 1000 mg in a targeted encapsulated form twice daily. However, neither method resulted in a significant reduction of *Ascaris suum* infection. The researchers proposed that the quick absorption of trans-cinnamaldehyde in the body might account for the lack of effectiveness observed (Williams et al., 2015).

Using a *Necator americanus* egg hatch inhibition assay, Chama et al. (2015) separated the chemical components of *Dichapetalum filicaule* to be examined for anthelmintic action. As a result, glycerol monostearate, dichapetalin X, and dichapetalin A were found to be active, together with a novel dichapetalin.

The key element responsible for the anthelmintic effects of *Thymus vulgaris* essential oil is thymol (Ferreira et al., 2016). This compound proves to be effective against all three critical stages of *Haemonchus contortus*: egg hatching, larval development, and adult forms.

Melaleuca alternifolia essential oil contains terpinen-4-ol, which has been demonstrated to have activity against the larvae and ova of *Haemonchus contortus* (Grando et al., 2016).

Wangchuk et al. (2016) employed xCELLigence technology to evaluate worm motility while screening four chemicals derived from *Ajania nubigena* on *Trichuris muris*. Among these, (3R, 6R)-linalool oxide acetate showed promising anthelmintic properties, but luteolin outperformed with the highest level of activity. Following this, luteolin was evaluated in a mouse model for its efficacy against *Trichuris muris* infection. A dosage of 100 mg/kg led to a 27.6% reduction in worm count, notably less effective than mebendazole, which achieved a 93.1% reduction (Wangchuk et al., 2016).

Dilrukshi et al. (2017) evaluated the motility of *Haemonchus contortus L3* larvae in order to study a natural product library. From this screen came deguelin, a derivative of rotenone. According to the authors, it demonstrated low toxicity against human NFF cells (IC50 > 50 μM) and high anthelmintic action (IC50 = 14.8 μM). According to a more recent study, deguelin modulates oxidative phosphorylation to mediate its anthelmintic action through the mitochondrial respiratory chain (Preston et al., 2017).

According to the research by Ortu et al. (2017), three specific chemicals present in the essential oil of *Ruta chalepensis* - namely 2-decanone, 2-nonanone, and 2-undecanone - demonstrated promising effectiveness against various gastrointestinal nematodes in sheep, such as *Trichostrongylus* spp., *Haemonchus contort.*

Coumarin (2H-chromen-2-one) was isolated through a bioassay-guided fractionation process from *Gliricidia sepium*, using an egg hatch assay to evaluate its effect on *Cooperia punctata*. The compound demonstrated an IC50 of 24 µg/mL (164.3 µM), effectively inhibiting both hatching and embryo development (von Son-de Fernex et al., 2017).

In a study using a mouse model, *Avenacoside B*, a saponin extracted from the green leaves of *Avena sativa*, was found to reduce the infectiousness of *Heligmosomoides bakeri* larvae. The application of Avenacoside B led to noticeable changes in larval morphology, an increase in the production of IL-4, and a reduction in glycoprotein pump (Pgp) activity (Doligalska et al., 2017).

Chlorogenic acid has been recognized as the effective anthelmintic compound, exhibiting an LC50 of 248 µg/mL in an in vitro study focused on the egg hatching and mortality of *H. contortus* larvae. This discovery was achieved through bioassay-guided purification from *Tagetes filifolia* (Jasso Díaz et al., 2017).

Castillo-Mitre et al. (2017) used an in vitro egg hatch inhibition assay for *H. contortus* to identify a number of caffeoyl and coumaroyl compounds from *Acacia cochliacantha*. The highest inhibition was achieved by caffeic acid at 1 mg/mL (98%), followed by methyl caffeate and methyl-p-coumarate (88%). An egg hatch suppression of 94% was also achieved by the fraction that included a combination of methyl ferulate + quercetin and p-coumaric acid + ferulic acid. The scientists came to the conclusion that plants belonging to the Leguminosae family might provide a different source for small ruminant gastrointestinal nematode control.

