Living with parasites

Natural remedies to control *Haemonchus contortus* and *Fasciola hepatica* parasitic infections in ruminants in the Netherlands

30-04-2021

By Julia Boeré, Elise Schuurman, Marije Steensma, Jialing Qian, Willeke Weewer, Jinyi Zhong

Special thanks

Without all the help we received we could not have written this report. We witnessed big hearts for animal production systems and a lot of enthusiasm on tackling parasite issues. Special thanks to Harm Ploeger, Adriaan Antonis, Jiaguo Liu, Dr. S. K. Kumar, Michael Walkenhorst, Hubert Cremer, Deyun Wang and Tedje van Asseldonk for explaining and discussing the issues and solutions regarding our topic. Special thanks to Klaartje van Wijk, Harmen van der Sluis, Jan Bruins, Jos Eldering, Chris Kennet, Karin Dijkstra, Frank Wennekers and Mara van den Berg for providing a practical perspective much needed for practical solutions.

Colophon

This report is produced by students of Wageningen University as part of their MSc-programme. It is not an official publication of Wageningen University or Wageningen UR and the content herein does not represent any formal position or representation by Wageningen University.

This report was made in consultation with experts and veterinarians, but the writers have no veterinary experience themselves. Always consult with your vet or advisor before implementing natural remedies for your livestock.

Cover page illustration: Boeré, J. (2016). Cow in a field in Germany.

Abstract

Parasitic resistance development has caused many difficulties for farmers trying to treat internal parasites in ruminants over the last decades. Natural remedies and practices against internal parasites in ruminants could provide a solution. However, a lot of knowledge on effective herbs has been lost in the Netherlands, which hampers implementation in Dutch agriculture. This study aimed to identify promising natural remedies and practices to control internal parasites in ruminants. In this report, the most promising natural remedies and practices against *Haemonchus contortus* (barber's pole worm) and *Fasciola hepatica* (liver fluke) were researched by literature study and interviews. Herbs which were shown to have significant effects *in vivo* and which are applicable in Dutch agriculture systems are considered promising herbs.

It is advised to increase species richness by implementing *Lotus corniculatus* (birdsfoot trefoil), *Cichorium intybus L*. (chicory) and *Onobrychis viciifolia* (sainfoin) to control *Haemonchus contortus*, and *Fumaria parviflora* (fineleaf fumitory) to control *Fasciola hepatica,* in the pasture to allow self-medication. Moreover, these herbs keep their anthelmintic properties when processed into hay or silage, which increases their availability throughout the year. Additionally, *Cymbopogon citratus* (lemongrass) essential oil against *Haemonchus contortus* and *Moringa oleifera* (drumstick tree) and *Nigella sativa* (black cumin) against *Fasciola hepatica* could be imported and added to the feed. Additionally, dried and fresh herbs could be combined into mixtures and be applied when infection rates are causing a burden to the animals. We recommend looking internationally, for example at the Chinese herb mixtures. Especially the Quchongxiehuosan and Ganzhisan mixture looked promising against *Haemonchus contortus* and *Fasciola hepatica*, respectively. Furthermore, applyingmedicinal herbs in combination with other alternative management methods could give more profound anthelmintic effects.

Contents

Introduction

Anthelmintic resistance development in all drug classes is increasing and has caused problems in livestock rearing for the last few decades (Kaplan, 2004; Kaplan & Vidyashankar, 2012). Multi-drug resistance in helminths of goats and sheep is a real threat to the viability of the sector. For horses and cattle, resistances in helminths are currently less significant, but their resistance is also increasing (Kaplan & Vidyashankar, 2012).

Helminths have an exceptionally high level of genetic diversity due to rapid rate of nucleotide sequence evolution and high levels of gene flow. In other words, they have a high mutation rate and fast exchange of genes (Anderson, Blouin, & Beech, 1998; Blouin, Yowell, Courtney, & Dame, 1995). The fast adaptation of the parasites combined with the ability to evade the immune systems in livestock animals and limited availability of drugs due to already developed resistances, gives livestock producers limited options for effective treatment against parasites.

For the majority of modern anthelmintics, their activity is targeted within only three biochemical/physiological areas (Martin, Robertson, & Bjorn, 1997; Wolstenholme & Rogers, 2005). These known target sites are all proteins which include ion channels, enzymes, structural proteins and transport molecules (Köhler, 2001). When exposed to these drugs, helminths can react by acquiring drug resistance through receptor loss or decrease of affinity for the drug on the target site. Once resistance in one target site appears, multiple drugs lose their effectiveness (Köhler, 2001; Martin et al., 1997). Since 1981, no new anthelmintic drugs were developed and approved, until in 2004 Monepantel was launched (Kaplan & Vidyashankar, 2012). However, this drug is not expected to solve the resistance problems, which are more deeply rooted in the way anthelmintic drugs are used and the ability of helminths to adjust to new situations. New anthelmintics with novel mechanisms could create new effective treatments against parasitic infections. However, development of these new drugs is extremely expensive, and there is the risk of losing revenue if the new drug is not shown to be effective (Kaplan & Vidyashankar, 2012). Therefore, a new approach is needed; learning to live with helminths in livestock and control infection rates without inducing further resistances.

In addition to problems with resistance, the use of anthelmintic drugs could affect soil health. Anthelmintic residues in the manure of treated animals may negatively affect earthworms populations (Goodenough, Webb, & Yardley, 2019). Some antiparasitic products were shown to negatively affect springtails, enchytraeids and earthworms (Diao, Jensen, & Hansen, 2007). Nevertheless Jensen, Diao, and Hansen (2009), tested another range of anthelmintic compounds and showed that these are unlikely to have a negative effect on soil life. However, Diao et al. (2007) and Jensen et al. (2009) both applied the anthelmintic to the soil after a dilution with acetone. Anthelmintic residues in manure from animals treated with the anthelmintic could result in different chemical and biological interactions as their structure could have been changed by the digestion system. In addition, the concentration of the chemical in the manure and in pastures is not considered in both studies. Goodenough et al. (2019) did consider concentrations of the anthelmintic drugs based on animal grazed pasture, but the drugs were only diluted with distilled water before applying to the soil. The negative effects of anthelmintics on soil life is therefore not broadly acknowledged and not enough research has been conducted. Nevertheless, while the debate on the exact effect of anthelmintics on soil health is still open, it may be wise to consider more sustainable alternatives to keep both animals and the soil healthy.

Health problems caused by internal parasites in ruminants may be prevented by changing management practices, and additionally use herbal remedies that focus on strengthening the immune system. Currently however, countries like the Netherlands have poor herbal knowledge,

which causes difficulties in using natural remedies in an effective manner. Farmers, veterinarians, and students are not usually taught about these practices. Other countries like India still have knowledge on traditional remedies and herbs (Nirala, 2019). It is thought that The Netherlands can learn from countries like India and apply natural remedies effectively to keep our livestock and environment healthy now and in the future. Nevertheless, it is not yet known how different herbal compounds work and work together, and how to apply herbs in Dutch agricultural systems in an effective and sustainable way.

Foundation Platform Natural Animal Husbandry (Stichting Platform Natuurlijke Veehouderij) wants to rediscover the knowledge on natural anthelmintic remedies and advise farmers to apply this knowledge in agricultural practices. In their ideal case: firstly, management practices are considered, such as changing pastures. Secondly, natural remedies should be applied, such as herbs. Finally, in case of serious disease synthetic remedies such as ivermectin can be effectively applied. This report is mainly focusing on the application of herbs for sheep and cattle in a Dutch agricultural context.

To determine an approach for the application, knowledge on herbs is needed to manage parasite infections effectively and sustainably in ruminants. However, this knowledge is currently fragmented, and not easily accessible for farmers and veterinarians in the Netherlands. An overview of the most effective herbs and how these can be applied is needed.

The main research question asked: "*Which natural remedies and practices for internal parasites in ruminants have the most potential, and how can these be applied by farmers in the Netherlands in the future?"*

Problems with parasitic infections and possible solutions touch on different disciplines and work fields. For example, immunology, animal behaviour, feeding strategies and breeding should be considered. Furthermore, this problem has a large ecological component, concerning soil health, soil food webs, and plant-, soil- and animal interactions. The social aspect, namely the willingness to implement alternative strategies, adaptive ability of the veterinary and agricultural sector, and awareness of the problem also plays a role. Finally, economics and feasibility, cost and return but also availability of herbs are key factors for the implementation of natural remedies to control parasites in livestock. Some of the mentioned aspects will be touched upon, but not all will be discussed in detail. The focus will be on herbs suitable for implementation and to identify issues hampering implementation. Further, there are numerous internal parasites that cause problems in livestock farming. This report only focuses on two parasites, namely *Haemonchus contortus* and *Fasciola hepatica*. Herbs can have different effects on various parasite species. Therefore, this report is a proof of concept, and applications for other parasite species may differ.

Methods

The current state of the problem was first assessed. Information on the most important parasites, including their pathology and life cycles, was gathered. Next, a list of 5-10 promising natural remedies against the two selected parasites was compiled.

To compile this information, a literature study was performed, as well as several interviews with experts on the field of parasitology, ethnobotany, soil health and ethnoveterinary medicine. A full list with the interviewed experts, their expertise and viewpoints can be found in the appendix.

Lifecycle and pathology of important livestock parasites

Haemonchus contortus (barber's pole worm)

Figure 1. *Haemonchus contortus*, with their twisted white ovaries creating the typical barber's pole pattern.

Introduction

Haemonchus contortus is the most economically important parasite in the Netherlands, and one of the most pathogenic parasitic nematodes targeting small ruminants (McLeod, 2004; Perry, 2002). The worms appear reddish with white ovaries that wind around the intestine in a spiral pattern, hence the name barber's pole worm (Taylor, Coop, & Wall, 2016) (Figure 1). The pathogenicity of *H. contortus* is due to its blood-feeding activity on its host and its capacity for rapid reproduction rate under favourable host and environmental conditions (Besier, Kahn, Sargison, & Van Wyk, 2016b). For the free-living stages of *H. contortus*, a relative humidity between 60–90% (Todd Jr, Levine, & Whiteside, 1970) and a temperature ranging anywhere between 15-30 °C are preferred (Todd Jr, Levine, & Boatman, 1976). Thus, parasitic infections are most common in the summer months. However. *H. contortus* is known to be adaptable to a wide range of environments and take advantage of short periods in which conditions are favourable for the free-living stages of the larvae (Waller & Chandrawathani, 2005). Because of its high level of polymorphism, *H. contortus* has a high level of genetic differences in environmental tolerance level, allowing the parasite to occur almost everywhere where small ruminants are reared (Besier, Kahn, Sargison, & Van Wyk, 2016a).

A high parasite load can lead to reduction in weight, quality of wool and hide, and milk production in the animal (Qamar, Maqbool, & Ahmad, 2011). Economic impact resulting from this parasitic infection is greatest when animals are managed under intensive conditions (Lindberg & Vatta, 2006). Besides sheep and goats, *H. contortus* can also infect cattle, deer, camels, llamas and alpacas, and can be found worldwide (Taylor et al., 2016).

Pathology

When sheep are infected, *H. contortus* is situated on the mucous layer of the abomasum, the fourth compartment of the rumen stomach (Taylor et al., 2016). The severity of the *H. contortus* infection is greatly dependent on the number of ingested infective larvae, and the ability of the host to reject the larvae and to replace the lost blood (Le Jambre, 1995).

During an acute infection, a single adult worm can consume up to 0.05 mL of blood per day, leading to anaemia (low number of red blood cells) when the parasite load is high. Furthermore, the presence of *H. contortus* causes lesions in the stomach wall (Figure 2), leading to further blood loss. Symptoms can be seen on the exterior of the sheep, since the testes, conjunctiva and gums of anaemic sheep appear pale or whitish (Figure 3) (Mehlhorn, 2016; Taylor et al., 2016). Additionally, prolonged anaemia may lead to oedema (build-up of fluid in the body's tissue, causing swelling). If ewes with suckling lambs are affected by the disease, milk production may halt, eventually leading to malnourishment or even death of the lambs (Taylor et al., 2016).

Especially in tropical regions with prolonged dry seasons, chronic *H. contortus* infections may occur (Taylor et al., 2016). The chronic low-level blood loss in combination with low nutritional value of the pastures can lead to weight loss, weakness and lack of appetite which could eventually lead to death.

Figure 2. (Left) *H. contortus* attached to the stomach wall.
Figure 3. (Right) Demonstration of the FAMACHA (FAffa MAlan CHArts) technique, conjunctival-colour index, in a llama to identify the level of severity of anaemia (blood loss).
Both images from 'Pathology of Haemonchus contortus in New World camelids in the southeastern United States: a retrospective review', by E. Edwards et al., 2016, Journal of Veterinary Diagnostic Investigation, *28,* p. 105-109.

Life cycle

H. contortus has a relatively simple and rapid lifecycle (Figure 4). Adult worms most commonly have sheep as their host. Female worms lay eggs that are passed in the faeces. The eggs hatch as first stage larvae (L1), then moults twice to become second (L2) and then third stage larvae (L3) in the faeces, which takes 4- 10 days in total. The infectious L3 migrate higher in the vegetation and can be eaten by the grazing sheep. After being eaten the L3 larvae migrate to the abomasum and develop through the fourth larval stage (L4) into adults in 2-3 weeks (Taylor et al., 2016). As blood feeders, the adults can cause severe disease. One adult can produce up to 10,000 eggs per day (Prichard, 2001), and a single animal can harbour thousands of worms in its lifetime. Development from egg to L3 larvae occurs most rapidly in warm, humid conditions. Under dry and cold conditions, the larvae either die in the field, arrest their development to overcome the cold or drought, or overwinter in the sheep host (in an arrested larval state) (Waller, Rudby-Martin, Ljungström, & Rydzik, 2004). Therefore, previously infected animals infect the clean pastures rapidly after winter, making it difficult to manage infection rate. This is mostly problematic for the young animals who have not yet developed a proper parasite immune defence (Parasitologist Dr. Harm Ploeger[*]). This problem occurs especially in high animal density grazing systems like in the Netherlands where land is limited.

[*] See appendix for the full interview

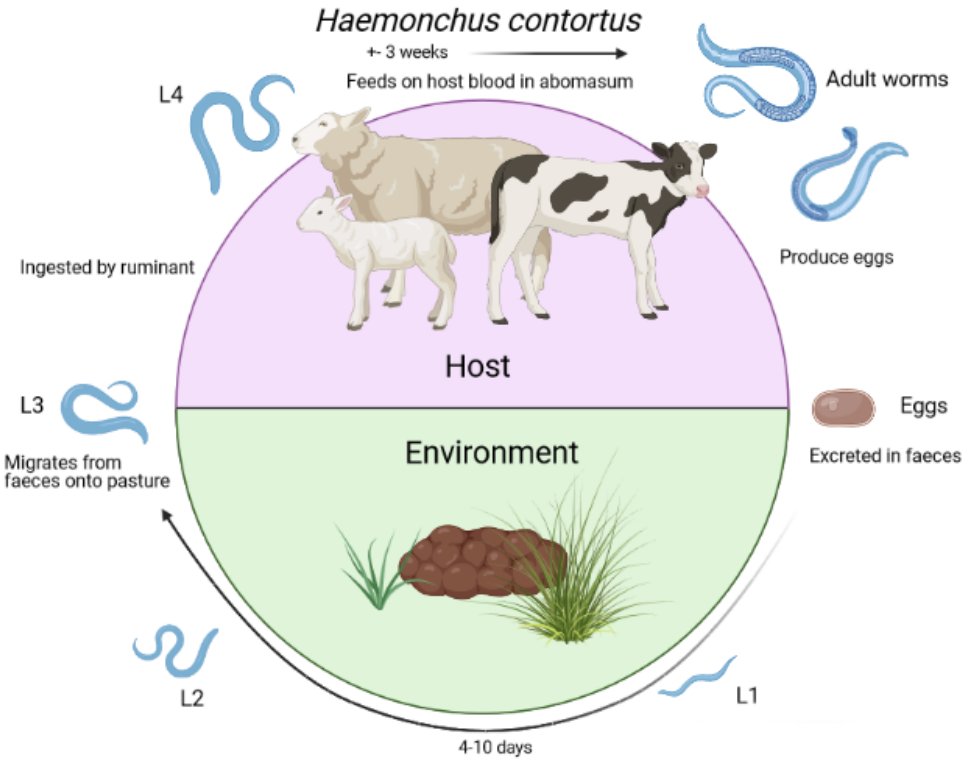


Figure 4. An illustration of the lifecycle of *H. contortus* in sheep and cattle. It takes 4-10 days (depending on the weather) for the eggs to develop into L3 larvae. After the L3 larvae are ingested, it takes approximately three weeks for the larvae to develop into the L4 and subsequently adult worms. The adult worms produce eggs, which starts the next cycle.

Current management

Common drugs used against *H. contortus* in sheep are Levamisole, albendazole, fenbendazole, oxfendazole, ivermectin, moxidectin, and closantel (Bowman, 1999). Because there is high similarity between the mechanism of different drug groups, resistance to these drugs is high. *H. contortus* has shown increased resistance to multiple anthelmintics, especially against albendazole and ivermectin (Castro-Arnáez, Montenegro, Vargas-Leitón, Álvarez-Calderón, & Soto-Barrientos, 2021).

The detection of impending *H. contortus* relies largely on periodic monitoring for anaemia levels through blood tests, or through the 'FAMACHA' conjunctival-colour index (Figure 3), or through faecal egg counts (Machen, Craddock, Craig, & Fuchs, 1998). One of the nonchemical management options that is currently used are grazing schedules. These schedules indicate the periods in which grazing ruminants should be excluded from the pasture. This type of management aims to minimise intake of infective *H. contortus* larvae and contamination of pasture with *H. contortus* eggs (Besier et al., 2016b). Other management options such as pasture rotation (Colvin, Walkden-Brown, Knox, & Scott, 2008) and alternation with other, less susceptible, ruminants (Southcott & Barger, 1975) are also effective. A great limitation for these management practices, however, is not only a lack of awareness of animal owners, but also the limited available pasture in intensive grazing situations (Colvin et al., 2008) (H. Ploeger[*]). If grazing management options are limited, the susceptibility of animal classes can be considered. The high-risk animals can be given priority in allocation to low-risk pastures (Morley & Donald, 1980). This, however, only partly solves the problem as the low-risk animals may still suffer if they get infected with a high parasite load. Furthermore, this approach requires more intensive management.

[*] See appendix for the full interview

Fasciola hepatica (common liver fluke)

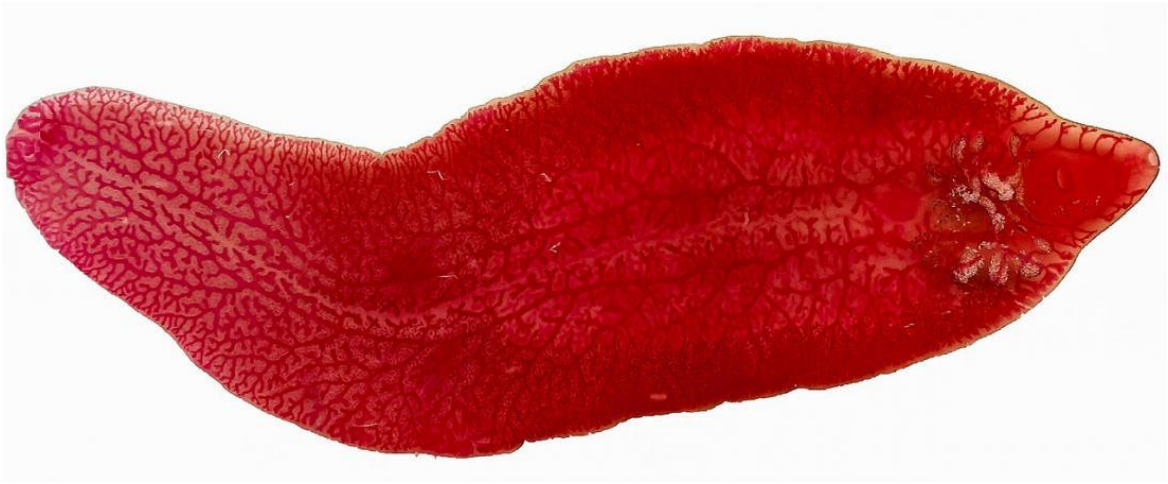


Figure 5: An adult liver fluke, with its head towards the right side.