On the basis of their composition of polyphenols, Soldera-Silva et al. (Soldera-Silva et al. 2018) hypothesized that avocado seeds would have potential for anthelmintic uses. They discovered anthelmintic substances that were more effective than rutin (EC50 = 30 µg/mL), such as epicatechin (EC50 = 10 µg/mL). Furthermore, despite the fact that Jasso et al. (2017) had previously discovered (although minor) anthelmintic activity, chlorogenic acid was also extracted and examined but did not demonstrate any notable effects.

Wanderley et al. (2018) found that cysteine protease derived from the latex of *F. benjamina* shows promise as an anthelmintic agent against H. contortus, making it a viable option for parasite management. Through the fractionation of latex protein extract (LPE) using ammonium sulfate and subsequent chromatography on CM-cellulose, they successfully isolated a cysteine protease known as FbP. This enzyme has a molecular weight of approximately 23.97 kDa and demonstrates stable proteolytic activity across a broad temperature spectrum and pH ranges of 6.0 to 10, with optimal performance at 60°C. The effective doses of FbP were determined to be 0.26 and 0.79 mg/mL, effectively inhibiting the growth and ensheathment of *H. contortus* larvae.

Calves have shown an interesting response to *Cooperia punctata*, where kaempferol 3-O-rhamnopyranosyl-(1→6)-β-D-glucopyranoside-7-O-rhamnopyranoside, also known as oxytroside, completely suppressed ensheathment. This compound was extracted from the leaves of *Gliricidia sepium* through a method called bioassay-guided purification, with a concentration of 2400 µg/mL (von Son-de Fernex et al., 2018).

In another study, procyanidin A2, a type of condensed tannin, was successfully isolated from the Australian plant *Alectryon oleifolius* using the same bioassay-guided approach. It displayed significant anthelmintic effects during larval development tests, achieving full inhibition at 50 µg/mL with an IC50 value of 12.6 µg/mL (Payne et al., 2018).

Additionally, *Baccharis conferta*, a plant native to Mexico, has provided the flavonol isokaempferide, which has recently been shown to have ovicidal effects on *H. contortus* eggs, demonstrated by an IC50 of 80 µg/mL. The researchers also isolated hydroxycinnamic acid and 4,5-di-O-caffeoylquinic acid from this plant, noting that a dose of 3 mg/mL resulted in complete inhibition of egg hatching (Cortes-Morales et al., 2019).

A mouse model infected with *Toxocara cati* resulted in good efficiency of Brazilian red propolis - IC50 = 300 µg/mL (Sinott et al., 2019).

In vitro ovicidal action against a number of cattle gastrointestinal parasitic nematodes is demonstrated by the bioactive compounds (gallic acid and an unidentified chemical) derived from *Caesalpinia coriaria*. According to García-Hernández et al. (2019), these galloyl derivatives showed 100% ovicidal activity against *Cooperia* spp., *Haemonchus* spp., *Ostertagia* ssp., *Oesophagostomum* spp., and *Trichostrongylus* spp. at 1000 µg/mL.

As used in Indian traditional medicine in India, the isolated in the study of Banerjee et al. (2019) andrographolide demonstrated significant anti-ovum (at 0.125 µg/mL) and anti-larvae (at 19 µg/mL) properties against *Ancylostoma duodenale*.

Castaneda-Ramirez et al. (2019) used bioassay-guided purification to isolate p-coumaric acid from *Senegali gaumeri* leaf extract. They come to the conclusion that while p-coumaric acid has anthelmintic qualities, it may also work in synergy with other substances.

Host resistance and resilience to helminth infections - defined as the ability to mount an effective immune response and to maintain productivity despite parasitic challenges - are largely influenced by genetic factors across various species, breeds, and individuals (Zvinorova et al., 2016; González et al., 2019). Improving resistance or resilience can enhance productivity in environments where the risk of parasitic infections is high and can reduce the need for treatments, thereby lessening the selection pressure for antibiotic resistance (AR). Consequently, research highlighting differences in resistance or resilience to bovine helminths can be instrumental in developing effective parasitism management strategies. By focusing on the use of resistant breeds and promoting the sustainable farming of indigenous breeds, we can improve outcomes (Zanzani et al., 2014).

When it comes to goats, they tend to be less effective than sheep at mounting immune responses against gastrointestinal nematodes (GINs), often resulting in higher infection levels (Hoste et al., 2010). Therefore, studies examining this issue in goats are particularly noteworthy. For instance, Alberti et al. (2014) explored the impact of natural GIN infections on milk yield and quality during an entire lactation season, comparing Alpine goats (AB), a breed known for its high milk production, with the Nera di Verzasca (NV), an Italian indigenous breed adapted to mountainous environments. Both breeds demonstrated reductions in milk output as well as protein and fat content due to GIN infections, but the decline was significantly more pronounced in the AB breed compared to the indigenous NV breed (Alberti et al., 2014). To delve deeper into the mechanisms driving the differences observed between goat breeds, a follow-up study was conducted involving AB and NV goats from the same farm. This study included both controlled nematode challenges and natural gastrointestinal nematode (GIN) infections in field conditions. It examined various factors related to fecal egg counts (FEC) and analyzed differences in anti-*T. circumcincta* antibody levels and packed cell volume (PCV) (Zanzani et al., 2020).

The findings consistently showed that NV goats exhibited lower mean EPG values during both experimental and natural GIN infections compared to AB goats. The study highlighted a significant difference in packed cell volume (PCV) between NV goats and their AB counterparts, with NV goats exhibiting notably higher values. Among AB goats, lower PCV values were linked to higher strongyle eggs per gram (EPG) counts, indicating that the decline in PCV during gastrointestinal nematode (GIN) infections likely stemmed from the presence of *H. contortus*. Furthermore, the levels of anti-*T. circumcincta* IgA were influenced by both strongyle EPG and breed, showing a negative correlation where higher EPGs were associated with lower IgA levels. Interestingly, NV goats displayed a more robust response to nematode infections compared to the AB breed. However, this increased resistance seen in NV goats was not connected to the circulating levels of T. circumcincta-specific IgA or IgE against L3 larvae. Both experimental and natural infections confirmed that NV goats are more resilient against GINs. Another study by Agradi et al. (2022) indicated that the hematological parameters of female NV goats differed from those of AB goats under similar rearing conditions, with NV goats typically showing higher red blood cell parameters. This discrepancy suggests that the local NV breed has developed a greater resistance to gastrointestinal parasites and a better adaptation to environmental stressors, likely