Introduction

Fasciola hepatica or common liver fluke is a species of parasitic Trematoda that can be found in wetland and other wet areas worldwide (Figure 5) (Taylor et al., 2016). It is a parasite that can infect cattle, sheep, goats, horses, deer and humans (Beesley, Williams, Paterson, & Hodgkinson, 2017b). While adult cattle are relatively resistant to serious disease but can get subclinical impairments caused by liver fluke, young calves and sheep are much more susceptible (Dixon, 1964; Forbes, 2020). The severity of the symptoms induced by the liver fluke depends on multiple factors, including the species of the host animal, the number of ingested fluke cercadia stage larvae, and the stage of parasitic development in the liver (Taylor et al., 2016). The resulting health problems have economic consequences. Infection results in lower production due to reduction in body weight, milk production, fertility, and an increase in mortality rate (Kaplan, 2001; Skuce & Zadoks, 2013).

Pathology

Acute infections with liver fluke in adult cattle rarely cause serious disease, and mostly cause problems in young calves or when the parasite load is very high in adult animals (Taylor et al., 2016). In sheep, however, acute infections with liver fluke can manifest as sudden deaths due to extensive liver damage and bleeding (Mulcahy et al., 1998). This usually occurs during the autumn and early winter (Taylor et al., 2016).

A subacute form of liver fluke infection also exists (Taylor et al., 2016). In sheep, this form can cause lesions in the liver that are similar but less severe than in the acute form. The subacute form can manifest as a rapid onset of anaemia (low number of red blood cells) and hypoalbuminemia (low level of albumin in the blood), which are signs of advanced liver disease and can lead to a high mortality rate. Both in the acute and subacute forms, the liver is enlarged. The subacute form usually occurs during the late autumn and in the winter.

Lastly, a chronic form of liver fluke infection can also occur, especially in the late winter and early spring (Taylor et al., 2016). This is the most common form of liver fluke infection in cattle. It is characterised by a loss of appetite, anaemia and hypoalbuminemia. This can lead to animals becoming emaciated over time, and an overall decrease in production due to reduced food intake. Liver fluke eggs can be found in the faeces of the infected animals. Chronic infection with liver fluke can cause liver damage, fibrosis (scarring of tissue) and haemorrhaging (bleeding). Furthermore, migration of the liver fluke to the bile ducts can lead to damage to the biliary (liver, gall bladder and bile ducts) tissue.

Life cycle

The liver fluke has an indirect life cycle which involves an intermediate host (Figure 6). The intermediate host in the Netherlands is *Galba truncatula* or the dwarf pond snail (van Andel & Ploeger, 2014). In other areas, different snails may be the most common intermediate host depending on climate and snail species prevalence. Infected animals excrete fluke eggs in their manure. When these eggs are washed out of the faeces, they start to develop in water. Under the influence of increased light and temperature, the eggs are hatched. From the eggs, miracidia, which are ciliated, nonfeeding larvae, are released (Williams, Howell, Graham-Brown, Kamaludeen, & Smith, 2014). These nonfeeding larvae feeding larvae require water to survive and find their suitable intermediate host. When they find a snail, they move in through the foot to the body cavity. Within the snail, the parasite then multiplies over a period of six weeks and develops into the next stage, which takes around two months (van Andel & Ploeger, 2014). Cercariae, the larval form developing within a sporocyst, are then released from the snail. One infected snail can produce up to several thousand cercariae. Free-swimming cercariae encyst themselves on the aquatic vegetation or grass around the water body, and form into the next stage of larvae: metacercariae (infective stage larvae). This stage of fluke larvae can be first expected in the Netherlands around August and can stay infectious for several months under wet conditions (van Andel & Ploeger, 2014). Grazing animals might eat vegetation contaminated with these infective larvae, which hatch inside the animal. The hatched excysted juveniles go through the gut wall and travel to the liver. The journey to the bile ducts takes about 10-12 weeks. Here, they mature and start producing eggs, starting the next cycle (Williams et al., 2014).

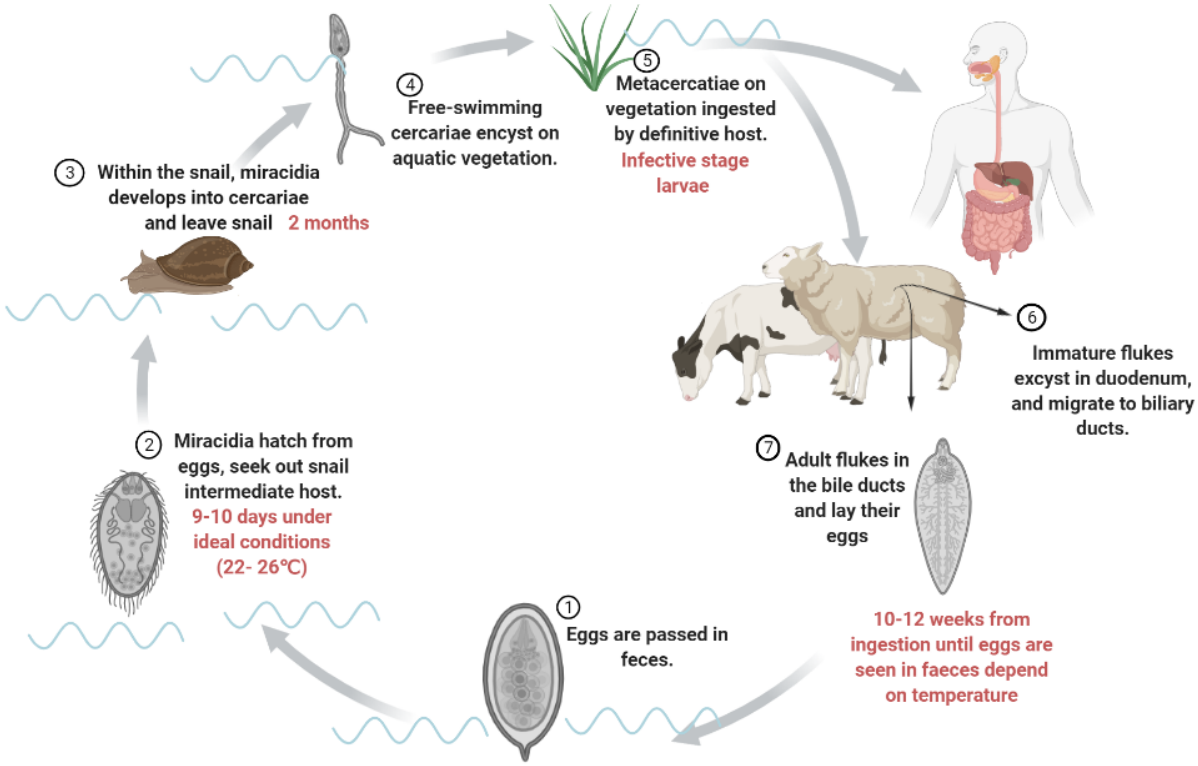


Figure 6. An illustration of the lifecycle of F. hepatica. It takes 6- 8 weeks for eggs develops into metacercariae (infective stage larvae) which can be ingested by grazing animals or human. After ingestion, it takes 10 –12 weeks (dependent on the weather) for these larvae excyst in duodenum and migrate to bile ducts. Then they mature and lay eggs, starting next cycle.

Current management

Reports of resistance to triclabendazole which is the most widely used in treating liver fluke and to other current drugs are increasing (Fairweather, Brennan, Hanna, Robinson, & Skuce, 2020). Moreover, liver fluke have the capacity to modulate the host's immune system, affecting susceptibility to and diagnosis of other pathogens. As of 2013, flukicide restrictions for lactating dairy cows only allow the use of albendazole and oxyclosanide for fluke control (Williams et al., 2014). Moreover, liver fluke have the capacity to modulate the host's immune system, affecting susceptibility to and diagnosis of other pathogens. As of 2013, flukicide restrictions for lactating dairy cows only allow the use of albendazole and oxyclosanide for fluke control (HPRA, 2013) However, those anthelmintics are only effective against the liver fluke in adult stages (Fairweather, 2011).

The infectious stage of larval development of the liver fluke can be found on grass in pastures where infected animals have grazed (Taylor et al., 2016). Studies in Switzerland indicated a reduction (32.5% to 12.5%) in the egg count of liver fluke in faeces of the eight farms which adapted pasture rotation system. Thus, the pasture rotation system is recommended for farms with snail habitats in pastures used for dairy cows (Knubben-Schweizer et al., 2010; Knubben-Schweizer & Torgerson, 2015). Overall, controlling snail host populations and restricting livestock access to snail habitats are highly important management practices, since liver flukes need their intermediate host to complete their life cycles (Fairweather et al., 2020).

Overview remedies

Based on ethnoveterinary knowledge, many different plant species have been seen to have an antiparasitic effect. Plants of interest are mostly selected by biological, chemical, biochemical and physical properties, for example plants that are rich in tannins. Another approach is to select plant species by detailed feeding observation studies in species rich areas who allow self-medication (Hoste et al., 2015). Evidence of self-medication of parasitized ruminants has been found, but the mechanism is unclear and beyond the scope of this project (Villalba, Miller, Ungar, Landau, & Glendinning, 2014). This report focuses on compiling the ethnoveterinary knowledge on herbs used against *Haemonchus contortus* and *Fasciola hepatica*. Self-medication by planting herbs in pasture can be a way to implement the herbs.

Effective compounds in herbs

Tannins

Tannins are secondary metabolites often found in plants. There are two groups of tannins: hydrolysable tannins and condensed tannins. Hydrolysable tannins are known to give rise to toxic compounds for ruminants when metabolised (Murdiati, McSweeney, & Lowry, 1991). Plant species that are considered toxic for ruminants by hydrolysable tannins include *Clidemia hirta* (harendog), *Quercus ilex* (oak), *Terminalia oblongata* (yellow wood), and *Ventilago viminolis* (supplejack) (Min & Hart, 2003). Condensed tannins, on the other hand, vary widely in chemical structure, resulting into different physical and biological properties. Generally, condensed tannins bind to protein and other molecules at a near neutral pH, as can be found in the rumen. At high concentrations, condensed tannins can inhibit carbohydrate fermentation in the rumen by binding to enzymes. Nevertheless, they are also often pointed out as an effective plant compound against gastrointestinal parasites (Min & Hart, 2003). Directly in multiple ways (Hoste et al., 2012): Firstly, by reducing the worm egg excretion and adult worm count. Secondly, by reducing the fertility of the worm. Thirdly, by lowering establishment success of L3 stage larvae in the host. Lastly, by inhibiting the development of eggs into third stage larvae. Furthermore, condensed tannins can increase the protein uptake in the small intestine. The condensed tannins bind to proteins in the rumen, decreases the protein solubilisation and degradation. Therefore, proteins would surpass the rumen that may result in a more efficient protein degradation and uptake in the small intestine in ruminants (Waghorn, 2008). The dietary effect of tannins on goats is seen to be less pronounced. This could be explained by the greater ability of their rumen's microbial population to degrade tannins (Brooker et al., 1994). The effectivity of condensed tannins is not only determined by their concentration in the plant, but also their chemical structure, where there is a lot of variation between plant species and varieties but also over the growing season (Häring et al., 2008). Interactions with other plant metabolites, like flavonoids or polyphenolic compounds, play a role as well (Akkari et al., 2014). To conclude, tannins can affect digestion and the presence of gastrointestinal parasites.

Herbal medicines against Haemonchus contortus in sheep

Table 1. Natural herbs which are effective against an infection with *Haemonchus contortus*. All experiments with herbs in this table are done in vivo. FEC = Faecal egg count. RBC = red blood cells. PCV = packed cell volume, a reduction means blood loss and can result in anaemia. Results on worm count, FEC and blood loss are compared to an infected untreated control group. Asterisk (*) indicates significant and (ns) indicates not significant ($\alpha < 0.05$).

Haemonchus contortus							
Herb	**Species**	**Application**	**Worm count**	**Feacal egg count**	**Blood loss**	**Other comments**	**References**
***Artemisia absinthium* (wormwood)**	Rasa Aragonesa lambs	20% in lucerne pellets	49%* reduction	73%* reduction	Higher compared to control diet		(Valderrábano, Calvete, & Uriarte, 2010)
***Cichorium intybus L.* (chicory)**	Swiss White Alpine x Swiss BlackBrown Mountain lambs	Fresh fodder, 84% chicory, ad libitum for 17 days	15% (ns) reduction	69%* reduction	Not studied	89%* reduction of total daily faecal egg output	(Heckendorn, Häring, Maurer, Senn, & Hertzberg, 2007; Heckendorn, Häring, Maurer, Senn, & Hertzberg, 2007)
***Cymbopogon citratus* nanoemulsion (lemongrass essential oil)**	10 – 16 month-old sheep	450mg/kg for 3 days	83.1%* reduction	Compared to control group reduction 17% (ns)	Not studied	Concentration essential oil in nanoemulsion was 20%'	(Macedo et al., 2019)
***Hedera helix* (hederae folium)**	6- to 8-month-old male indigenous Ethiopia sheep	Aqueous extract of ripe fruits applied by a drench.	637 worms 44%* Reduction	Lower day 2 (46%*, egg count 625), Lower 7(38%*, egg	Untreated group higher decrease in PCV than	Cultured faeces: reduction of the eggs hatched (36%),	(Eguale, Tilahun, Debella, Feleke, & Makonnen, 2007)

				count 6892). day 14 (ns)	before treatment. *H.helix* group no change in PCV before and after treatment.	compared to 60% hatching untreated group	
***Hedysarium coronarium* (sulla)**	Rasa Aragonesa lambs	Pellets, 20% lucerne	8% (ns) reduction	22% (ns) reduction	Not measured	Reduced egghatching rate*, but L3 stage higher in faeces*	(Valderrábano et al., 2010)
***Lotus corniculatus* (birdsfoot trefoil)**	Swiss White Alpine x Swiss BlackBrown Mountain lambs	Fresh fodder, 68% Birdsfoot Trefoil, Ad libitum for 17 days	49% (ns) reduction	58%* reduction	Not measured	63%* reduction of total daily faecal egg output	(Heckendorn, Häring, et al., 2007)
	Suffolk-Hampshire crossbred lambs	Birdsfoot trefoil-enriched pasture, 15kg/ha	Therapeutic treatment: 88.6%* reduction by 4 week Preventive treatment: 4 times higher FEC than therapeutic measurement, however peak was 48% (ns) less than therapeutic FEC peak.	Not studied	Therapeutic: 3%* lower at wk6 compared to control, PCV increased throughout experiment Preventive: 6%* lower at wk6 compared to control		(Mata-Padrino et al., 2019)
***Lespedeza cuneata* (Chinese bush-**	4-month-old ewe lambs Study 1	Fed as hay in excess	67% (ns) reduction compared to control	98%* reduction at day 7	PCV 4%* higher than control group	Study the effect of L. cuneata on natural	(Lange et al., 2006)

clover)						infection and reinfection	
	4 – month old ewe lambs Study 2	Fed as hay in excess	26% (ns) reduction compared to control	67%* - 82%* reduction from day 28-49	PCV similar compared to control	Study the effect of L. cuneata on reinfection after natural infection was removed	(Lange et al., 2006)
	Merino ewes	Leaf part fed as hay ad libitum for 35 days	Not studied	51%* reduction at day 35	Not studied	Higher rectal temperatures suggesting lower risk anaemia	(Erika et al., 2017)
Onobrychis viciifolia **(Sainfoin)**	Sheep yearlings and ewes	Hay and silage ad libitum	Not studied	30-40%* Reduction	Not studied		(Ploeger, Verkaik, Bokma-Bakker, & Antonis, 2021 in review)
	Swiss White × Swiss Black-Brown Mountain lambs	Hay and silage ad libitum	50%* reduction	Hay 34%* Silage 49%*	Hay increases blood loss but within acceptable levels		Heckendorn et al. (2006)
	Rasa Aragonesa lambs	20% in ground lucerne pellets	13% (ns) reduction	54%* reduction	Higher compared to control diet	Reduced egg hatching rate*	(Valderrábano et al., 2010)
	Swiss White Alpine × Swiss BlackBrown Mountain lambs	Fresh fodder, 61% Sainfoin ad libitum for 17 days	35% (ns) reduction	62%* reduction	Not studied	63%* reduction of total daily faecal egg output	(Heckendorn, Häring, et al., 2007)

Table 2. Natural herbs which are effective against an infection with *Haemonchus contortus* or against internal parasites in general. All experiments with herbs in this table are done in vitro. FEC = Faecal egg count. Results on worm count, FEC and blood loss are compared to an infected untreated control group unless otherwise indicated. Asterisk (*) indicates significant.

Haemonchus contortus							
Herb	**Species**	**Study**	**Application**	**Worm count**	**Feacal egg count**	**Other comments**	**References**
***Artemisia absinthium* (wormwood)**	Sheep	*In vitro* *In vitro*	Aqueous extract2mg/ml Ethanolic extract 2mg/ml	71%* reduction after 24h 100%* reduction after 24h	100%* reduction 100%* reduction		(Akkari et al., 2014)
	Female lambs	*In vitro*	Aqueous extract stem 3.76mg/ml	Not studied	100%* reduction	0.35g/kg flavonoids, 6.48 g/kg phenolic acids	(Mravčáková et al., 2020)
***Malva sylvestris* (mallow)**	Female sheep	*In vitro*	Aqueous extract flower 5.89mg/ml	Not studied	100%* reduction	6.50g/kg flavonoids, 0.66 g/kg phenolic acids	(Mravčáková et al., 2020)
***Punica granatum* (pomegranate)**	Sheep	*In vitro*	Methanolic peel and root extract	100%* (peel), 90%* (root) killing after 7 hours (10 mg/ml extract)	94.63%* (peel), 90.33%* (root) egg hatch inhibition (1 mg/ml extract)	Plant material identified by local livestock raisers	(Aliyi Hassen et al., 2020)
***Artemisisa herba-alba* (white wormwood)**	Sheep	*In vitro*	Methanolic flower and aerial extract	100%* killing after 7 hours (10 mg/ml extract)	98.67%* (flower), 88.3%* (aerial) egg hatch inhibition (1 mg/ml extract)	Plant material identified by local livestock farmers	(Aliyi Hassen et al., 2020)
***Ruta chalepensis* (fringed rue) essential oil**	Sheep	*In vitro* *In vitro*	Flower essential oil (1mg/ml) Leaf essential oil (1mg/ml)	87.5%* killing after 8hrs 75%* killing after 8hrs	Not studied Not studied	100%* egg hatch inhibition 99%* egg hatch inhibition	(Akkari et al., 2015)

Table 3. Herb mixtures which are effective against an infection with *Haemonchus contortus*. All experiments with herbs in this table are done in vivo. FEC = Faecal egg count. Results on worm count, FEC and blood loss are compared to an infected untreated control group. RBC = red blood cells. PCV = packed cell volume, a reduction means blood loss and can result in anaemia. Asterisk (*) indicates significant.

Haemonchus contortus						
Herb mixtures	**Species**	**Application**	**Worm count**	**FEC**	**Blood loss**	**References**
Herb mix 1	Female lambs	100g/DM/d	67%* lower	>70%* reduction between day 49-70	Positively affected*	(Váradyová et al., 2018)
Herb mix 2	Female lambs	100g/DM/d	Not studied	63%* reduction between day 32-60	No significant effect	(Váradyová et al., 2017)
Herb mix 3	Female lambs	100g/DM/d	27%* lower	58%* reduction between day 44-70	Not studied	(Mravčáková et al., 2019)
Herb mix 4	Female lambs	100g/DM/d	13% lower	52%* reduction between day 44-70	Not studied	(Mravčáková et al., 2019)
Quchongsan	Lambs	Sheep > 50kg: 55g sheep < 50kg: 27.5g	Not studied	98%* reduction on day 7	Not studied	(Liu, Fan, Chen, & Zhong, 2011)
Quchongxiehuosan	Sheep from 30-40 kg	40g	99%* lower on day 21 for all Nematode	87%* reduction on day 14; 100%* reduction on day 28	Not studied	(Li et al., 2016)
Bio-dewormer	2–3-year-old goats	G1: 1400 mg/kg G2: 1200 mg/kg G3: 1000 mg/kg Every once a week	Not studied	From day 0-15: G1: 62% * reduction G2: 52% * reduction G3: 33% (ns) reduction From day 0-30: G1: 91%* reduction G2: 84%* reduction G3: 40%* reduction Compared with control group, G1, G2 were significantly lower on day 15. G1, G2, G3 were significantly lower on day 30.	G1: 7.2%* higher PCV, 21.6%* higher haemoglobin concentration on day 30. G1 & G2: significantly higher RBCs and lower white blood cells compare to control group. Positive effect on serum.	(Abbas et al., 2020)

Promising herbs against *Haemonchus contortus*

Artemisia absinthium (wormwood) and *Malva sylvestris* (mallow)

Introduction

Artemisia absinthium (left), also called wormwood, is a perennial shrubby plant. It is native to temperate regions of Asia, Europe, Northern Africa, and can also be found in the United States and Canada (Nin & Bennici, 2001). Nowadays, the plant is used as herbal medicine in Unani and Ayurveda practice for anti-inflammatory, antioxidant and antimicrobial purposes, and can also affect the GI tract and urinary system(Bhat et al., 2019; Canadanovic-Brunet, Djilas, Cetkovic, & Tumbas, 2005).
Malva sylvestris L. (right), also called common mallow, is a plant native to Europe, North Africa and Asia (Barros, Carvalho, & Ferreira, 2010). The whole plant has been used for therapeutic applications, but most effects are reported on the leaves and flowers, as these are used for anti-ulcerogenic, antioxidant, and anti-inflammatory purposes (Chiclana, Enrique, & Consolini, 2009).

Effectivity in ruminants

Mravčáková et al. (2020) showed a strong ovicidal effect of aqueous extracts of both wormwood and mallow on *H. contortus* in the *in vitro* egg hatch test (Table 2). However, *in vivo* studies of treatments with one or a mixture of these plants showed no clear effect on egg and worm reduction (Mravčáková et al., 2020). The *in vivo* results show that wormwood and mallow are not responsible for egg reduction in the lambs by themselves.

However, Váradyová et al. (2017), Váradyová et al. (2018) and Mravčáková et al. (2019) have shown an increase in resistance of lambs against H. contortus. These studies used mixtures of 9 – 13 dry medicinal plants, which include *A. absynthium* and *M. sylvestris* (Table 3). This suggests that the effect of medicinal plants depends on the synergy and variety of plant polyphenols and the combination of bioactive compounds. Furthermore, it seems that mixtures of herbs do not have a direct anthelmintic effect, but rather show an increase in resistance of lambs against *H. contortus*.
This was confirmed by Mravčáková et al. (2021), who studied the addition of methanolic extracts of *A. absynthium* stems and *M. sylvestris* flowers to diets of lambs infected with *H. contortus*. Treatment with *M. sylvestris* alone or in combination with *A. absynthium* increased the resistance of lambs against an infection with *H. contortus*, as these treatments influenced antioxidant activity by reducing oxidative stress in abomasal mucosa, thereby reducing inflammation and enhancing local immune responses (Mravčáková et al., 2021).

Valderrábano et al. (2010) showed that feeding *A. absynthium* leads to a reduction in adult worm numbers and faecal egg count (Table 1), which is a promising result. In addition, there was a significant higher daily weight gain for infected lambs fed with *A. absynthium* compared to the control. This study identified that the risk of *H. contortus* infection decreases with 73% when feeding *A. absynthium*.

Application *Artemisia absinthium* (wormwood)

A. Absinthium can be grown in the Netherlands and is often used as an ornamental pant. It can withstand cold winters and dry conditions. The palatability of fresh *A. absynthium* is low, but when ensilaged and mixed with grass the intake can be even higher than from normal silage (Beigh & Ganai, 2017). No studies on the effect of whole plant application, fresh or as silage, against parasites can be found. Only studies on extracts are available. Fresh leaf acquisitive extracts with sugar could be used as a drench. Furthermore, pellets could be made from the extract after being dried as done by (Beigh & Ganai, 2017; Valderrábano et al., 2010). *A. absynthium* should be approached with caution; wrong dosing can stimulate undesirable side effects. To ensure safe application, more studies in a Dutch livestock context are need.

Cichorium intybus L. (chicory)

Introduction

Cichorium intybus L. (chicory) is a perennial herb of the family *Asteraceae*, and it is a resident plant in Europe, many parts of Asia, Africa and America. Next to that, chicory is a part of the natural grassland around the world and has been planted as a forage crop and as a component of perennial pastures, because of its high nutrition value and digestibility (Li & Kemp, 2005).

In addition, chicory has high amounts of minerals which can increasing resistance against nematodes (Athanasiadou et al., 2007). Altogether, this makes chicory a potential plant against worm infection.

Effectivity in ruminants

Previous studies have shown grazing chicory was able to lower the abomasal nematode worm count and faecal egg count in deer, cattle and lambs with a stable liveweight gain (Hoskin, Barry, Wilson, Charleston, & Hodgson, 1999; Pena-Espinoza, Thamsborg, Desrues, Hansen, & Enemark, 2016; Scales, Knight, & Saville, 1995; Tzamaloukas, Athanasiadou, Kyriazakis, Jackson, & Coop, 2005). Condensed tannins are considered as the effective anthelmintic compounds in herbs, but chicory has relatively low condensed tannins, instead flavonoid, coumarins, caffeic acid derivatives and specifically sesquiterpene lactones are the main plant secondary metabolites in chicory, in which flavonoid has anthelmintic activity and sesquiterpene lactones can inhibit the motility of gastrointestinal larvae. Besides, the plant structure also showed a reduced motility of larvae (Molan, Duncan, Barry, & McNabb, 2003). Which may explain why grazing chicory showed a generally good anthelmintic effect against gastro-intestinal parasite.

Heckendorn, Häring, et al. (2007) proved chicory was effective against *H. contortus* in sheep (Table 1). However, lambs did not show any gain in live weight at the end of the chicory feeding experiment, because of a limitation of dry matter intake. This limitation was due to the higher water content of chicory and the small rumen size of lambs (Heckendorn, Häring, et al., 2007). Chicory also shows good anthelmintic performance against gastro-intestinal nematodes in general. Compared to non-anthelmintic grass, chicory induces significant decreases in total abomasal worm count and faecal egg count. In addition, chicory showed a significant reduction of faecal egg account of *T. circumcincta* as well (Veldhuis, 2009). Since *H. contortus* was also present in abomasum, it is worthwhile to study the anthelmintic effect of chicory specifically against *H. contortus*. Moreover, live weight gain of lambs was increased in the chicory group (Athanasiadou et al., 2007; Marley, Cook, Keatinge, Barrett, & Lampkin, 2003; Veldhuis, 2009). However, 4.16%* higher worm count was observed in small intestine compared to control group (Marley et al., 2003). Therefore, grazing chicory may need to combined with another method to achieve the best efficacy.

Application

Chicory grows well under dry conditions, especially in early spring and summer, and is dormant in winter. Its nutritional value is highest in summer, because the reproductive stem grows fast during this period. As *H. contortus* is most problematic in June, July and August according to researcher Adriaan Antonis[*], it is possible to supply chicory to the main diet to reduce the nematode infection.

Furthermore, chicory grows well in the first two years, but after that, nitrogen fertilizer is needed to maintain high production of this pasture. Therefore, it could be combined with an nitrogen-fixing species, such as sainfoin or birdsfoot trefoil, to ensure N-supply and thereby the productivity. In total, chicory can be productive for 4-6 years under grazing (Veldhuis, 2009). Chicory keeps its anthelmintic properties when ensilaged and is resilient to grazing and tramping (Heckendorn, Häring, et al., 2007; Pena-Espinoza et al., 2016). The palatability of chicory is comparable with a *Lolium perenne* white clover mix, which is quite high (Häring et al., 2008). The chicory leaves have a better nutrition value than the stem. Therefore, management needs to focus on maximizing leaf growth and minimizing stem growth. In addition, animals sometimes refuse to eat the stem. To ensure minimized stem growth and maximize leaf intake, the pasture should regularly be grazed or mown (Li & Kemp, 2005; Veldhuis, 2009). Furthermore, due to the broad leaves, chicory takes a relatively large amount of space. So, to ensure a highly productive pasture, the sowing density of chicory in the mixture should be adjusted. Moreover, there are various species of chicory available. The variations with a high level of sesquiterpene lactones like the Puna varieties, often used in New Zealand, are promising regarding there anthelmintic performances (Li & Kemp, 2005; Veldhuis, 2009).

[*] See appendix for the full interview

Cymbopogon citratus nano-emulsion (lemongrass essential oil)

Introduction

C. citratus, commonly known as lemongrass, is a perennial grass grown in tropical aeras. It has been widely used as an essential oil, food, and perfume. It is known as a traditional medicine in Southeast Asia, West Africa and South America (Adeneye & Agbaje, 2007; Blanco, Costa, Freire, Santos Jr, & Costa, 2009; Manvitha & Bidya, 2014; Negrelle & Gomes, 2007; Shah et al., 2011). Lemongrass has anti-inflammatory, anti-oxidant, anti-bacterial, anti-anxiolytic and anti-fungal functions (Mirghani, Liyana, & Parveen, 2012; Olorunnisola, Asiyanbi, Hammed, & Simsek, 2014).

Effectivity in ruminants

In a study by Macedo et al. (2019), the anthelmintic effect of both lemongrass free essential oil and in nano-emulsion was studied against *H. contortus* in sheep. The major component of this oil is citral, and in this study, the concentration of citral was 36.75%. This study shows that the nano-emulsion had better anthelmintic effects than the free essential oil. Therefore, only the effect of the nano-emulsion is described in this report. The nano-emulsion of lemongrass essential oil showed a numerical but not significant reduction in faecal egg count after 15 days compared to the control group (Table 1). Faecal egg count did significantly decrease (47%) comparing day 0 to day 15 of the nano-emulsion lemongrass oil group. Furthermore, when comparing adult worm count after slaughtering of the control and experimental group, a significant worm count reduction is seen after treatment (Table 1). To conclude, this oil a promising remedy to control *H. contortus* burden. The anthelmintic effects are not only dependent on the concentration of citral, but are also influenced by the synergy between essential oil constituents.

Application

It is possible to add fresh and dried lemongrass in the feed as a general health enhancer (Olorunnisola et al., 2014). However, no studies on lemongrass grazing or feeding it as hay have been found. Therefore, the palatability and effectiveness regarding this application remains unknown. Another studied option is to add essential oil as a part of the herb mixtures, as lemongrass essential oil has shown promising anthelmintic effects (Macedo et al., 2019). Moreover, it has a pain relief function and can improve feed utilization. Adding lemongrass did not affect feed intake, but rather improved nutrient digestibility and ruminal health of the animal. Improved milk yield and better milk quality was observed as well (Kholif et al., 2020). Therefore, lemongrass is a good candidate to be added as a supportive component to an herb mixture. In addition, large amounts of lemongrass essential oil are produced yearly in subtropical countries (Haque, Remadevi, & Naebe, 2018). Another approach of using essential oil is to add it in a lick block (Junkuszew et al., 2015). Adding essential oils to the lick block has been seen to have a mild anthelmintic effect, reducing coccidia infection and improving healthy weight gain without increasing adiposity. Studies under more intense worm infection situation and against *H. contortus* are needed to investigate anthelmintic properties on this application, but the palatability was not affected by adding lemongrass for any of the methods described above. Furthermore, lemongrass is readily available in Netherlands.

Hedera helix (Hederae folium)

Introduction
Hedera helix is an evergreen climbing plant that is found in the Dutch forests, which can also grow as groundcover. It grows well on most soils, in both sunny and shaded conditions. When not controlled, it can outcompete other plant species. Traditionally, the leaves and fruits of *H. helix* have been used against diseases in the gastrointestinal tract. *H. helix* has been reported to have anthelmintic properties *in vivo* and *in vitro* (Eguale et al., 2007; Urban, Kokoska, Langrova, & Matejkova, 2008) Chemicals such as alkaloids, saponins and flavonoids that are present in *H. helix* could be the basis of its anthelmintic effects (Debella, 2002; Eguale et al., 2007).

Effectivity in ruminants
In a study in Ethiopia, sheep were artificially infected with *H. contortus*. Four weeks later, a drench with an aqueous extract of the ripe fruits of the H. helix was tested (Eguale et al., 2007). The faecal egg count was significantly lower in the *H. helix* treated group than the untreated group (Table 1). However, at day 14, no difference was seen in faecal egg count. Nevertheless, the adult worm count at day 15 of the *H. helix* treated sheep was significantly lower (Table 1). However, the synthetic drug was still more effective as it showed a decrease in egg count to 0 eggs at day 14 and no worms found at day 15.

In an *in vitro* faeces culture experiment the *H. helix* group showed a reduction in the percentage of eggs hatched, compared to the untreated group (Table 1)(Eguale et al., 2007). The blood levels of the *H. helix* treated sheep did not change after treatment, but *H. helix* showed some effect as the untreated sheep showed an increase in blood loss. Nevertheless, the blood levels of the synthetic group showed a decrease in blood loss, which indicates a higher effectivity of the synthetic drug compared to *H. helix* (Table 1)(Eguale et al., 2007).

To conclude, extracts of *H. helix* were able to reduce faecal egg count, worm count, blood loss and infectivity of male indigenous Ethiopian sheep compared to nontreated animals. However, *H. helix* was not nearly as effective as the synthetic drug. Nevertheless, at this dose of *H. helix* no clinical signs of toxicity were found in the sheep. Therefore, to increase the effectiveness of this herb, the dose could be increased. Alternatively, repeated treatment for a few days could be implemented (Eguale et al., 2007). Further study is necessary to look at the effectiveness of *H. helix* with different applications, concentrations, and their possible toxic effects.

Application

H. helix was mentioned as a potential useful plant against internal parasites by Jaring Brunia, a Dutch organic dairy farmer. Eguale et al. (2007) did show a moderate anthelmintic effect of *H. helix* against *H. contortus*. In addition, it could easily grow in Dutch pastures on the ground and against fences and trees. This would allow an opportunity for self-medication by the cattle or sheep to improve and sustain their health (Villalba et al., 2014). However, *H. helix* can be difficult to control, and it can crowd out other plants (Ringold, Magee, & Peck, 2008). Therefore, farmers need to be aware of the growth rate and extent of the crowding of *H. helix* before they implement this. In addition, we also need to consider if it is even desirable to implement such a competitive plant. Furthermore, there are no studies found that apply the plant to livestock by self-medication and its palatability is unknown. And we were not able to find a study conducted in Europe on Dutch breeds, or a study testing the fresh plant and other plant tissues as in practise extracts of herbs are generally less effective than fresh material (Ethnoveterinary specialist Dr. Kumar[*].

[*] See appendix for the full interview

Hedysarium coronarium (sulla)

Introduction

Hedysarium coronarium (sulla) can be grown in temperate climates and is palatable for both cows and sheep (Niezen, Robertson, Waghorn, & Charleston, 1998). Additionally, it is beneficial for animals' total production performance, for example worm resistance, milk quality, meat quality, liveweight gain and feed intake (Di Trana et al., 2015).

Effectivity in ruminants

In the past, (Niezen et al., 1998) showed a reduction in faecal egg and worm count of gastrointestinal parasites in sheep when feeding sulla and it showed a positive effect on daily weight gain. Nevertheless, no clear numbers were given in this study. A more recent study from (Valderrábano et al., 2010), showed that feeding sulla to sheep leads to a reduction in worm and faecal egg count, which is a promising result (Table 1). However, this reduction was not significant compared to the control group. In addition, the study showed, when feeding sulla the risk of *H. contortus* infection is 2.5 times higher than feeding the control diet. This is mainly due to the higher amount of L3 larvae found in faeces of sheep fed sulla. Therefore, no clear conclusion can be drawn on the anthelmintic properties of sulla against *H. contortus*. Further research is needed to confirm the promising effects (Niezen et al., 1998) showed. Therefore, we will not expand on the application of sulla in this report.

Lotus corniculatus (birdsfoot trefoil)

Introduction

Lotus corniculatus, or birdsfoot trefoil, can be found in many regions, including Europe, Asia, Africa, Australia and North and South America (Lonngren et al., 2020). Research has shown that birdsfoot has anthelmintic effects against gastrointestinal parasites in general (Marley et al., 2003).

Effectivity in ruminants

Heckendorn, Häring, et al. (2007) showed significant reductions in faecal egg and worm count of *H. contortus* when feeding sheep birdsfoot (Table 1). Furthermore, Mata-Padrino et al. (2019) studied both the preventative and therapeutic effect of birdsfoot on lambs infected with *H. contortus*. The preventative experiment included lambs which grazed one week on the birdsfoot trefoil-enriched pasture before they were infected with *H. contortus*, whereas the therapeutic experiment included lambs that were infected with *H. contortus* 4 weeks prior to grazing the birdsfoot trefoil-enriched pasture. Birdsfoot trefoil-enriched pasture seems a good therapeutic treatment as it increased packed cell volume (PCV) with time (23% at the start, 29% at end of 6 weeks) and faecal egg count decreased (Table 1). For the preventative treatment, the faecal egg count increased with time and the PCV decreased with time (Table 1). However, the peak of faecal egg count was much lower than the peak measured in the therapeutic group. In addition, the PCV for preventive treatment was decreased to 26% at the end of the 6-week experiment, which was still higher than the PCV (23%) at the beginning of the experiment for the therapeutic group. These results indicate that birdsfoot has anthelmintic effects on *H. contortus* in sheep.

Furthermore, Mata-Padrino et al. (2019) suggested that a PCV lower than 20% indicates that sheep need treatment with anthelmintic drugs. The PCV for both preventive and therapeutic treatment did not become lower than this limit. Therefore, birdsfoot can contribute to lower the risk of sheep suffering from anaemia and thereby control to a 'healthy' number of parasites in the gut (Mata-Padrino et al., 2019). In the study from Mata-Padrino et al. (2019), the cultivar Bruce was used, which could contribute to the promising anthelmintic effects as this cultivar has a high condensed tanning content.

Application

Birdsfoot trefoil is a slower establishing legume naturally occurring in more nutrient poor systems (Fort et al., 2015). Due to slow establishment, it can be easily suppressed in mixtures with more competitive grasses (Heckendorn, Häring, et al., 2007; Weewer, 2021). This may be overcome by a higher seeding density. Once established, birdsfoot is relatively resilient against flooding and droughts and tolerates acid soils (Ramírez-Restrepo, Kemp, Barry, & López-Villalobos, 2006). In monoculture, a yield of 5.3 - 8.5 tDM/ha/year yield can be reached in New Zealand climates, comparable to the Netherlands (Ramírez-Restrepo et al., 2006). Weeding is needed for birdsfoot to reach its full potential (Weewer, 2021). Furthermore, as birdsfoot is a legume, it could increase the nitrogen input in the system without the need of artificial fertilizer. However, the rooting of birdsfoot is not considered optimal for nitrogen transfer between legumes and non-legume species (Pirhofer-Walzl et al., 2012). Birdsfoot is suitable for grazing, making hay and silage, but no studies are available on the anthelmintic effect after preserving. The palatability is slightly low (Scharenberg et al., 2007) .

Lespedeza cuneata (Chinese bush-clover)

Introduction

L. cuneata is a shrubby, deciduous perennial legumes which has length of 0.5 - 1.5 metre (Ohlenbusch & Bidwell, 2007). *L. cuneata* is a native species from Asia and in the 20th century which is introduced in America for forage and to control erosion. In America it is seen as an invasive species, as this legume can alter soil conditions and thereby strengthen its own growth (Coykendall & Houseman, 2014).