due to centuries of specific adaptation strategies to their breeding context.
To investigate the differences between breeds, Zanzani et al. (2020) analyzed factors connected to fecal egg counts (FEC) as well as variations in anti-*T. circumcincta* antibody and packed cell volume (PCV) levels in AB and NV goats from the same farm. This was done under both controlled nematode challenges and natural gastrointestinal nematode (GIN) infections in field conditions. They found that the mean egg per gram (EPG) values in NV goats were notably lower compared to those in goats experiencing both natural and experimental GIN infections. The higher nutritional value (NV) of NV goats compared to AB goats significantly influenced their packed cell volume (PCV). In AB goats, there was a notable connection between lower PCV values and elevated strongyle eggs per gram (EPG), suggesting that the decline in PCV during gastrointestinal nematode (GIN) infections was likely due to *Haemonchus contortus*. Both the breed and the strongyle EPG levels affected the antibodies against *Teladorsagia circumcincta*, with IgA levels showing an inverse relationship with EPG. While NV goats exhibited a more effective response to nematodes than the AB breed, the levels of circulating *T. circumcincta*-specific IgA or IgE against L3 larvae did not indicate a direct correlation with the NV breed's increased resistance to GIN infections. Overall, NV goats showcase a greater resilience against GIN infection, as evidenced by both natural and experimental challenges. Another study (Agradi et al., 2022) showed that, under the same rearing conditions, reference haematological intervals in female goats of the local breed (NV) and the cosmopolitan breed (AB) differed, with the majority of red blood cell parameters being higher in NV than in AB. These differences indicated that the local breed was more resistant to gastrointestinal parasites and more adapted to environmental stressors, likely as a result of centuries-old breeding context-specific adaptation strategies.
To explore the differences in GIN reinfection between local and cosmopolitan goat breeds, along with the effect of specific risk factors like sampling, breed, and number of births, Zanzani et al. (2019) examined the qualitative and quantitative variations in fecal egg counts (FEC) after anthelmintic treatment in a naturally infected herd of three dairy goat breeds: one local breed (Orobic) and two cosmopolitan breeds (Alpine and Saanen). The study revealed that cosmopolitan goats showed higher FEC values in multiparous individuals compared to primiparous ones. In contrast, Orobic goats displayed the opposite trend in reinfection rates. This difference could be attributed to the stronger acquired immunity of local goats compared to their cosmopolitan counterparts.
Genetic diversity is evident among various sheep breeds and individual animals, leading researchers to propose that selectively breeding sheep with better resistance to gastrointestinal nematodes (GINs) could be a viable and sustainable way to tackle parasite-related issues (Bishop & Morris, 2007). The farming practices surrounding the Sarda sheep range from semi-extensive to semi-intensive, making GIN infections quite common due to their reliance on natural grasslands and feed crops (Casu et al., 2022). Interestingly, a study by Aguerre et al. (2018) found a significant correlation between fecal egg count (FEC) during both artificial and natural infections. Given this insight and the heritability estimates from a substantial experimental group in Italy, genetic selection for parasite resistance appears to be a promising avenue for the Sarda breed (Casu et al., 2022). This research also highlighted potential genes and polymorphisms that could be explored in further validation studies.
The two main animal health issues in dairy sheep that have a significant effect on productivity are nematode parasites and mastitis. To select for greater resistance to subclinical mastitis, somatic cell count (SCC) has been employed as an indicator (Barillet, 2007). Understanding the connections between these important health characteristics is necessary for the implementation of nematode resistance and SCC in breeding programs. Dairy sheep in Italy were studied to assess the genetic (co)variances related to resistance against nematode parasites, measured through fecal egg counts (FEC), in natural infection and somatic cell count (SCC) contexts (Sechi et al., 2009). To isolate specific environmental effects

and gather accurate lactation data for both traits used in the genetic analysis, records for somatic cell score (SCS) and FEC from each subpopulation were carefully processed within a sample of 949 backcrossed ewes and their 806 daughters. The heritability coefficients for FEC and lactation SCS (LSCS, which is the average of test days during each lactation) were 0.19 and 0.16, respectively. The genetic correlation between the two traits was found to be 0.21, while the phenotypic correlation was minimal at 0.01. Based on these heritability estimates, both traits can be evaluated with traditional quantitative methods. Furthermore, selecting for one trait is not expected to adversely affect the other, given the anticipated genetic relationship between LSCS and FEC.

Thus, the use of breeding techniques that create animals with a naturally low vulnerability to nematode infections may become more and more crucial in the integrated management of these helminths in small ruminants. Local goat breeds in Italy have shown higher resilience to GIN, and selection for greater resistance to GINs and subclinical mastitis in dairy sheep has been assessed. A list of potential genes and polymorphisms for the Sarda breed has been provided, which may be utilized in future research (Maurizio et al., 2023).