Effectivity in ruminants

Lange et al. (2006) studied the anthelmintic effect of *L. cuneata* on both natural infection with *H. contortus* in sheep and possible reinfection (study 1) and reinfection with *H. contortus* in sheep after removing natural infection with synthetic drugs (study 2). *L. cuneata* turned out to be more effective in reducing naturally existing parasites (study 1) than establishing parasites (study 2) (Table 1). One explanation of this difference might be that the condensed tannins present in the plant influence the abomasal environment. Establishing parasites entering the already changed environment did not experience the change in abomasal environment and may therefore be not affected by this change. Furthermore, both studies showed a reduction in faecal egg count (Table 1), which might benefit sheep by reducing the pasture contamination with *H. contortus*. Moreover, the reduction in faecal egg count observed by Lange et al. (2006) were confirmed by a study by Erika et al. (2017). This study showed reduction on faecal egg count and possible lower risk of anaemia when feeding *L. cuneata* (Table 1).

Application

L. cuneata can be considered as an alternative for viable forage. Namely, it flourishes on eroded, infertile and poor soils. In addition, *L. cuneata* is disease, drought and insect resistant and thereby provides high yield. Furthermore, *L. cuneata* has a high litter input and therefore can increase soil quality (Lange et al., 2006). Moreover, it is commercially available and applied in pastures. In addition, as the legume has nitrogen-fixing abilities, it establishes well in low nitrogen input pasture (Erika et al., 2017). However, in parts of Southeast US it is considered as a noxious weed. First, it needs to be considered why it is such a noxious weed in this region, before it can be implemented in the Netherlands. Furthermore, one point to consider is that cattle have been seen to select out *L. cuneata* when grazing (Ohlenbusch & Bidwell, 2007), goats are willing to graze *L. cuneata* (Min, Pomroy, Hart, & Sahlu, 2004), and sheep and goats consume it as hay (Shaik et al., 2006). The anthelmintic properties of *L. cuneata* have been shown to be preserved during hay production (Lange et al., 2006). The drying process of hay causes oxidative changes in tannins and improves the palatability of the forage (Goldstein & Swain, 1963). In addition, converting *L. cuneata* grass to hay will make the plant storable and available at the time when it is needed. Moreover, hay can be further processed into pellets, ground meal or even incorporated into feeding programs (Lange et al., 2006). For management implementation, it is important to consider that sheep are 'browsing' the legume and thereby only eat the leaves and avoid the stems.

Punica granatum (pomegranate)

Introduction

P. granatum L. is an ethnoveterinary medical plant native to Italy. This plant has been studied as a therapeutic compound to improve health, for example on prevent and treatment of cancer and it has an anti-inflammatory and anti-infective effect (Arun & Singh, 2012; Bassiri-Jahromi, 2018). Furthermore, pomegranate is well-known for its anthelmintic activity in in vitro studies (Ahmed et al., 2020; Jabeen, Anwar, Mahmood, Zia, & Murtaza, 2015). It is believed that pelletierine, a molecule belonging to the class of alkaloids, is the active substance of the plant. Pelletierine can be found in the bark of the root of *P. Granatum* (Jayaprakash, 2017).

Effectivity in ruminants

The peel of the pomegranate fruit and the root of the tree are traditionally used as holistic and natural medicine against tapeworms. Aliyi Hassen et al. (2020) found that an extract of the peel and root of *P. granatum* had promising effects on the egg hatch inhibition and adult motility of *H. contortus in vitro* (Table 2). These anthelmintic effects increased in a time and dose-dependent manner. Further *in vivo* research needs to be done to confirm the anthelmintic effects of *P. granatum* in sheep and to identify possible side effects. As *P. granatum* is part of the herb mixture Quchongxiehuosan from China, which is also described in this report, it is quite promising that this herb also shows some anthelmintic effect *in vivo* through this mixture. In addition, Boonmasawai, Sungpradit, Jirapattharasate, Nakthong, and Piasai (2013) showed a 55% significant reduction in FEC of gastrointestinal nematodes at day 1 of feeding alcoholic peel extract of pomegranate (300mg/kg) in goats. Further *in vivo* research is needed on the anthelmintic activity of pomegranate peels specifically against *H. contortus* to make it a promising application.

Application

Pomegranate peel as a by-product of fruit juice factories could be made into pellets or powders to apply by feeding as supplementary feed. Nevertheless, the cost and feasibility of this application is unclear. Besides, it is possible to add Pomegranate peel in the herb mixture as it has already used in the formula described in (Li et al., 2016).

Onobrychis viciifolia (sainfoin)

Introduction

Onobrychis viciifolia, or sainfoin, is a drought tolerant and palatable forage legume. It used to be widely spread throughout Europe before the use of commercial fertilizer. As sainfoin is a nitrogen fixer, it is suppressed in nitrogen rich systems. Its tannin and polyphenol composition are thought to increase protein utilization, prevent bloating, reduce greenhouse gas emissions by reducing methane emission, and hold anthelmintic properties (Carbonero, Mueller-Harvey, Brown, & Smith, 2011). Multiple studies have been studying the effect of sainfoin on different helminths.

Effectivity in ruminants

Heckendorn et al. (2006) studied the effect of feeding lambs with silage and hay sainfoin on *H. contortus*. The lambs had not yet encountered parasites before and where infected with *H. contorus and* 28 days after infection the treatment started. The treatments contained 16 days of feeding with unlimited concentrate and silage sainfoin, or hay sainfoin, or with a ryegrass clover hay combined with maize lucerne silage. There were no differences in daily weight gain between the treatments. Sainfoin hay decreased *H. contortus* faecal egg count (FEC) compared to the control group (Table 1). However, it should be noted that the dry matter percentage of the faeces from the animals fed sainfoin increased. The decrease in FEC resulted mainly from the reduction in worm count measured (Table 1). Nevertheless, blood loss has no difference between the groups, except the hay fed sainfoin group (Table 1). To conclude, sainfoin hay and silage can reduce the infectious level of the animals and the worm burden, but it did not reduce the blood loss. Moreover, another study from Heckendorn, Häring, et al. (2007) showed promising anthelmintic effects in egg and worm count when feeding sainfoin to lambs.

In addition, Valderrábano et al. (2010) showed that feeding sainfoin to sheep leads to a reduction in worm and faecal egg count, which is a promising result (Table 1). However, only the reduction in egg count was significant. In addition, there was a significant higher daily weight gain for infected lambs fed with sainfoin compared to the control. This study identified that the risk of potential *H. contortus* infection was reduced with 34% when feeding sainfoin. This reduction is mainly caused due to a decrease in egg hatching rate.

In a not yet published study by Ploeger et al. (2021 in review) experiments showed that feeding sainfoin at 40% ratio of total feed resulted in a decreased egg count of *H. contortus* in sheep (Table 1). Looking at all the results, sainfoin looks like a promising remedy.

Application

Sainfoin keeps its anthelmintic properties when dried or ensilaged (Häring et al., 2008; Heckendorn, Häring, et al., 2007). Therefore, sainfoin could be easily stored to be used indoors and flexible throughout the year. Even though sainfoin has a relatively high tannin concentration, sainfoin is highly palatable for sheep (Häring et al., 2008). Legume species, like sainfoin and birdsfoot trefoil, can use fixed nitrogen provided by bacteria in their root nodules. However, in nitrogen rich environments legume species are suppressed, as they have less advantage of their nitrogen fixation (Kirwan et al., 2007). Under low fertilization, a greater establishment of sainfoin has been seen in Switzerland in mixture with *Festuca parensis* (meadow fescue) (Häring et al., 2008). The establishment is reflected into the harvest, in monoculture a harvest of 13 t DM/ha/year was reached, in the mixture with *F. parensis* 16.5 t DM/ha/year was reached (Häring et al., 2008). The differences between monoculture and mixture were explained by the weed suppression by *F. parensis* (Häring et al., 2008). However, (Häring et al., 2008) also mentioned fluctuation in tannin richness of sainfoin when grown in mixtures, but how this affects the anthelmintic properties is unclear. Sainfoin is likely to grow well in relative nitrogen poor pastures in combination with relatively slower growing grass varieties like *F. parensis. Lollium perenne,* known as a common grass, is likely to be too competitive (Weewer, 2021). When Sainfoin is used at Dutch farms, it is often sown in monoculture in ungrazed fields, to later be harvested and ensilaged (Parasitologist H. Ploeger[*]). These fields are ungrazed, as sainfoin is vulnerable to trampling (H. Ploeger[*]). To conclude, sainfoin can be ensilaged and stored as hay or can be applied on separate fields that are unsuitable for grazing, in combination with a relatively low competitive grass like *F. parensis.*

[*] See appendix for the full interview

Promising herb mixtures against *Haemonchus contortus*

Herb mixtures

Váradyová et al. (2017), Váradyová et al. (2018) and Mravčáková et al. (2019) studied the anthelmintic effect of different non-commercial herb mixtures (Table 4). The total egg and worm count were reduced in the infected lambs which received herb mix 1 (Table 3), which is a promising result (Váradyová et al., 2018). The total faecal egg counts of infected lambs receiving herb mix 2 were also reduced (table1) (Váradyová et al., 2017). Furthermore, the study by Váradyová et al. (2018) showed that providing organic zinc alone or in combination with the herb mix provides antioxidants that minimize the oxidative stress in infected lambs. The herb mix 1 with different botanical families slowed down the infection dynamics and improved production indicators in lambs (Váradyová et al., 2017).
Mravčáková et al. (2019) showed that methanolic extracts had better anthelmintic effects then the aqueous extracts had *in vitro*. Both herb mix 3 and 4 look promising as they lowered the faecal egg count. A significant decrease in abomasal worm count was observed in infected lambs treated with herb mix 3 compared to untreated infected lambs (Table 3). No effect on the beneficial microbiota of the lambs was seen after treatment with the herbal mixtures. Of all the 4 mixtures, only herb mix 2 caused a significantly higher body weight and live weight gain in treated lambs compared to untreated lambs (Mravčáková et al., 2019; Váradyová et al., 2017; Váradyová et al., 2018).

Mravčáková et al. (2019) showed that the 4 herbs, *A. absinthium, M. chamomilla, F. officinalis and M. sylvestris,* had strong anthelmintic effects *in vitro* and that herb mixtures should be enriched with herbs with high anthelmintic properties. In summary, herb mix 3 had better *in vivo* anthelmintic effects than herb mix 4 and can cause a decrease in helminth infection intensity in the lambs. The stronger effects of mix 3 could be due to the higher content of phenolic acids and flavonoids. Furthermore, these studies show that the *in vitro* results show anthelmintic effects, but *in vivo* results show that this effect is not sufficient to eliminate parasites completely. However, the decreases in egg and worm count suggest that these herbal mixtures could have indirect anthelmintic effects *in vivo* and increase resistance of lambs against *H. contortus* for the long term (Mravčáková et al., 2019; Váradyová et al., 2018).

Application of the herb mixes

All 4 herb mixtures described above (Table 4) are non-commercial herb mixtures. All dry herbs in the mixture were obtained from commercial sources: AGROKARPATY in Slovak Republic and BYLINY Mikeš in Czech Republic (Váradyová et al., 2017; Váradyová et al., 2018). It is possible to buy the single herbs from these commercial sources, however, making the specific herb mixture needs expertise. Therefore, it is suggested to try to produce these herb mixtures commercially and make them available in The Netherlands.

Table 4. The composition of different herb mixtures tested on their anthelmintic and antioxidant effect in lambs infected with *H. contortus.* All herb mixtures were fed in the same amount: 100g/DM/d/animal.

Herbmix 1 (Váradyová et al., 2018) +Ad libitum meadow hay, concentrate of 500 g DM/d 3.55mg/g phenolic acid, 9.96 mg/g flavonoids				**Herbmix 2** (Váradyová et al., 2017) +600 g DM/d meadow hay, concentrate of 350 g DM/d			
Species	Family	Parts	%mix	Species	family	Parts	%mix
Althaea officinalis	*Malvaceae*	Root	11.8	*Althaea officinalis*	*Malvaceae*	Root	8.55
Petasitus hybridus	*Asteraceae*	Root	11.8	*Chamomilla recutita*	*Asteraceae*	Flower	8.55
Inula helenium	*Asteraceae*	Root	11.8	*Fumaria officinalis*	*Papaveraceae*	Stem	8.55
Plantago lanceolata	*Plantaginaceae*	Leaf	11.8	*Hyssopus officinalis*	*Lamiaceae*	Stem	8.55
Rosmarinus officinalis	*Lamiaceae*	Leaf	11.8	*Inula helenium*	*Asteraceae*	Root	8.55
Solidago virgaurea	*Asteraceae*	stem	11.8	*Malva sylvestris*	*Malvaceae*	Flower	8.55
Fumaria officinalis	*Papaveraceae*	stem	11.8	*Melissa officinalis*	*Lamiaceae*	Stem	8.55
Hyssopus officinalis	*Lamiaceae*	stem	11.8	*Petasitus hybridus*	*Asteraceae*	Root	8.55
Foeniculum vulgare	*Apiaceae*	Seed	5.6	*Plantago lanceolata*	*Plantaginaceae*	Leaf	8.55
				Rosmarinus officinalis	*Lamiaceae*	Leaf	8.55
				Solidago virgaurea	*Asteraceae*	Stem	8.55
				Foeniculum vulgare	*Apiaceae*	Seed	5.0
				Artemisia absinthium	*Asteraceae*	Stem	1.0
Herbmix 3 (Mravčáková et al., 2019) 57.3 mg/g phenolic acid, 41.5 mg/g flavonoids				**Herbmix 4** (Mravčáková et al., 2019) 22.2 mg/g phenolic acid, 29.5 mg/g flavonoids			
Species	Family	Parts	%mix	Species	Family	Parts	%mix
Matricaria chamomilla	*asteraceae*	Flower	13.4	*Matricaria chamomilla*	*asteraceae*	Flower	12.4
Fumaria officinalis	*papaveraceae*	stem	13.4	*Fumaria officinalis*	*papaveraceae*	flower	12.4
Hyssopus officinalis	*lamiaceae*	stem	13.4	*Calendula officinalis*	*asteraceae*	Flower	12.4
Malva sylvestris	*malvaceae*	Flower	13.4	*Malva sylvestris*	*malvaceae*	stem	12.4
Melissa officinalis	*lamiaceae*	Stem	13.4	*Achillea milefolium*	*asteraceae*	Stem	12.4
Plantago lanceolata	*plantaginaceae*	Leaf	13.4	*Cichorium intybus*	*asteraceae*	Stem	12.4
Solidago virgaurea	asteraceae	stem	13.4	*Hypericum perforatum*	*hypericaceae*	Stem	12.4
Foeniculum vulgare	*apiaceae*	seed	5.0	*Urtica dioica*	*urticaceae*	stem	12.4
Artemisia absinthium	*asteraceae*	stem	1.0	*Artemisia absinthium*	*asteraceae*	stem	1.0

Herb mix from China 驱虫散 (Quchongsan)

Quchongsan has been commercially and widely used against all kinds of parasites, especially nematodes, in China. Various veterinary drug companies are selling this mix, but the formula differs between companies. Below is one example of one commercially available formula (Table 7).
Liu et al. (2011) studied the anthelmintic effect of a commercial recipe of Quchongsan compared with the potent synthetic drug albendazole. After feeding the herb mix for 7 days to sheep, the FEC was significantly reduced (Table 3), which is a promising result. Furthermore, it is shown that Quchongsan was 5.7% (98.3% vs. 92.6%) more effective in reducing FEC than Albendazole on nematodes, 4.2% (100% vs. 95.8%) on tapeworm and 79.3% (98.9% vs. 19.6%) on *coccidia*. The main ingredients of this recipe can be found in Table 5.
However, in this study, toxicity (half lethal dose) and the influence on pregnant animals were not investigated. Thus, more research is needed. Study design of this experiment also needs improvement since no negative control group was set. Although this recipe was designed against all parasites, the author also mentioned its specificity against *H.contortus.* Therefore, an experiment should be designed with only *H.contortus* infection in sheep.
Additionally, Li et al. (2016) tested an updated recipe of Quchongsan named Quchongxiehuosan (Table 6), they compare the anthelmintic activity of this drug with synthetic drug, of which the main effective compound is Ivermectin. In this study, medicine particle diameter was micron-sized less than 100μm in order to enhance the effectiveness, as this is believed to improve absorption. Both FEC and worm count were significantly lower compared to the control group (Table 3), which makes this mix a good therapeutical medicine. Moreover, the deworming performance of Quchongxiehuosan is almost the same as Ivermectin. In addition, Quchongxiehuosan group showed 4% higher of weight gain than Ivermectin group. Nine types of nematodes were detected in the sheep, but no *H.contortus* was found. However, due to high similarity of different nematode species, there is a reason to believe Quchongxiehuosan would also work against *H.contortus*.
In summary, Quchongsan showed promising anthelmintic activity against almost every tested nematode species, and it has a comparably strong anthelmintic effect as synthetic drugs. Furthermore, it has positive effect on live weight gain as well (Li et al., 2016; Liu et al., 2011). Therefore, it could be used it as a general prevention against internal parasites as suggested by Jiaguo Liu (see appendix for interview).

Application of the herb mix Quchongsan
The herb mixture is widely commercially available online. However, the import policy should be considered. In addition, the ingredients of the recipe are also available in traditional Chinese medicine shop, but to maximize the effect and avoid the toxicity, doses need to be strictly obeyed. Therefore, an expert is needed to adjust the formula according to the clinical performance (Professor Wang*; Dr. Kumar*). Herbs available in traditional Chinese shops can contain seeds. It should be investigated if theses seeds can germinate after passing through the gastro-intestinal tract to reduce the risk of introducing an invasive species. A way to overcome invasion risk could be by grinding the herbs into a powder before application.
Another approach would be to look for European recipes, as the ingredients can be produced locally and thereby used as easily available fresh herbs. Fresh herbs in India are recommended over dry herbs (Dr. Kumar*). Lastly, the cost of herbs will be lower if they do not need to be imported.

* See appendix for full interviews

Table 5. The main ingredients of formula Quzhongsan which are tested on their anthelmintic effect and compared with albendazole (Liu et al., 2011).

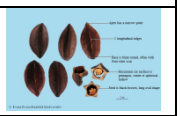
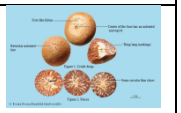
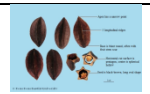
Chinese name	Latin name	English name	Parts	Processing	Functions	Picture
鹤虱	*Carpesii Fructus*	Common Carpesium Fruit	Fruit and seeds	The dried mature fruit o*f Carpesium abrotanoides L.(Asteraceae)*	Kills worms, resolves toxin, promotes spleen, harmonizes stomach. Apply to stomach pain due to worm accumulation, trichomonas vaginalis.	
使君子	*Quisqualis Fructus*	Rangooncreeper Fruit	Fruit and seeds	The dried mature fruit of *Quisqualis indica L.(Combretaceae)*	Kills worms, eliminates accumulation. Apply to abdominal pain due to worms.	
槟榔	*Arecae Semen*	Betelnutpalm Seed	Fruit and seeds	The dried mature seed of *Areca catechu L. (Arecaceae)*	Kills worms, against worm stasis, abdominal swelling pain.	
雷丸	*Omphalia lapidescens Schroet.*	Stone-like omphalia	Fungus	Dry sclerotia of *Omphalialapidescens Schroet.*	Against parasitosis	

Table 6. The main ingredients of formula Quzhongxiehuosan which are tested on their anthelmintic effect and compared with Ivermectin (Li et al., 2016).

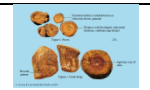
Chinese name	Latin name	English name	Parts	Processing	Functions	Picture
瑞香狼毒	*Euphoriae Ebracteolata Radix*	Fischer Euphorbia Root	Roots and rhizomes	The dried root of *Stellera chamaejasme L.(Thymelaeaceae)*	Kills worms. Apply to parasitic malnutrition, abdominal mass, cardiac and abdominal pain	
诃子	*Chebulae Fructus*	Medicine Terminalia	Fruits and seeds	The dried mature fruit o*f Terminalia chebula Retz.(Combretaceae)*	Strength lung and intestines and resolves toxin. Apply to lingering diarrhea, lingering dysentery, anal prolapsed,	
川楝子	*Toosendan Fructus*	Szechwan Chinaberry Fruit	Fruits and seeds	The dried mature fruit of *Melia toosendan Sieb. et Zucc.(Meliaceae)*.	Soothes the liver, relieves pain, expels worms.	

槟榔	*Arecae Semen*	Betelnutpalm Seed	Fruit and seeds	The dried mature seed of *Areca catechu L. (Arecaceae)*	Kills worms, against worm stasis, abdominal swelling pain.	
石榴皮	*Punica granatum L.*	Pomegranate peel	Bark	Dried bark of Pomegranate	Against diarrhea, dysentery, metrorrhagia and metrostaxis, leukorrhea, enterozoic abdominalgia.	
百部	*Stemonae Radix*	Japanese Stemona Root	Roots and rhizomes	The dried tuberous root of Stemona sessilifolia (Miq.) Miq., Stemona japonica (Bl.) Miq., Stemona tuberosa Lour. (Stemonaceae).	Kills worms. Externally applied to take care of head lice, body lice, enterobiasis.	
大黄	*Rhei Radix et Rhizoma*	Rhubarb Tangute Rhubarb	Roots and rhizomes	The dried root and rhizome of Rheum palmatum L. or Rheum tanguticum Maxim. ex Balf, or Rheum officinale Baill. (Polygonaceae).	Relieves toxin. Apply to damp-heat diarrhea, edema and abdominal fullness.	

Table 7. One example of a commercial Quchongsan and its main composition (Dingniu, 2021 viewed)

Chinese name	Latin name	English name	Parts	Processing	% mix	Functions	Picture
鹤虱	*Carpesii Fructus*	Common Carpesium Fruit	Fruit and seeds	The dried mature fruit of Carpesium abrotanoides L.(Asteraceae)	15%	Kills worms, resolves toxin, promotes spleen, harmonizes stomach. Apply to stomach pain due to worm accumulation,	
使君子	*Quisqualis Fructus*	Rangooncreeper Fruit	Fruit and seeds	The dried mature fruit of Quisqualis indica L.(Combretaceae)	10%	Kills worms, eliminates accumulation. Apply to ascariasis, enterobiasis, abdominal pain due to worms.	

槟榔	*Arecae Semen*	Betelnutpalm Seed	Fruit and seeds	The dried mature seed of Areca catechu L. (Arecaceae)	10%	Kills worms, against worm stasis, abdominal swelling pain.	
绵马贯众	*Dryopteridis Crassirhizomatis Rhizoma*	Male Fern Rhizome	Roots and rhizomes	The dried rhizome and remnants of leaf stems of Dtyopteris crassirhiaoma Nakai.(Dryopteridaceae)	Unknown	Resolves toxin, expels worms, stops blood. Apply to abdominal pain, metrorrhagia.	
干姜（炒）	*Zingiberis Rhizoma*	Dried Ginger (fried)	Roots and rhizomes	The dried rhizomes of Zingiber officinale (Willd.) Rosc.(Zingiberaceae). (Processed by frying)	Unknown	Apply to abdominal cold pain, vomiting, diarrhea, yang exhaustion and acute thoracic pain.	
附子（制）	*Aconiti Lateralis Radix Praeparata*	Prepared Common Monkshood Daughter Root (processed, probably hei shunpian)	Roots and rhizomes	The processed product prepared from the daughter root of Aconitum carmichaelii Debx.(Ranunculaceae).	Unknown	Relieves pain. Apply to vomiting and diarrhea, cold limbs and faint pulse, chest and abdominal cold pain, cold diarrhea, and diarrhea due to deficiency and cold,	
乌梅	*Mume Fructus*	Smoked Plum	Fruits and seeds	The dried nearly mature fruit of Prunus mume (Sieh.) Sieb. et Zucc.(Rosaceae). Harvested in summer when the fruit is nearly ripe, dried in low temperature and then covered until it turned black.	Unknown	Strength lung, and intestines, calms worms. Apply to vomiting and abdominal pain due to ascariasis, ascariasis of creeping figary tract.	
诃子	*Chebulae Fructus*	Medicine Terminalia	Fruits and seeds	The dried mature fruit of Terminalia chebula Retz.(Combretaceae)	5%	Strength the lung and intestines, resolves toxin. Apply to lingering diarrhea, lingering dysentery,	

						anal prolapsed,	
大黄	*Rhei Radix et Rhizoma*	Rhubarb Tangute Rhubarb	Roots and rhizomes	The dried root and rhizome of Rheum palmatum L. or Rheum tanguticum Maxim. ex Balf, or Rheum officinale Baill. (Polygonaceae).	8%	Relieves toxin. Apply to damp-heat diarrhea, edema and abdominal fullness.	
百部	*Stemonae Radix*	Japanese Stemona Root	Roots and rhizomes	The dried tuberous root of Stemona sessilifolia (Miq.) Miq., Stemona japonica (Bl.) Miq., Stemona tuberosa Lour. (Stemonaceae).	5%	Kills worms. Externally applied to take care of head lice, body lice, enterobiasis.	
木香	*Aucklandiae Radix*	Costusroot	Roots and rhizomes	*The dried root of Aucklandia lappa Decne.(Compositae)*	*3%*	*Stops pain, resolves stasis, warms middle, digests food.*	
榧子	*Torreyae Semen*	Grand Torreya	Fruits and seeds	The dried mature seed of *Torreya grandis Fort.(Taxaceae).*	*3%*	*Kills worms, Apply to abdominal pain due to worm distention.*	

Bio-dewormer

Abbas et al. (2020) tested the anthelmintic activity of a plant mixture they formulated against *H. contortus* and *F. hepatica*. The containing plants in this herbal mixture were selected through various plant families which are proven to have anthelmintic efficacy (Santos, Cerqueira, Branco, Batatinha, & Botura, 2019). Plants used in the study were bought in the local market, then grinded and mixed and added it with a certain percentage to the mixture. The composition of the herb mixture, named bio-dewormer, can be found in Table 8.

In this study, goats with similar faecal egg count per gram were selected and fed bio-dewormer in different doses. At day 0, 15 and 30, faeces and blood sample were collected. Egg reduction rate in faeces was calculated to see the effectiveness. Moreover, hematology and serum biochemistry were analysed to investigate the toxicity to animals. It is shown that bio-dewormer has a very promising performance against *H. contortus* (Table 3). Furthermore, the tested concentrations show no toxicity to the host, seen by the blood samples. Moreover, all 3 doses of bio-dewormer fed groups, had higher weight gain compared to the control group. From a high to low dose of bio-dewormer, the weight gain increased with 4.05%, 2.54% and 1.36%, respectively.

Application of bio-dewormer

The availability of different herbs in the herb mixture needs to be investigated in the Dutch market. Furthermore, in the study it is unclear how they applied the mixture, we assume they mixed it through the feed. More research into this mixture is needed as well, as this is the only study test about this specific formula published. Besides, there are only 84% known ingredients in given formula, the authors need to be contacted to figure out the rest of the recipe.

Table 8. Composition of bio-dewormer studied by (Abbas et al., 2020), the plants were bought from their local market.

Family name	Scientific name	English name	Weight (%)
Lamiaceae	Mentha *spicata*	Mint	2
Combretaceae	Combretum indicum	Chinese honeysuckle	2
Combretaceae	Terminalia chebula	Chebulic myrobalan	2
Rosaceae	Rosa sericea	Rose	2
Apiaceae	Foeniculumvulgare	Fennel	18
Apiaceae	Trachyspermumammi	Bishop's weed	18
Apiaceae	Cuminumcyminum	Cumin	3
Fabaceae	Glycine soja	Wild soybean	10
Fabaceae	Sansevieriatrifasciata	Viper's bowstring hemp	10
Fabaceae	Casia fistula	Golden rain tree	2
Gentianaceae	Swertia L.	Felworts	1
Linaceae	Linumusitatissimum	Linseed	2
Nitrariaceae	Peganumharmala	Harmel	2
Asclepiadaceae	LeptadeniaReticulata	Beaumont root/ black root	3
Theaceae	Camellia sinensis	Camilla	4
Brassicaceae	Lepidium sativum	Garden cress	1
Cucurbitaceae	Citrulluscolocynthis	Korrtumma	2

Complications for *Fasciola hepatica* treatment

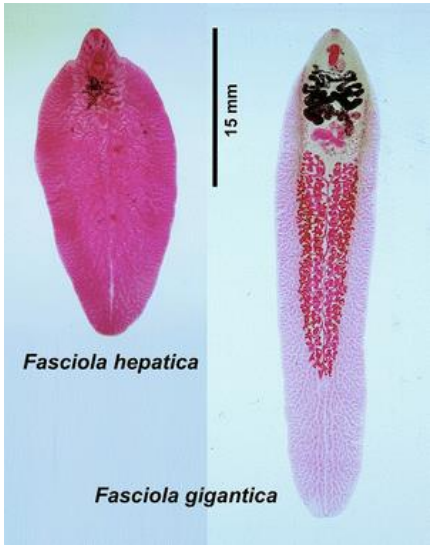


Figure 7: Images of the adult stages of *F. hepatica* (left) and *F. gigantica* (right), with a bar for scale. *F. gigantica* is longer than *F. hepatica* and lacks distinct 'shoulders'. Adapted from *Helminth Infections and their Impact on Global Public Health* (p. 95), by Fabrizio Bruschi, 2014, Wien: Springer. Copyright [2014] by Springer-Verlag Wien.

Location of the parasite

As mentioned before, ingested *F. hepatica* larvae migrate to the liver, where they mature and produce eggs to start the next cycle of development (Taylor et al., 2016). Their location makes the parasite more difficult to reach using orally fed herbs, in contrast to *H. contortus*, which lives in the abomasum and is thus exposed to anything consumed by the host animal (Veterinarian and researcher Michael Walkenhorst[*]). Thus, animals infected with *F. hepatica* may benefit most from herbs with compounds that are taken up into the systemic circulation, or herbs that boost the general immune system of the host animal. Due to this complication, most research into herbal remedies to treat *F. hepatica* have been performed *in vitro*. When considering these remedies for *in vivo* use, it is important to consider whether these herbs or herbal compounds can readily be digested and absorbed by the host animal.

Fasciola hepatica* versus *Fasciola gigantica

A close relative of *F. hepatica* is the larger *F. gigantica* (Figure 7)(Taylor et al., 2016). *F. hepatica* can be found in more temperate zones, including Europe, Oceania, North- and South America, and the more temperate zones of Asia and Africa (Castilla Gómez de Agüero et al., 2020). In contrast, *F. gigantica* only occurs in more tropical zones, such as Sub-Saharan Africa and parts of Asia. Thus, in the Netherlands, only *F.* hepatica occurs naturally. While they are distinct separate species, they have been known to hybridise (Agatsuma et al., 2000; Saijuntha et al., 2018). These hybrids are usually infertile and thus cannot reproduce (Hayashi et al., 2017; Ichikawa et al., 2011). However, the ability of these two species to hybridise together points to high genetic similarity. Thus, research into the effect of herbal remedies against *F. gigantica* have also been included in this overview. Furthermore, as *F. gigantica* is typically larger than *F. hepatica*, we expect the doses mentioned in studies on *F. gigantica* to also be effective for *F. hepatica.* However, more in-depth *in vitro* and *in vivo* studies on the differences between the two species' reactions to herbs may be helpful to confirm their applicability.

[*] See appendix for the full interview

Table 9. Natural herbs which are effective against an infection with *Fasciola hepatica*. All experiments with herbs in this table are done *in vivo*. FEC = Faecal egg count. Results on worm count, FEC and clinical symptoms are compared to an infected untreated control group. Asterisk (*) indicates significance ($\alpha < 0.05$).

Fasciola hepatica						References
Herb	Species	Application	Worm count	Faecal egg count (FEC)	Clinical symptoms	
Albizia anthelmintica (worm-cure Albizia)	*F. gigantica* in Goats	Bark water with a (high) dose of 9 g/kg BW	95.5% reduction of adult worms	Significant reduction, not mentioned how much*		(Koko, Galal, & Khalid, 2000)
Allium sativa (garlic)	*F. hepatica* in sheep	Oral ingestion of garlic powder with a dose of 5mg/animal		Reduction in worm burden of 8, 16 and 21% on day 3, 7 and 14 respectively. The drug albendazole sulphoxide showed a reduction of 67, 90 and 98%* on the same days.		(Ijaz et al., 2009)
Balanites aegyptiaca (Desert date)	*F. gigantica* in Goats	9 g/kg BW fruit mesocarp	93.2% reduction of adult worms	Significant reduction, not mentioned how much*		(Koko et al., 2000)
Bio-dewormer	2–3-year-old goats	1400 mg/kg BW Weekly treated for 4 weeks		82.35% reduction at day 30	Significant weight gain and no side effect on liver or kidney.	(Abbas et al., 2020)
Caesalpinia crista (crested fever nut/nicker nut)	*Fasciola sp.* In Buffaloes	Oral ingestion of grinded *C. Crist*a seeds in a 2% gum solution with a dose of 50 mg/kg body mass.		86.5%* reduction at day 28 after two doses		(Maqbool, Hayat, & Tanveer, 2004)

	Fasciola sp. in Sheep	Oral ingestion of grinded *C. crista* seeds mixed with wheat bran with a dose of 80 mg/kg body mass		87.3% reduction at day 28 after 2 doses.		(Mushtaq et al., 2015)
Fumaria parviflora (fineleaf fumitory*)*	*Fasciola sp.* in buffaloes	F. parviflora aerial parts mixed in a 2% gum solution with a dose of 60 mg/kg body mass.		95.7%* reduction at day 28 after 2 doses		(Maqbool et al., 2004)
	Fasciola sp. in Sheep	Dried grinded leaves of F. parviflora mixed wheat bran with a dose of 80 mg/kg body mass.		Reduction of 91.8%* at day 28 after 2 doses		(Mushtaq et al., 2015)
	Fasciola sp. In cattle	Fed to the cows the aqueous extracts twice with a dose of 120 mg/kg body mass.		90.48%* reduction at day 28 after 2 doses	Body condition of the cows improved, cows turned into healthy without side effects	(RANA, 2015)
Ganzhisan (herb mixture)	Sheep	Orally drench with warm water	100% reduction *	15 days after feeding Ganzhisan, 100%* reduction	7 days after orally fed with Ganzhisan in summer, 12 out of 14 sheep were fully recovered with regards to the body temperature, appetite, rumination and activity*.	(Zhong, 2012)

	Cattle	After feeding 250 g honey, feed the decocted herbmix		100%* reduction	Animals recovered	(Zhou & Han, 2004)
Genistein (some fabacae)	*F. gigantica* in rabbits	Soybean extract	Fewer pathological signs of *F. gigantica* infection in rabbits' livers	FEC reduction of 95.8%* on day 7, five out of six rabbits had stool samples negative for *F. gigantica* eggs		(Nassef, El-Kersh, El Sobky, Harba, & El Refai Khalil, 2014)
Moringa oleifera (drumstick tree)	*F. gigantica* in sheep	150 mg/kg BW aqueous extract every 48 hours		100% reduction in lightly infected sheep* In vitro: strong ovicidal effects	Improved body weight gain*	(El Shanawany et al., 2019)
	F. hepatica in rabbits	150 mg/kg BW every 24 hours	No adult flukes found after autopsy of treated group, untreated group had 3.2 on average	100%* reduction	Improved condition of liver and gallbladder	(Kandil et al., 2018)
Nigella sativa (black cumin)	*Fasciola* sp. in buffaloes	Seeds powdered for drenching, 50 mg/kg BW		88.2%* and 81.25%* reduction on day 15		(Kailani, Akhtar, & Ashraf, 1995)
	Fasciola sp. in buffaloes	Two doses of seeds powdered for drenching, 50 mg/kg BW		81.25%* reduction on day 28 (after 2 doses)		(Maqbool et al., 2004)

*Saussurea lappa (*costus/ kuth*)*	Buffaloes	Oral ingeston of grinded *S. lappa* roots in a 2% gum solution with a dose of 200 mg/kg body mass		78.7%* reduction at day 28 after 2 doses.		(Maqbool et al., 2004)

Table 10. Natural herbs which are effective against an infection with *Fasciola hepatica*. All experiments with herbs in this table are done *in vitro*. FEC = Faecal egg count. Results on worm count, FEC and clinical symptoms are compared to an infected untreated control group. Asterisk (*) indicates significance ($\alpha < 0.05$).

Fasciola hepatica					References
Herb	Species	Application	Worm count	Faecal egg count (FEC)	
Areca catechu (betel nut)	*F. gigantica*	Betel nut extract	At a concentration of 2.5% and 5%, *A. catechu* caused mortality within 30 seconds. At a concentration of 1%, flukes died after 2 minutes		(Jeyathilakan, Murali, Anandaraj, & Abdul Basith, 2010)
	F. gigantica	Betel nut extract	At BNE extract concentrations of 2.5, 5, and 10 ml BNE/ 25 ml nutrient broth, motility time until death was 0.22, 0.07 minutes, and no movement at all, respectively		(Yamson, Tubalinal, Viloria, & Mingala, 2019)
Azadirachta indica (Neem tree)		Parasites were immersed in different concentratios of crude extract of shade dried leaves (for 1 hour duration, then 2, 3, 4, 5)	Mortality of larvae was 100% at an extract concentration of 4mg/ml after 4 hours		(Ibekwe, 2019)

Cuminum Cyminum (cumin)	*F. hepatica*	Essential oil of *C. cyminum,* 0.06-2.06 mg/mL		Strong ovicidal effect, 0% hatch rate Low toxicity for bovine cells (bovine kidney cell line MDBK)	(Machado Pereira da Silva et al., 2020)
*Genistein (*some *fabacae)*	*F. gigantica*	Soybean extract	Damage to tegument and signs of apoptosis induction after 24 hours		(Nassef et al., 2014)
	F. hepatica	0.27 mg/mL genistein	Reduced activity after 30 minutes, all flukes stopped moving after 3 hours		(Toner, Brennan, Wells, McGeown, & Fairweather, 2008)
Moringa oleifera (drumstick tree)	*F. gigantica*	Aqueous and ethanolic ectrscts		Strong ovicidal effect	(Hegazi et al., 2018)

Table 11. Herbs used on snails, either to kill the larvae inside the snails, or to kill the snails as intermediate host.

Host snail species of *F. hepatica*					References
Herb	Species	Application	Stage of larvae	Study	
Allium sativum (garlic)	*Lymnaea acuminata* snails	Exposed to allicin (active compound)	Redia and cercaria lethal value redia= 0.01 mg/L) and cercaria= 0.009 mg/L in vitro. in vivo toxicity of allicin in redia= 0.66 mg/L and cercaria= 0.38 mg/L. Snails did not die	*In vivo* and *in vitro*	(Sunita & Singh, 2011)
Areca catechu (betel nut)	*Lymnaea acuminata* snail	Treatment with sublethal concentrations of arecoline, the active component of A. catechu seed, on certain enzymes in the nervous tissue of *Lymnaea acuminata*	Targeted at snails: Treatment caused significant inhibition of acetylcholinesterase, and acid and alkaline phosphatase in the *L. acuminata*'s nervous tissue. Causing snails to die	*In vivo*	(Jaiswal & Singh, 2008)
Azadirachta indica (Neem tree)	*Lymnaea acuminata* snails	Exposed to azadirachtin	Mortality of redia stage larvae at a lethal value of 0.11 mg/L and cercaria at 0.05 mg/L after 8 hours. Snails did not die	*In vivo*	(Sunita & Singh, 2011)
Cuminum cyminum (Cumin)	*Radix peregra*	Essential oil of *C. cyminum*	Strong molluscicidal effect on both snail eggs and adult snails, egg hatchability severely reduced	*In vivo*	(Sousa, Rosa, Cunha, & Fernandes-Ferreira, 2017)

Promising herbs tested in vivo

Albizia anthelmintica (worm-cure Albizia)

Introduction

Albizia anthelmintica, also known as worm-cure Albizia, is a is a thorny, deciduous, medium canopied tree growing to about 8m. It occurs in semi-arid areas of Africa and can thrive in areas where the average annual temperature is up to 40°c and an annual rainfall between 400-1,000 mm (Dery & Otsyina, 2000). The plant is widely used in East Africa for the control of helminth parasites (Desta, 1995; Gradé & Longok, 2000; Koko et al., 2000).