The industry of small ruminant animals, particularly in tropical and subtropical regions, must be alarmed by this phenomenon. Natural immunity is weakened in developing countries due to insufficient natural nutritional resources (Knox et al., 2006). The wormer specialists use several methods to carry out their actions. All items in the same class, however, operate in the same way. As a result, other items in the same class suffer when there is opposition to one of the products in the class. The veterinarian must determine whether the antiparasitic medication is appropriate based on the animals' health, quality, and parasitic load (quantitative parasitological analyses). In any event, the growing prevalence of AR compels us to search for substances other than pharmaceutical treatments (Ferreira et al., 2016). Additionally, this emergency must force many veterinarians and farmers to address preventative medicine, including integrated care against parasites (Lambertz et al., 2019). Alternative approaches to parasite management must be considered in conjunction with the administration of anthelmintics. From this vantage point, medicinal plants have long been utilized in pharmaceuticals. However, their applications have always been limited to the traditions of confined groups, and neither the potential of such cures nor non-compounds/mixtures have ever been completely explored. To treat parasitic nematode infections, such as those caused by *Strongylus, Parascaris,* and *Ascaris species* in monogastric animals, including humans, chenopodium oil has been a longstanding treatment option in the UK (Githior et al., 2006). This oil is extracted from the *Chenopodium ambrosioides* plant. Various parts of the plant, particularly the dried leaves and flowers, have also been utilized as anthelmintics since the early 1900s. In Latin America, *Chenopodium continues* to be employed for similar purposes (Potawale et al., 2008). Research by Maqbool et al. (2017) indicates that other species, including the male fern Dryopteris filix-mas and several *Artemisia* species, have shown effectiveness against nematodes from the *Ascaridia* genus in poultry as well as certain cestodes from the *Moniezia* genus in ruminants. Numerous plants that have the potential to be employed as anthelmintics have been found through recent investigations. The scientific community is being pushed to reexamine ethnopharmacological traditions in order to find alternative medications and treatments due to the current scarcity of chemotherapeutic medicines, which is also connected to mechanisms related to resistance. In a review paper, Liu et al. 2020 provided an overview of botanically derived chemicals that have been published since 2002 – a review of a larger amount of compounds that include information about the molecule's class, potential mechanism of action, effectiveness against the parasite model, and plant of origin. Only a small number of those mentioned, though, have undergone in vivo testing. According to thorough research published in 2020 by Garcia-Bustos et al. (2020), numerous natural items have anti-parasitic properties for both humans and other animals. Regarding the chemical structure, this review does a decent job of classifying and describing the most significant compounds.

The southern Italian area of Calabria offers a wide variety of plants in addition to a very rich ethnobotanical and ethnopharmacological past. The region's high degree of geographical variability is associated with botanical richness. A vast number of ecological niches that can support this kind of variety have been made possible by the significant height difference between Southern Italy and the Mediterranean's center. Because of this heterogeneity, non-native plants have been able to colonize the area and have been assimilated into the local ethnopharmacological knowledge over the years. *Punica granatum* is one of these that was domesticated in the Mediterranean region as early as the fifth millennium BC. Gallic acid, ellagic acid, phenolic punicalagins, and other fatty acids are among the components of the pomegranate that are known to exist. Other components include flavonones, flavones, tannins, anthocyanidins, catechin, rutin, quercetin, and other flavonols. Although the synergistic impact of the phytocomplex's ingredients must always be taken into account, alkaloids and tannins are the pomegranate constituents with the strongest anthelmintic effects (Villalba et al., 2017). Parasiticidal action has been emphasized in vitro in other investigations (Abozeid et al., 2020, Kaiaty et al., 2021).

CONCLUSIONS

Thus, it is vital to conduct more precise trials on the action of plant anthelmintics. Using natural antiparasitic plant supplements can enhance the quality of animal products in the market. This approach not only improves animal health but also contributes to safer food for human consumption. As an advanced strategy, it's essential to explore the functional effects of these supplements. Additionally, further research on polyphenols should be conducted to provide more evidence of their benefits for livestock health. Furthermore, to create the most appropriate ratio between polyphenol extracts and plants from which they can be best used depending on the characteristics to be improved, the quality of polyphenol preparations is also an essential topic of research, in view of their efficacy of application. For better animal health, lifestyle and food safety, the addition of polyphenols can be tested and practically implemented with a free choice of feeding approach. These properties encourage research and the use of various biologically active components as a natural means to improve the quality of their production.

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