Effectivity in ruminants

One study can be found on the effectiveness against gastrointestinal nematodes such as liver fluke. An experiment was carried out by Koko et al. (2000) to assess the effectiveness of traditional medicine commonly used in Sudan against *F. gigantica* burdens in goats. They compared the effectiveness of *A. anthelmintica* with that of the commercially used drug albendazole. Two weeks after treatment, adult liver fluke numbers were significantly reduced. These results are promising, but more research is needed to determine the correct dosage and treatment strategy (Table 9).

Application

Although *A. anthelmintica* showed a strong anthelmintic effect specifically against liver flukes as well as various type of worms (Githiori, Höglund, Waller, & Baker, 2003; Gradé, Tabuti, Van Damme, & Arble, 2007), it seems it has poor palatability for animals and is not available in any form in the Netherlands.

Allium sativum (garlic)

Introduction

Allium sativum, also known as garlic, is cultivated all over the world, and has been used for medicinal purposes for thousands of years, even going back to the ancient Egyptian time (Londhe, 2011). Garlic has many active constituents, which can help by lowering cholesterol concentrations, lowering blood pressure, inhibiting platelet aggregation and enhancing fibrinolytic activity (Tattelman, 2005). When the garlic bulb is damaged by cutting or crushing, the enzyme allinase is activated within *A. savitum*, metabolizing alliin to allicin. Allicin is one of the biologically most active compounds in *A. savitum*, and can be used against helminths (Dausch & Nixon, 1990). Garlic is most often administered in a powdered form.

Effectivity in ruminants

In a study by Ijaz et al. (2009) for example, garlic powder efficacy to decrease worm burden in sheep was investigated (Table 9). Worm burden was decreased in the group treated with garlic powder, but this decrease was not statistically significant. The main trematode species that the sheep were infected with in this study were *F. hepatica* and *Cotylophoron cotylophorum*. This study however only used 4 sheep per treatment group, and selected animals that were positive for helminths by counting the eggs per gram of faecal samples, which means different animals could have had differing parasite pressure per gastrointestinal tract parasite (GIT) species. Dairy farmer Karin Dijkstra* recommends combining garlic with parsley, as this is said to enhance its effectivity.

Effectivity in snails

Sunita and Singh (2011) studied the effect of garlic on *Fasciola gigantica* in infected *Lymnaea acuminata* snails, which are intermediate hosts, *in vitro* and *in vivo*. Toxicity of allicin against redia stage larvae and cercaria stage was highest under *in vitro* conditions. *In vivo* toxicity of allicin in *L. acuminata* required higher dosages (Table 11). In 2013, Sunita, Kumar, and Singh (2013) studied the effect of garlic on *F. gigantica* infected *L. acuminata*, both *in vitro* and *in vivo* over the different months over the year. They found that the effectivity of *in vivo* application was greatly dependent on the abiotic factors in the aquatic ecosystem. At higher temperatures in the summer months, more snails were infected by *F. gigantica,* but allicin was also found to be more toxic. In the winter months, the water temperature was low, and toxicity of allicin was low as well (Table 11).

* See appendix for full interview

Application

Although an *in vivo* study was performed to assess the effectivity of garlic powder in sheep, the results of this study (Ijaz et al, 2009) were not sufficiently significant to advise the use of garlic powder for its antiparasitic activity. In order to make a definitive assessment, further research is needed. Garlic was found to be effective against *F. gigantica* larvae in snails. Thus, implementation in the diet or pasture may help to control snail population levels. Furthermore, many general health benefits have been ascribed to garlic, and it has been used as a poultry feed additive (Kothari, Lee, Niu, & Kim, 2019). Implementation in the pasture may allow animals to self-medicate and improve their general health, while potentially helping to control aquatic snail levels.

Balanites aegyptiaca (desert date)

Introduction

Balanites aegyptiaca, also known as desert date, is a slow-growing, evergreen or semideciduous, spiny tree and can grow up to 10 metres or taller. It is one of the most common wild plant species of the arid areas of Africa and South Asia (Hall, 1992). Different parts of the plant are widely used throughout East Africa as anthelmintic (Nkunya, Weenen, & Bray, 1990).

Effectivity in ruminants

One study can be found on the effectiveness against gastrointestinal nematodes such as liver fluke. An experiment was carried out by (Koko et al., 2000) to assess the effectiveness of traditional medicine commonly used in Sudan against *F. gigantica* burdens in goats. They compared the effectiveness of *B. aegyptiaca* with that of the commercially used drug albendazole. When they determined the percental reduction in fluke counts from the liver 2 weeks after the start of the treatment, the efficacy of the mentioned therapeutics was 93.2 and 97.7%, respectively. These results are promising, but more research is needed to determine the correct dosage and treatment strategy (Table 9).

Application

For *B. aegyptiaca*, only essential oil is purchasable in the Netherlands, at a high cost. Therefore, the implementation of this herb will not be further discussed in this report, as there are currently too many hurdles to overcome.

Bio-dewormer

Abbas et al. (2020) showed that a mixture of plants, of which the composition is shown in table 8, is effective *in vivo* for managing *F hepatica* in goats. Generally, a significant reduction of faecal egg count with no side effects on liver or kidney was observed. In addition, a dose-dependent response to this herbal mixture was noticed. The highest efficacy for reducing faecal egg count with maximum weight gain at a certain dose of herbal mixture was indicated in Table 9. The anthelmintic activity might be caused by a combination of anthelmintic compounds in the plants, such as tannins, saponins and flavonoids. Also, synergy of the components of different herbs used in the mixture might increase anthelmintic activity. The results of this study indicated that this herbal mixture is promising (Table 9). The application of this herbal mixture can be found in the *H. contortus* part, as the bio-dewormer was also tested on this parasite species.

*Caesalpinia crista (*crested fever nut/nicker nut*)*

Introduction

Caesalpinia crista, commonly known as crested fever nut or nicker nut, is a climbing shrub which can climb to 10 meters in length (Li, Zhang, Li, & Chen, 2004). This species is widely distributed throughout India and the (sub)tropical regions of Southeast Asia (Das et al., 2010). Different parts of *C. Crista* are used in traditional medicine; the seeds are considered to be effective for helminthic infection in sheep (Akhtar & Aslam, 1989).

Effectivity in ruminants

An *in vivo* study by Maqbool et al. (2004) studied the efficacy of *C. crista* seeds on buffaloes naturally infected with fasciolosis (Table 9). At a dose of 30 mg/kg body mass, a decrease in Eggs count per gram faeces of 48.9% and 80.8% was shown on day 18 (after one dose) and day 28 (after two doses). At a dose rate of 50 mg/ kg body mass, the drug was 57.7% and 86.5% effective at one- and two dose levels, respectively. Another in vivo study by Mushtaq et al. (2015) studied the efficacy of finely grinded seeds of *C. crista* on sheep naturally infected with fasciolosis (Table 9). In sheep, at a dose of 80 mg/kg body weight, reduction in faecal egg count was shown to be 58.7% and 87.3% on day 18 (after one dose) and day 28 (after two doses), respectively.

Application

For the *in vivo* study by Maqbool et al. (2004), the optimal dose of *C. crista* seeds was 50 mg/kg body mass for buffaloes. The seeds were dried, grounded into a powder form and suspended in 100 ml of 2% gum solution. The solution was orally ingested. For the *in vivo* study by Mushtaq et al. (2015), the optimal dose was 80 mg/kg body mass. For sheep, the dosage is thus known. For calves, the dose should be adjusted. Since this plant only grows in (sub)tropical climates, this herb has to be imported. This herb however is not easily obtainable for import, thus practical application of this herb in the Netherlands does not seem feasible.

Fumaria parviflora (fineleaf fumitory)

Introduction

Fumaria parviflora, commonly known as fineleaf fumitory, is an annual herb that is indigenous in Europe, Africa and Asia, and most diversely in the Mediterranean region and Indo-Pakistan subcontinent. It can also be found in Australia and North and South America (Hayashi et al., 2017). It is mainly used in traditional medicine throughout the Indo-Pakistan subcontinent for its anthelmintic properties (Al-Shaibani, Phulan, & Shiekh, 2009; Hördegen, Hertzberg, Heilmann, Langhans, & Maurer, 2003). Fumaria parviflora contains various alkaloidal chemical compounds majorly were protopine and adlumidiceine. The leaves of *Fumaria parviflora* could be used in powders which have anti-inflammatory properties by prohibiting cytokines and anti-oxidation (Rizvi et al., 2017). In Turkish tradition, it was used for hepato-biliary dysfunction (Jameel, Ali, & Ali, 2014).

Effectivity in ruminants

An *in vivo* study by Maqbool et al. (2004) studied the efficacy of *F. parviflora* aerial parts on buffaloes naturally infected with fasciolosis. 28 days after treatment, faecal egg counts were significantly reduced (Table 9). Another *in vivo* study by Mushtaq et al. (2015) studied the efficacy of dried grinded leaves of *F. parviflora* in sheep naturally infected with fasciolosis. This study showed similar results to the study in buffaloes, as faecal egg counts were strongly reduced after 28 days (Table 9).

An *in vivo* study that compared the therapeutic effect of *Fumaria parviflora* and Triclabendazole against Fasciolosis was conducted on cows. 10 healthy cows were the control group. Efficacy was determined by the EPG of before and after treatment (RANA, 2015). The results indicated that *Fumaria parviflora* aerial parts had a high efficacy on reducing egg count (Table 9). Also, the body condition of the cows improved with no side effect of the herbs observed. Although Triclabendazole reached 100 % reduction on faecal egg count, the efficacy of this herb is comparable with this standard drug.

Application

Fumaria parviflora is native in Europe, Asia and Africa and could be found in the Netherlands. This plant can successfully grow on different soils and thrive on loam and sandy soils with neutral pH under sunny weather. In order to preserve the high concentrations of alkaloids in the plant, the plant should be harvested before withering. There are commercial products in form of powders of this herb or raw herb named ‘pitpapra’, which could be delivered all over the world. The powders could be mixed in with wheat bran and then fed to animals.

Chinese Herbmix: Ganzhisan (肝蛭散)

Introduction

Ganzhisan is a Chinese herbal mixture which could be used for treating liver flukes in China (Veterinarian Jiaguo Liu*). The doses of different herbs in the mixture are different for sheep and cattle, as shown in table 12 and 13.

Effectivity in ruminants

The clinical experiment investigating the anthelmintic effects of Ganzhisan on metacercaria and *F. hepatica* adults in the sheep was done by Zhong (2012). This experiment consisted of two parts, which are carried out in summer and spring respectively. In summer, no fluke eggs were found in the faeces, since acute disease in summer is caused by ingested metacercaria which have not yet matured and started to produce eggs yet. Sheep infected by liver fluke were fed with Ganzhisan and the clinical recovery of sheep were studied. In spring, eggs were found in the faces of untreated infected sheep, indicating that the metacercaria had developed into adult fluke in the bile duct and caused chronic disease. In the Ganzhisan treated group, no eggs were found. The results showed that Ganzhisan was highly efficient for killing adult flukes and larvae as well as contributing to sheep health (Table 9).

Zhou and Han (2004) used Ganzhisan with the herbmix 2 recipe (Table 13) during their study. The Ganzhisan was decocted with water and the warm extraction was fed to cattle infected by liver fluke on the farm. Faecal egg counts were significantly reduced, and clinical recovery of cattle was observed (Table 9).

Application

These herbal powders could be bought from Chinese medicine stores in the Netherlands and then mixed according to the correct dose. In addition, commercial products could also be bought from Chinese medicine companies. However, the dose of each herb in the commercial products is slightly different from those used in the experiments, which showed 100% efficiency for killing *F. hepatica*. As a result, further studies on the efficiency of Ganzhisan commercial products are still needed.

* See appendix for the full interview

Table 12: Herbmix 1: Ganzhisan for sheep

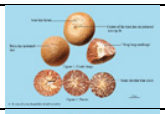
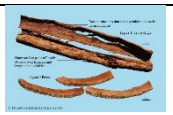
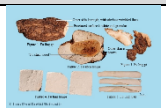
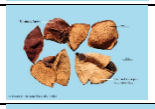
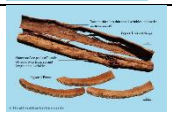
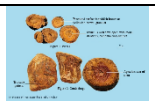
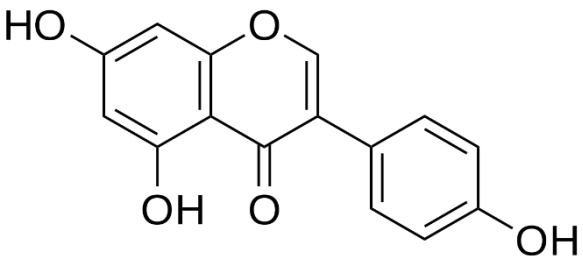
Chinese name	Latin Pharmaceutical Name	English name	Parts	Functions for sheep		Dose for sheep (gram)
茯苓	*Poria*	Indian Buead Tuckahoe	Fuling lumps	leaches out dampness, fortifies the spleen		12
苏木	*Biancaea sappan*	Sappen wood	Wood	Quickens the blood, relieves pain.		12
槟榔	*Arecae Semen*	Betel nut	Seed	kill worms		15
贯众	*Cyrtomium fortunei*	Cyrtomium rhizome	Root	Kill worms		15
甘草	*Glycyrrhizae Radix et Rhizoma*	Liquorice Root	Root	Supplements the spleen; harmonizes the nature of other medicinals		10
厚朴	*Magnoliae Officinalis Cortex*	Officinal Magnolia Bark	Root barks and tree barks	Moves qi, disperses stagnation		10
龙胆	*Gentianae Radix et Rhizoma*	Chinese Gentian Root	Root	Clears heat, dried dampness, drains the liver		15
木通	*Akebiae Caulis*	Akabia Stem	wood	leaches out dampness, fortifies the spleen		6
泻泽	*Alismatis Rhizoma*	Oriental Waterplantain Tuber	Root	leaches out dampness, fortifies the spleen		15
肉豆蔻	*Myristicae Semen*	Nutmeg	Fruit and seed	Apply to diarriha		10

Table 13: Herbmix 2: Ganzhisan for cattle

Chinese name	Latin Pharmaceutical Name	English name	Parts	Functions for cattle		Dose for cattle (gram）
茯苓	*Poria*	Indian Buead Tuckahoe	Lumps	leaches out dampness, fortifies the spleen		50
苏木	*Biancaea sappan*	Sappen wood	Wood	Quickens the blood, relieves pain.		20
槟榔	*Arecae Semen*	Betel nut	Seed	kill worms		40
贯众	*Cyrtomium fortunei*	Cyrtomium rhizome	Root	Kill worms		50
苦参	*Sophorae Flavescentis Radix*	Lightyellow Sophra Root	Root	Anti-inflammatory		40
陈皮	*Citri Reticulatae Pericarpium*	Tangerine Peel	Peel	Kill worms		40
厚朴	*Magnoliae Officinalis Cortex*	Officinal Magnolia Bark	Root barks and tree barks	Moves qi, disperses stagnation		30
龙胆	*Gentianae Radix et Rhizoma*	Chinese Gentian Root	Root	Clears heat, dried dampness, drains the liver		40
大黄	*Rhei Radix et Rhizoma*	Rhubarb	Root	Anti-inflammatory		6
泽泻	*Alismatis Rhizoma*	Oriental Waterplantain Tuber	root	leaches out dampness, fortifies the spleen		30
肉豆蔻	*Myristicae Semen*	Nutmeg	Fruit and seed	Apply to diarrhoea		20

Genistein (some *fabacae* species)



Introduction

Genistein is an active compound of some plants in the *fabacae* family, including soybeans, *Psoralea* and *Pueraria* (Table 14) (Kaufman, Duke, Brielmann, Boik, & Hoyt, 1997). Genistein has been found to have antiparasitic properties against various nematodes, trematodes and cestodes (Toner et al., 2008). Genistein has been found to have antiparasitic properties against various nematodes, trematodes and cestodes (Toner et al., 2008).

Effectivity in mammals

An *in vivo* study treated *F. gigantica* infected rabbits with soybean extract, which is a rich source of genistein (Nassef et al., 2014). Faecal egg count significantly decreased, with 5 out of 6 treated rabbits testing negative for *F. gigantica* eggs on day 7 (table 9). It should be noted that this study included a positive control with triclabendazole, which was able to produce a 100% faecal egg reduction and cure rate by day 3. Furthermore, upon autopsy the livers of soybean extract treated rabbits showed fewer pathological signs of *F. gigantica* infection, such as necrosis, polyp formation and congestion of the veins and sinusoids. The reduction of pathological signs was similar to that produced by triclabendazole treatment. Overall, this study demonstrates the high efficacy of soybean extract to treat *F. gigantica* in rabbits, although the effect of triclabendazole was somewhat faster and more pronounced.

***In vitro* effectivity**

In an *in vitro* study, adult *F. hepatica* were exposed to genistein (Toner et al., 2008). The *F. hepatica* flukes showed disruption in the tegument in the form of blebbing, swelling and general damage to the surface of the flukes (Table 10). Furthermore, fluke motility decreased until the worms stopped moving completely. Another *in vitro* study incubated *F. gigantica* worms with soybean extract, with triclabendazole as a control (Nassef et al., 2014). After 24 hours, DNA extraction and DNA gel electrophoresis was performed. Both the triclabendazole and soybean extract groups showed significant signs of apoptosis induction (Table 10). These two studies point to the flukicidal activity of genistein, both as a soybean extract and as a purer form.

Application

Genistein has been shown to have strong antiparasitic properties against various parasite species, including *F. gigantica* in rabbits. However, an *in vivo* study in sheep or cattle is needed to assess effectivity and the correct dose. The high genistein content of soybeans and other fabacae plants is promising. Furthermore, *Trifolium*, or clovers, have been found to have a high genistein content (Table 14). Clovers have already been implemented in pastures in the Netherlands for some time, as they are palatable and nutrient-rich herbs for cattle and sheep (Meijer & Visch, 1995).

Table 14. Some plant species with a high genistein content (Kaufman et al., 1997).

Plant species	Genistein (mg/kg dry weight)
Psoralea corylifolia (babchi)	2151.0
Glycine max (fermented soybean miso)	427.6
Pueraria lobata (kudzu)	316.9
Baptisia australis (baptisia)	350.7
Styphnolobium japonicum (chinese scholar tree)	181.6
Trifolium sp. (clover)	110.0
Lupinus albus (lupine)	101.1

Moringa oleifera (drumstick tree)

Introduction

M. oleifera, or drumstick tree, is a slender tree that can grow extremely fast (Radovich, 2011). It can reach heights of up to 10 meters, but usually remains a bit smaller. Almost all parts of the tree are used for their nutritional or medicinal value. Different parts of *M. oleifera* such as seed, leaves and roots etc., have various functions like antiparasitic activity (Hegazi et al., 2018; Kaur, Kaur, Singh, & Singh, 2014) and water purification for reducing the number of helminth eggs (Sengupta et al., 2012). Annually, 1.2 million metric tonnes of *M. oleifera* fruit is produced in India (Radovich, 2011).

Effectivity in ruminants

A study in sheep investigated the effect of *M. oleifera* leaf extract on animals co-infected with *F. gigantica* and the bacterium *Clostridium novyi* (El Shanawany et al., 2019). The infected sheep were divided into a heavily infected, lightly infected and mixed infected control group. The first two groups were orally treated with aqueous *M. oleifera* leaf extract. After treatment, the heavily and lightly infected groups showed improved body weight gain (Table 9). The body weight gain observed in this study may have been caused directly by the high nutritional value of *M. oleifera* leaves, or indirectly by the decreased parasite load. Additionally, the heavily and lightly infected groups showed a reduced faecal egg count when compared to the untreated control group. Furthermore, eggs isolated from the faeces showed signs of degeneration, with eggs having darkened or no embryos inside.

Thus, the *M.* Oleifera leaf extract significantly impacted *F. gigantica* egg viability. An *in vitro* study found similar ovicidal results, where non-embryonated and developed eggs were most susceptible to aqueous and ethanolic extracts, respectively (Hegazi et al., 2018) (Table 10).

Effectivity in other mammals

Another in vivo study of the anthelmintic effect of *M. oleifera* seed extract on rabbits which experimentally infected with *F. hepatica* was done by (Kandil et al., 2018). A 100 % FEC reduction was observed 7 days after the treatment, and no flukes could be found after autopsy (Table 9). A positive contribution to the recovery of the liver and gallbladder of rabbits was also noticed, which might be caused by regenerated liver parenchyma and improved liver function (Hodžić et al., 2013). This study showed promising anthelmintic activity of *M. oleifera* seed extract. However, this study was conducted on rabbits. Further in vivo studies on sheep or cattle with variable doses of the extract are needed to ensure the efficiency and safety as well as the applicability.

Application

M. oleifera leaf and seed extract was shown to be effective *in vivo* in both sheep and rabbits. Both faecal egg counts and adult fluke numbers were reduced, which points to potent ovicidal and flukicidal properties. Furthermore, the high nutritional value of *M. oleifera* can help affected host animals to regain their strength and body weight. The climate in the Netherlands is not suitable for growing *M. oleifera* outside on a large scale. However, the plant is produced on a large scale in warmer countries, such as India and the northern countries of Africa and South America. Thus, it is possible to import either powdered *M. oleifera* leaves or extracts. These can then be added as supplemental feed.

Nigella sativa (black cumin)

Introduction

Nigella sativa, or black cumin, is an annual flowering plant that is native to western Asia and eastern Europe (Thulin, 1993). It has been cultivated since ancient times, as the plant's black seeds have been used for both culinary and medicinal purposes. The plant is produced on a large scale in Asia, Africa and parts of the Mediterranea. While the common name for this plant resembles that of *C. cyminum*, the two plants are not closely related.

Effectivity in ruminants

Two *in vivo* studies on *Fasciola* sp. infected buffaloes investigated the anthelmintic effects of *N. sativa* seeds (Kailani et al., 1995; Maqbool et al., 2004). Both studies observed a significant decrease in faecal egg count (Table 9). However, the studies disagreed on the correct dosage and administration frequency. Thus, more research is needed to determine these parameters for optimal faecal egg count reduction. Both studies did not observe any side effects or toxicity upon treatment with the *N. sativa* seeds.

***In vitro* results**

The effects of the main active compound of *N. sativa* seeds, thymoquinone, were investigated in an in vitro study using *F. gigantica* (Ullah et al., 2017). Adult *F. gigantica* specimens showed reduced motility and damage to the tegument and spines on the surface of the animals (Table 10). Additionally, enzymatic disruption had occurred.

Application

In the studies by Kailani et al. (1995) and Maqbool et al. (2004), *Nigella sativa* seeds were dried, finely powdered, and then suspended in fluid and administered orally by drenching. While (Kailani et al., 1995) saw promising results on day 18 after a single dosage of 25 mg/kg BW, (Maqbool et al., 2004) only saw similarly promising results on day 28 after two dosages of 50 mg/kg BW. As both studies did not observe any side effects or toxicity, it is most likely safe and effective to use the dosage of 50 mg/kg BW. However, further research should be performed in cows and sheep to determine the correct and most effective dose.

Saussurea lappa (costus/ kuth)

Introduction

Sausserea lappa, commonly known as costus or kuth, is a perennial herb that grows up to 1-2 meters. The species is endemic to a limited part of the Himalayas (Shah, 2006). Due high demand for the plant and limited distribution, this plant is now a critically endangered species and is now prioritized for conservation of endangered medicinal plants (Kuniyal, Rawat, Oinam, Kuniyal, & Vishvakarma, 2005). The plant is widely used in both traditional and modern medicine against chronic skin diseases, rheumatism, inflammation and if used for its anthelmintic properties (Tsarong, 1994; Zahara et al., 2014).

Effectivity in ruminants

An *in vivo* study by Maqbool et al. (2004) studied the efficacy of *S. lappa* roots on buffaloes naturally infected with fasciolosis (Table 9). A decrease in faecal egg count was noted, but this decrease was not as strong as for *Nigella sativa*, which was researched in the same study.

Application

For anthelmintic application in the in vivo study by Maqbool et al. (2004), the optimal dose of S. lappa roots was 200 mg/kg body mass for buffaloes. The roots were dried, ground into powder form and suspended in 300-500 ml 2% gum solution, because of its low solubility and high dosage level. The solution was orally ingested.

S. lappa was enlisted as an endangered species at the Convention on International Trade in Endangered Species of Wild Fauna and Flora. Because this herb is an endangered species, cultivating it to use the roots for anthelmintic application is unrealistic.

Promising herbs from in vitro studies

Areca catechu (betel nut)

Introduction

Areca catechu L., also known as betel nut, is mainly grown in Sout/Southeast Asian countries and its seeds are commonly used in herbal medicine for, inter alia, its laxative, digestive, anti-diarrheal and anthelmintic properties (Amudhan, Begum, & Hebbar, 2012; Jaiswal, Kumar, Singh, & Singh, 2011; Keshava Bhat, Mythri, & Ashwin, 2016).

***In vitro* results**

In vitro studies focusing on the effectiveness of *Areca catechu* against liver fluke seem very promising. An in vitro study by Jeyathilakan et al. (2010) studied the effect of betel nut extract on *Fasciola gigantica*. *A. catechu* extract was able to cause mortality within 30 seconds to 2 minutes, depending on the concentration (Table 10). Another *in vitro* study by Yamson et al. (2019) compared the effectiveness of betel nut extract with that of the commonly used anthelmintic drug Albendazole. In this study, betel nut extract thus showed a shorter time until cessation of movement and death than Albendazole.

Effectivity in snails

An *in vivo* study by Jaiswal and Singh (2008) studied the effects of arecoline, the active compound of *A. catechu* seed on certain enzymes in the nervous tissue of *Lymnaea acuminata*, the intermediate host of *F. gigantica.* In this study, treatment with sublethal concentrations of arecoline caused significant inhibition of enzymes in the *L. acuminata*'s nervous tissue (Table 11). This component could thus be applied to control vector snail populations to solve large-scale problems with fascioliasis. Methods to kill the intermediate hosts of *Fasiola spp*. should however be implemented with care, as snails are important components of an aquatic ecosystem (Sunita & Singh, 2011).

Application

The *in vitro* results of *A. catechu* showed that the extract was able to cause mortality in *F. gigantica* after only a short period of time. Furthermore, the active compound arecoline was able to cause mortality in snails. Thus, this extract could be used to control snail populations in the pasture. However, it is difficult to determine the correct dosage in a larger body of water. *A. catechu* powder and extract can be purchased in the Netherlands, but for correct implementation, further research into the correct dosage is needed.

Azadirachta indica (neem tree)

Introduction

Azadirachta indica, also known as neem tree, is a fast growing, evergreen tree found in most tropical countries (Asif, 2012). Neem has been used extensively as a homeopathic remedy in countries such as India as a treatment for inflammation, infections, fever, skin conditions, and dental problems (Asif, 2012; Lakshmi, Krishnan, Rajendran, & Madhusudhanan, 2015). Throughout history, extracts from bark, leaves, fruits and root have been applied as treatment for leprosy, intestinal helminthiasis and respiratory disorders in children (Van der Nat, Klerx, Van Dijk, De Silva, & Labadie, 1987).

***In vitro* results**

In an *in vitro* study by Ibekwe (2019), anthelmintic effect of leaf extract of *A. indica* was tested on *Paramphistomum cervi* and *Fasciola hepatica*. For *F. hepatica*, mortality was shown to be 100% at an extract concentration of 4mg/ml after 4 hours. The *in vitro* results seem very promising (Table 10).

Effectivity in snails

Other applications of *A. indica* seem more promising: Sunita and Singh (2011) studied the effect of *A. indica* on *F. gigantica* in infected *Lymnaea acuminata* snails *in vitro* and *in vivo*. *In vivo* treatment with azadirachtin, an active component of *A. indica*, caused high toxicity against redia stage and cercaria larvae (table 11). These concentrations were high enough to kill the parasites, but not the intermediate hosts. This is important, as snails are important components of aquatic ecosystems and thus, their eradication is not desirable.

Application

Azadirachta indica has a promising result *in vitro* studies (Ibekwe, 2019). However, when looking at various *in vivo* studies, leaves of *A. indica* do not reduce faecal egg count for various gastrointestinal nematodes (Costa et al., 2006; Githiori, 2004; Hördegen et al., 2003). Thus, more *in vivo* studies are needed to find out the anthelmintic effectiveness part of the plant and the safe doses.

Apart from that, Neem tree can also use to treat on snails to kill the infectious larvae, but since it only used active compound but not the herb itself, how to apply it in practice need to be study. Another approach is to used essential oil, as Neem tree oil is available in the Netherlands.

Cuminum cyminum L. (cumin)


Introduction

Cuminum cyminum is a slender, herbaceous annual plant that is thought to be native to Syria, Egypt, and Turkestan (Divakara Sastry & Anandara, 2013). It is closely related to parsley. The seeds of the plant, cumin seeds, are among the earliest known spices, having been used in the ancient Egyptian mummification process. Nowadays, cumin is produced in high quantities, mainly in Central and South Asia. An estimated 300.000 tons are produced annually worldwide. *C. cyminum* grows best on well-drained sandy loam soils, with optimal growth temperatures ranging from 25-30 °C. The plant is sensitive to rain and frost damage, which can reduce productivity. *C. cyminum* can grow in the Netherlands, if weather conditions are favourable.

***In vitro* results**

In an *in vitro* study by da Silva et al. (2020), essential oil of *C. cyminum* was shown to have a strong ovicidal effect (Table 10). To assess the toxicity of the essential *C. cyminum* oil, a cell viability assay was performed on bovine cells. The essential oil was found to have relatively low toxicity.

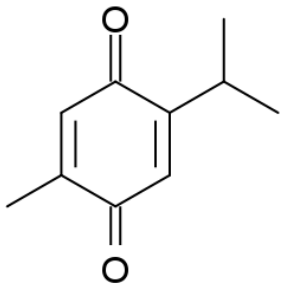
Effectivity in snails

Essential oil of *C. cyminum* was found to have a strong molluscicidal effect on both eggs and adults of the snail species *Radix peregra* (Sousa et al., 2017). This snail species is an intermediate host in the life cycle of *F. Hepatica* and can be found in Europe and North Asia (Relf, Good, McCarthy, & de Waal, 2009). Furthermore, *R. peregra* can live in dryer, more acidic environments that the most known intermediate host snail, *G. truncatula.* Thus, infection of *R. peregra* with *F. hepatica* can lead to an expansion of the areas where fascioliasis is prevalent. In the study by Sousa et al. (2017), essential oil of *C. cyminum* had a strong negative effect on egg hatchability (Table 11). The essential oil was also able to cause lethality in both juvenile and adult *R. peregra*. These effects can most likely be attributed to the compound cuminaldehyde. Although more research is needed, the molluscicidal effect of *C. cyminum* essential oil may help to control *R. peregra* and possibly other *F. hepatica* host snail species, thus hindering the lifecycle of the parasite. However, it should be considered that snails are an important part of the aquatic ecosystem. Thus, the focus should be on control rather than eradication.

Application

The effectivity of *C. cyminum* has not yet been assessed in an *in vivo* study. Thus, before this herb is used as a preventative herbal remedy, more research is needed to establish the correct dose. However, the strong *in vitro* ovicidal effect is quite promising. As cumin can grow in the Netherlands if weather conditions are favourable, it may be possible to implement the herb in the pasture. This approach allows the animals to self-medicate. Furthermore, the highly concentrated essential oil was found to have relatively low toxicity. Thus, toxicity for the whole plant is likely even lower, when it is not consumed in excess. The presence of *C. cyminum* in the pasture may also affect aquatic snail populations, either directly or via residues in the manure. A subsequent reduction in this intermediate host may hinder the lifecycle of *F. hepatica*.

Nigella sativa/thymoquinone



In an *in vitro* study, the active compound of *Nigella* sativa, thymoquinone, was shown to cause enzymatic disruption in *F. gigantica* (Ullah et al., 2017). Interestingly, one of the disrupted enzymatic pathways was that of free radical scavenging antioxidant molecules. As parasites live in a hostile environment in their host, they need strong antioxidant activity to protect themselves from reactive oxygen species (ROS) damage caused by immune cells and inflammation. Disrupting *F. gigantica* or *F. hepatica*'s antioxidant activity may make them more vulnerable to their host's immune system. A recent *in vitro* study incubating *F. gigantica* with thymoquinone observed a significant increase in ROS generation (Rehman et al., 2020). Additionally, they found a similar downregulation of antioxidant enzymes. DNA disruption and the presence of apoptosis markers were also observed upon thymoquinone treatment.

Thymoquinone has been found to have a protective effect on the liver (Darakhshan, Bidmeshki Pour, Hosseinzadeh Colagar, & Sisakhtnezhad, 2015). Interestingly, it does this by enhancing antioxidant activity. A possible explanation for this is the difference in physiology and molecular pathways between mammals and trematodes. Thymoquinone also inhibits hepatotoxicity, protects against necrosis, and induces cell proliferation to repair tissue damage. Thymoquinone has been shown to have very little adverse effects and toxicity. Thus, *N. sativa* is a safe herb that may not only be useful to treat fasciolosis by negatively impacting *F. gigantica* or *F. hepatica*, but also by protecting against liver damage caused by the parasites.

Discussion

Excessive use of anthelmintic drugs has induced resistance development in parasites and is thought to have negative effects on soil health. To overcome these problems, a new approach is needed to control internal parasites in ruminants. The main question asked in this study was: "*Which natural remedies and practices for internal parasites in ruminants have the most potential, and how can these be applied by farmers in the Netherlands in the future?*" The main goal of this approach is to avoid high parasite burdens, and to strengthen the immune system without inducing resistance development of the parasites. This study's focus is on herbal medicines. Next to herbal medicine, several managing options are identified which could be applied to the farm system. Combining these options is expected to have an enhanced effect in controlling internal parasite infections.

Alternatives for anthelmintics

1. Coexistence between parasite and host

Eradication of all internal parasites in the animal is not the best approach (biologist Tedje van Asseldonk[*]; parasitologist Dr. Harm Ploeger [*]; researcher Adriaan Antonis[*]). The parasite itself is not the problem, but the overgrowth of the parasite is. Thus, coexistence of parasites and animals is important. Therefore, T. Van Asseldonk[*] prefers herbs with relatively low toxicity, to control rather than eradicate parasitic infections. This opinion is shared by biological farmer, Klaartje van Wijk [*]. She does not feed herbs to treat or prevent parasitic infections, but to maintain balance of the immune system and host-parasite interactions. Additionally, H. Ploeger [*] confirms this; we do not need to focus on parasite extinction, but more on controlling the infection. By focusing on reducing parasite burden, the host still gets infected and develops immunity without suffering from a severe infection. This idea is also shared by A. Antonis [*], who suggests that animals need to get infected by parasites to build natural resistance, which is consistent with the old friend hypothesis. Parasite infections are most common in young animals. The old friend hypothesis states that when young animals do not encounter parasites in their first year of life, they lack resistance later in life and therefore will get more severe infections once infected in a later stage of life. This is confirmed by Jackson, Friberg, Little, and Bradley (2009), who suggest that parasite infections are important for normal development of the immune system of the host. Helminths have anti-inflammatory effects in the host which can reduce the host's susceptibility to allergies (Schabussova & Wiedermann, 2014). In addition, it is believed that getting rid of one parasite species in the host creates space for another, even more harmful, parasite species. Herbs can positively contribute to the coexistence between parasite and host, as they have anthelmintic effects, but do not usually lead to complete parasite eradication.

[*] See appendix for the full interview

2. Pasture rotation / mixed grazing

Living with parasites requires maintenance of the complex balance between hosts and parasites. A. Antonis* believes that controlling parasitic infections is a multidisciplinary approach. This not only involves treating animals with herbs, but also pasture rotation to lower infection risk. A. Antonis* suggested to move sheep to a different field every 2-3 weeks, as *H. contortus* larvae reach the infective stage L3 in this period of time (Eekeren & Eysker, 2004). After the land has been grazed, the field can be mowed, as this removes the parasites which were left on the land. As for *F. hepatica,* controlling the intermediate host snails of the parasite species is also highly important (Knubben-Schweizer et al., 2010).

It is also suggested to let different animal species graze on the land, called mixed grazing (A. Antonis*). In the case of *F. hepatica* and *H. contortus*, it needs to be considered that both cattle and sheep can be infected by these parasites (Beesley, Williams, Paterson, & Hodgkinson, 2017a; McLeod, 2004; Perry, 2002). Therefore, an animal species that is not susceptible for these parasites would be preferable for grazing before and after susceptible species. For example, horses are not susceptible to *H. contortus* and can therefore 'clean' the field before farmers let the sheep and cows graze on the field (McLeod, 2004; Perry, 2002).

3. Grass management

It is important to keep enough grass on the land for the animals or keep the animal density on grassland low. When sufficient grass is available, animals usually do not graze near manure (Hutchings, Athanasiadou, Kyriazakis, & Gordon, 2003). However, if there is not enough grass left, animals start to eat the grass near their own manure, where most infectious larvae are present (Hutchings et al., 2003). This idea is shared by H. Ploeger*, who mentions that raising awareness of correct management practices has more impact than feeding medicinal herbs.

Furthermore, it has been suggested to let the ruminants graze on smaller pastures. This requires more labour-intensive rotation, but this will shorten the time of ruminants eating the same grass and thereby lower the infection risk (H. Ploeger*). Moreover, A. Antonis* highlighted the need to ensure favourable circumstances for dung beetles and fungi in the ground, as they are part of a food web and can kill nematodes. In order to provide a suitable environment for dung beetles and fungi, farmers need to use fewer synthetic nitrogen fertilizers, or even none at all, to limit nitrogen levels in the soil (Hsueh, Mahanti, Schroeder, & Sternberg, 2013). Namely, when the nitrogen level is too high in the soil, fungi are not able to use their nematode trapping systems (Hsueh et al., 2013). However, pasture production still needs to be ensured, and thus a balance is needed between production and fertilization input. Additionally, the microbiome of the animal needs to be stable. The microbiome can be influenced by both parasites and herbs. However, (Mravčáková et al., 2019) showed only weak changes in the microbial community of infected lambs which were fed herbal mixtures.

4. Breeding

A. Antonis* suggested that some animal breeds or certain animals are more susceptible for parasitic infections than others. This is confirmed by Preston and Allonby (1979) which showed that Scottisch Blackface, Finn Dorset and Merine sheep homozygous for haemoglobin A were less susceptible for *H. contortus* infection. Namely, these sheep developed smaller parasitic burdens compared to sheep homozygous for haemoglobin B and heterozygous sheep. Therefore, farmers could choose to only breed with animals which are less susceptible to parasitic infections. For this overview, we did not study this approach in further detail, as it is beyond the scope of our project.

5. Medicinal herbs

Herbs have been shown to be effective in reducing the adult worm and faecal egg count of internal parasites. However, as shown from the results, most herbs do not have 100% effectivity. Synthetic drugs, on the other hand, often show an effectivity of (close to) 100% in reducing the adult worms and faecal egg count (Eguale et al., 2007) at least if no parasite resistance develops. By reducing the worm count, there is a lower worm burden in the livestock, reducing the severity of health problems. Meanwhile, by reducing the egg count, there will be lower future pasture infectivity for livestock.
Herbs can positively contribute to controlling parasitic infections, as they have anthelmintic effects, but do not usually lead to complete parasite eradication. Moreover, researcher S.K. Kumar[1] says that herb mixtures not only cure diseases, but also maintain the general health of the animal. This is confirmed by (Mravčáková et al., 2021) who showed evidence that herb mixtures do not have a direct anthelmintic effect, but rather increase resistance of lambs against gastrointestinal nematodes by their antioxidant effect.

Most promising herbs for *Haemonchus contortus*

After considering the criteria (*in vivo* evidence, effectiveness, accessibility) for plants to qualify as a promising herb, *Cichorium intybus L.* (chicory), *Lotus corniculatus* (birdsfoot trefoil), *Lespedeza cuneata* (Chinese bush-clover) and *Onobrychis viciifolia* (sainfoin) are most suitable for Dutch agriculture systems. These herbs can be grown in the pasture in the Netherlands and can be ensilaged. It stood out that chicory showed high anthelmintic effects, especially in summer. The infection risk of *H. contortus* is highest in summer and therefore makes chicory promising to decrease *H. contortus* worm burden. A disadvantage of chicory are the broad leaves that take a relatively large amount of space which could hamper productivity of the pasture (Veldhuis, 2009). *Lotus corniculatus* (birdsfoot trefoil) shows promising anthelmintic effects and is resilient against flooding, droughts and acidic soils (Ramírez-Restrepo et al., 2006). However, birdsfoot trefoil is a slow establishing legume which normally occurs in nutrient poor systems (Fort et al., 2015). Therefore, this herb can be easily suppressed in mixtures with more competitive grasses and weeds (Heckendorn, Häring, et al., 2007; Weewer, 2021). Furthermore, the *L. cuneata* (Chinese bush-clover) showed promising anthelmintic effects, especially to treat already existing parasites rather than preventing reinfections. This herb is resistant against disease, drought and insects and it can grow best on eroded, infertile and poor soils (Lange et al., 2006). It also has a nitrogen-fixing ability (Erika et al., 2017). However, it needs to be considered if implementation of this herb is desirable as it is described as a noxious weed in parts of the US (Ohlenbusch & Bidwell, 2007). Next to that, it has low palatability and ruminants do not select to eat them in pasture. However, when fed as hay, sheep and goats do eat it (Shaik et al., 2006). Sainfoin is

* See appendix for the full interview

another nitrogen-fixing species, which flourishes under low fertilization conditions, probably in combination with a less competitive grass like tall fescue (Kirwan et al., 2007);(H. van de Sluis[*]). When implementing sainfoin in pasture, farmers need to be aware that this herb is vulnerable to trampling (H. Ploeger[*]). Therefore, grazing and mowing management should be combined (H. van de Sluis[*]). How plants can be implemented into Dutch pastures is further discussed in the chapter on self-medication.

Other herbs also seem promising, but need further research to consider in pasture application or other ways of implementing. *Hedysarium coronarium* (sulla), for example, can be easily grown in the Dutch pasture, but showed no concrete results. Only Niezen et al. (1998) mentioned anthelmintic effects of sulla, but no quantitative data were mentioned. In this case, further experiments with sulla needs to be done to confirm the anthelmintic properties (Niezen et al., 1998). Looking at *Artemisia absinthium* (wormwood), only studies on pellets from dried extracts are available which show promising anthelmintic effects in worm and faecal egg count. In addition, fresh wormwood has a low palatability, and is therefore not suitable for in pasture implementation. Furthermore, T. van Asseldonk[*] mentioned dosing of wormwood can be tricky. Herbal extracts of Hederae folium (*Hedera helix)* also show anthelmintic properties, but further studies need to be done on their anthelmintic effects when considering whole plant application. Hederae folium can outcompete other plant species, thus research needs to be done on their growth in the pasture with other plants, before implementation as a herb for ruminants to self-medicate (Ringold et al., 2008). *Cymbopogon citratus* (lemongrass) essential oil extract shows promising anthelmintic effects, especially for adult worm count reduction. It is a good candidate to add as a supportive component to herb mixtures, as it also has a positive effect on rumen health, nutrient digestibility and has a pain relief function (Kholif et al., 2020). However, to implement lemongrass in the pasture more needs to be done on the effectivity of

When looking at the herb mixtures, all 4 mixtures described in Table 4 look effective. However, these are non-commercial mixtures and therefore it is not easily available for Dutch farmers. The Chinese herb mixture Quchongsan looks promising, especially the updated recipe named Quchongxiehuosan (Table 3). This mixture is commercially available online, and the individual ingredients are available in traditional Chinese shops. However, dosages need to be strictly obeyed to maximize their effect and avoid toxicity. The anthelmintic properties of the herb mixture called bio-dewormer look promising (Table 3), but more evidence for this recipe is needed, as well as availability on the Dutch market. The knowledge on how to combine different herbs and how to dose is on the edge of extinction in the Netherlands and for most farmers and veterinarians not accessible (H. Cremer[*]).

In summary, there are many plants with anthelmintic effects against *Haemonchus contortus* which can be grown in the Dutch pasture. However, we need to be aware of their growth rate, crowding ability, fertilization needs and palatability in order to create an herb-enriched pasture for self-medication. These plants can also be fed as hay or silage which makes them storable and increases its availability throughout the year, without losing their anthelmintic properties. Moreover, herb mixtures look promising, but they are not yet easily available for the Dutch market. In addition, combining herbs and oil extracts in mixtures might be interesting to study as this practice increases the diversity of active compounds.

[*] See appendix for the full interview

Most promising herbs for *Fasciola hepatica*

For liver fluke specifically, only *Fumaria parviflora* (fineleaf fumitory) was found to fit all set criteria of a promising herb which might be applied in the pasture. This plant species was very effective in *in vivo* experiments for sheep and cows and is indigenous to Europe (Mushtaq et al., 2015; RANA, 2015). Since this herb can already be found in the Netherlands, albeit in small numbers (Floron, 2021), it may be feasible to grow this herb in the pasture, but the palatability still needs to be investigated.

Promising natural herbs which could be implemented in the feed are *F. parviflora*, *Moringa oleifera* (drumstick tree) leaves and seeds, and *Nigella sativa* (black cumin) seeds. These three herbs can be dried and used in powdered form or as extracts and could be added as supplements to the feed. *M. oleifera* and *N. sativa* are shown to be effective in extract or powdered form in sheep and buffaloes, respectively. Meanwhile, reliable results from fresh herbs, however, have not been found. Additionally, these two plants grow in tropical environments, and thus need to be imported. This means that they cannot be implemented in the Dutch pasture.

For treatment of liver fluke infection, most herbs recorded to have a significant effect on liver fluke burden are from tropical areas. Most herbs indigenous to tropical or subtropical areas are unable to grow in Dutch climate conditions. Some of these herbs, however, are already widely grown for commercial use in other countries. These herbs could thus be easier to obtain, such as *Nigella sativa* or *Moringa oleifera*. If these herbs are to be implemented, contact should be made with international dealers to obtain this herb in dried, powdered or oil form. Not all exotic plants with shown anthelmintic properties however are easily available for commercial use in the Netherlands, which makes them not feasible under current conditions. Examples of these are *Albizia anthelmintica, Balanites aegypiaca,* and *Caesalpinia crista*. Plants which are enlisted as endangered species, such as *Saussurea lappa*, are also excluded, as there are even more difficult to obtain and not sustainable.

The Chinese herbal mixture Ganzhisan showed high efficacy for treating *F. hepatica,* both in acute and chronic infections. There are already commercial products of this mixture available online in China, according to Chinese veterinarian Jiaguo Liu[1]. The mixtures are available in powder forms. In addition, the powders of each herb in this mixture could be bought from Chinese medicine store in the Netherlands, and mixed based on the given doses under instructions (Table 12).

Allium sativum (garlic), was very promising in *in vitro* studies, and is often mentioned as a useful herb for overall health. However, *in vivo* studies in sheep using garlic did not show significant reductions in parasite burden. While in *F. hepatica*'s intermediate host, the aquatic snail, garlic did show a significant decrease in parasite burden. Snail management involving application of herbs could thus also be a viable option. Another herb that was effective against liver fluke larvae in snails was *Azadirachta indica*. Both herbs only killed the larvae inside the snail and left the snails alive (Sunita & Singh, 2011). *Areca catechu* and Cuminum cyminum were found to directly target the intermediate host, stopping the life cycle of the fluke by limiting the number of available intermediate hosts. This manner of intervening in the fluke's life cycle also could be effective, but methods to kill the intermediate hosts of *Fasiola spp*. should be implemented with care, as snails are important components of an aquatic ecosystem (Jaiswal & Singh, 2008; Sunita & Singh, 2011). Finding a way to implement herbs in snail management, however, is still in development. Those beforementioned In vivo studies so far have fed the herbs directly to snails or incubated the snails with herb extracts. As of now, this method of application is not a realistic approach in the pasture, since snails will have sufficient opportunity to evade these herbs or herb extracts.

The bio-dewormer formula of (Abbas et al., 2020) was shown to be very effective *in vivo* against liver fluke. However, the availability of these different herbs on the Dutch market is uncertain. Since not all ingredients are mentioned in the study, the formula cannot easily be reproduced, which makes applicability more difficult.
Genistein, an active anthelmintic compound found in various plants, was shown to be very effective in reducing parasite burden in rabbits. Although this does seem promising, no further studies with genistein have been performed on sheep or cattle. Since the results cannot be predicted, plant species possessing a high genistein content are not included in the final list.

When assessing the activity of an herb against liver fluke, it is important to note that much more research has been done on *Fasciola gigantica* species than *Fasciola hepatica*. As *F. gigantica* only occurs in tropical and subtropical areas (Castilla Gómez de Agüero et al., 2020), most herbal remedies that are discovered for this liver fluke also only grow in (sub)tropical regions. Exotic plants are difficult to implement in the Netherlands, as they can become invasive species and pose a threat to indigenous species. On the other hand, these plants may not be able to grow in more temperate climates at all. More temperate zones that deal with *F. hepatica* do not use as many herbal remedies as in the more tropical climates. This could be because of the limited number of plant species with anthelmintic properties in temperate climates, or because of a lack of research.

Extracts vs whole herb application

In some of the studies that were consulted for this overview, extracts of plants were used, while other studies show the effects of whole plant application. An advantage of using extracts, mentioned by S.K. Kumar[*], is that dosing the remedy can be done more precisely. Furthermore, when extracts are made, the active compound of herbs can be identified. Moreover, herb extracts may be easier to transport and incorporate in commercially available products. However, plant isolates and extractions are likely more susceptible for resistance development, but further research is needed to be done to confirm this. In addition, other methods of plant extractions could give different results. Eguale et al. (2007) and Mravčáková et al. (2019) showed that alcoholic extracts had better *in vitro* effects in killing nematodes than aqueous extracts. This could be due to a difference in absorption into the body of the parasite. Thus, the type and method of extraction can have a great influence on the effectiveness of herbal medicines. An advantage of using whole plants over extracts, is that these can be grown in the pasture to allow self-medication. In addition, S. K. Kumar[*] mentioned that whole plants are more effective than their extracts.
Apart from herbs, it seems quite promising to investigate the anthelmintic effects of essential oils derived from plants. A study of Macedo et al. (2019) showed significant reduction in *H. contortus* worm load when feeding lemongrass essential oil nano-emulsion (Table 1). In addition, a study by Aquino Mesquita et al. (2013) showed that *Eucalyptus staigeriana* essential oil nano-emulsion lead to 83.7% reduction in parasite load of *H. contortu*s. It was shown in rats that a dose of lemongrass essential oils till 1000mg/kg showed no toxic effects. However, further research needs to be done to confirm these effects in ruminants. Furthermore, nano-emulsions of essential oils seem to be less toxic than the free oils of plants as nano-emulsions allow formation of nanoparticles of the chitosan matrix. This leads to a better anthelmintic activity and decreased toxicity (Macedo et al., 2019). In addition, T. van Asseldonk[*] suggests to make strong essential oil out of useful herbs, because this is easy to restore. According to Swiss vet and researcher Michael Walkenhorst[*], to reach a high effectiveness against parasites, it is important to combine compounds which act via very different mechanisms. For example, he suggested to combine oil with tannin compounds as they can have quite different modes of action.

[*] See appendix for the full interview

Herbs vs herb mixtures

Both single herbs and herb mixtures can be effective in controlling parasitic infections. Out of our results, it seems that herb mixtures are more effective than single herbs. This can be due to the synergy of herbs when mixing them (Mravčáková et al., 2019; Váradyová et al., 2017; Váradyová et al., 2018). In addition, Mravčáková et al. (2021) suggested that herb mixtures do not have a direct anthelmintic effect, but rather increase resistance of lambs against gastrointestinal nematodes. This is due to the antioxidant effect by reducing oxidative stress in the abomasal mucosa, thereby reducing inflammation and enhancing local immune response. M. Walkenhorst*, confirmed this; when using different botanical families, the diversity of the mixture can be increased further. Mixing herbs also increases the diversity of active compounds. Adding herbs that are known to have an immunoboosting effect can help to control parasitic infections both directly and indirectly. These compounds may complement each other or reduce each other's toxicity. For example, tannin rich plants and chamomile can be combined to treat diarrhoea, as the tannins reduce binding of *e. coli* to the gastrointestinal wall, while chamomile reduces inflammation. S.K. Kumar* mentioned that the balance of herb mixtures is also important for the effectivity. For example, garlic has a hot character and should be combined with a cool herb. Dairy farmer Karin Dijkstra* mentioned that garlic should be combined with parsley. Some compounds in the formula are acting, others are working synergistically or maintaining homeostasis in the formula/counteracting the negative effects of other compounds. For example, in the Chinese medical mixture which used for *F. hepatica,* betel nut is used for killing worms while another herb has function on clearing the livers. Because of the balance it has, herb mix not only cure diseases, but also maintain the general health of the animal. Therefore, it is suggested to add single herbs to the feed or plant them in pasture to increase general health of the animal and reduce parasitic infections. However, when infections are more severe and real control is needed, specific herb mixtures are advised. Although exotic herbal mixtures have better performance than single synthetic drugs in practice in their home country, they only have best efficacy under local theoretical instruction (S.K. Kumar*; J. Liu*; D. Wang*).

Self-medication

By increasing functional biodiversity, an opportunity for self-medication can be created for the grazing livestock. In this way, farmers would be able to manage parasitic infections to some degree with relatively low labour intensity (Villalba et al., 2014). Veterinarian M. Walkenhorst* mentioned that many animals are known to self-medicate. A sheep and cattle farmer from New Zealand, Chris Kennet, mentioned a case of self-medication; he had a sick cow that the vet did not know how to cure. He let the cow graze in a species rich garden. The cow started to eat from all different kind of plants and its condition subsequently improved (Personal communication, 17 April 2021).

The effects of whole herbs that have been studied in this report and could be implemented into the pasture are chicory, sainfoin, birdsfoot trefoil, *Hedera helix* and sulla. However, establishment of sainfoin and birdsfoot trefoil could be challenging, as these herbs do not fit into a competitive grass pasture (M. Walkenhorst*; H. Ploeger*). Nevertheless, a commercial seed mixture is available containing sainfoin and birdsfoot trefoil amongst other herbs and grasses. Sainfoin and birdsfoot trefoil do consist within the pasture for at least 2 years but are often the first ones to disappear (Technical specialist roughage Barenbrug, Harm van der Sluis*). Management is key to ensure herb persistence, fertilization especially should be lowered when nitrogen fixation herbs are sown. Due to the difficulties

* See appendix for the full interview

in pasture establishment, sainfoin is in the Netherlands often sown on fields that are unsuitable for grazing and made into hay or silage fed to young lambs and to ewes during and after lambing (H. Ploeger*). Generally, it could be more effective to first screen the environment on herbs occurring naturally and connect herbal implementation closely to the landscape to ensure successful establishment (T. van Asseldonk*). There is also a risk related to increasing herb density in pasture is related to animal health and productivity. Not all herbs have a medicinal and nutritional value by increasing the density of non-nutritional herbs productivity would decline (Herb specialist Hubert Cremer*). Furthermore, it should be always ensured that the animals are not pushed to eat the herbs. When being pushed they could overdose themself in the same way animals can be pushed to eat too close to manure dumps (Hutchings et al., 2003);(H. Cremer*). Therefore, the animal density and the herb density should be in balance.

For preventive application by herbal hay or silage, palatability should be considered. Palatability of for example nettle its improved in hay and silage compared to fresh nettle (Humphries & Reynolds, 2014); (H. Cremer*). However, for self-medication palatability is less of concern. For example, surveys showed that parasitized goats eat non-palatable plants that contain anthelmintic properties (Gradé, Tabuti, & Van Damme, 2009). Biological dairy farmer K. Dijkstra* also mentioned that cows sometimes eat from plants in the pasture that are not indicated as nutritious, like *Rumex acetosa* (sorrel). Therefore, a low density of unpalatable but effective plants for self-medication would already be sufficient.

It should also be considered that the herbs have their own growing cycle and are not always available throughout the year in the pasture. Furthermore, their chemical composition changes over the season (Häring et al., 2008). This makes the exact effectivity at each given moment difficult to predict. Nevertheless, by making silage and or hay anthelmintic and immune system stimulating properties are often conserved (Häring et al., 2008). The effectiveness is thus not affected, and the seasonal reliance reduced.

Chinese bush clover was seen to be promising against *H. contortus,* but it is also known to be highly invasive in the United States. Before introducing a non-native plant species its invasion risk should be considered and investigated.

Increasing functional diversity could enhance the ability of livestock to manage their parasitic infection rate via self-medication. Nevertheless, implementation of herbs can be challenging as most herbs favour a more nitrogen-poor systems. Invasion risk should be investigated before implementation of foreign plants.

Side effects

Up until now, no evidence has been found on parasitic resistance development against medicinal herbs and negative effects on soil health. Still, farmers and veterinarians need to be aware of possible resistance development against medicinal herbs. However, even as there is lack of evidence at the moment, this does not mean that resistance against herbs will never occur in the future if herbs are applied more often. Resistance against medicinal herbs may be built by parasites, but it would likely take a long time to develop. In addition, it is less likely that resistance will be generated against herb mixtures and herb-rich pastures when compared to using only one single herb (H. Ploeger*). S.K. Kumar* believes that the use of herbal extracts has a higher risk of resistance development. In contrast, it is unlikely that resistance against medicinal herbs will be developed, as herbs contain many different active compounds, especially when using mixtures of herbs (M. Walkenhorst*). Nevertheless, there is

currently not enough data on resistance development available to draw a conclusion on this topic. Once herbal remedies are implemented on a larger scale, research can be done on resistance development against medicinal herbs. In the *in vivo* studies that were consulted for this overview, no toxicity of the experimental doses of medicinal herbs was observed in the animals. However, it is important to keep in mind that if the dosages of herbs are increased, some toxic effects could occur. So, before implementing higher doses of medicinal herbs than those used in the studies, research on toxicity with that specific dose needs to be done. It is suggested that mixing several herbs will reduce toxicity, as the herbs can complement each other or even reduce each other's toxic effects (T. van Asseldonk*, M. Walkenhorst*). In addition, S. K. Kumar* mentioned that it is believed that Ayurveda formulas (traditional Indian recipes) do not have many side-effects, as most ingredients are also suitable for human consumption. However, the dose needs to be obeyed correctly (D. Wang*). In addition, veterinarian J. Liu*, confirmed that medicinal herbs are less toxic than the correspondent synthetic drugs.

Different results between *in vitro* and *in vivo*

In literature, many effective *in vitro* studies can be found regarding promising herbal remedies for internal parasites. However, the *in vivo* results of herbs found to be promising *in vitro*, differ quite a lot. Mainly, *in vivo* studies of herbs seem to be less effective than their *in vitro* practices. The difference in results between *in vitro* and *in vivo* experiments could be due to the pharmacology of host animals, experimental conditions, metabolism of bioactive compounds by microbiota in rumen or different bioavailability of enzymes (Mravčáková et al., 2020). Therefore, we need to take in mind that the effective herbs *in vitro* need further *in vivo* research on the effectiveness, palatability and application before these herbs can be implemented in the Dutch farmer's system.

Limitations of alternatives for anthelmintics

A multifactorial approach with high emphasis on pasture management and providing medicinal herbs looks promising for controlling parasitic infections. However, this approach includes some limitations, and is not easy to apply for all farmers. Namely, a questionnaire with 450 sheep farmer respondents showed that pasture management is the main bottleneck for farmers who have difficulties with parasite control. The majority of farmer respondents were willing to improve their pasture management, but were unable to do so due to limited pasture size and amount of available grass. In addition, farmers need to follow rules when their livestock is grazing on dykes or land that has also been appointed for nature preservation. This limits farmers in ensuring proper pasture rotations (Ploeger, Antonis, Verkaik, Vellema, & Bokma-Bakker, 2016). Furthermore, T. van Asseldonk* mentioned that it is impossible to supply herbs for all livestock in the Netherlands, as the number of animals is too high for that. Additionally, legislation could be a limit in the development of natural herbs. Moreover, the palatability of herbs needs to be considered. Farmers are sometimes not willing to feed effective herbs to cows, since most effective herbs are woody, but cows prefer tender plants (A. Antonis*). A. Antonis* the idea of pastures with multiversity plants, but he said that it is difficult to grow such herb-rich farms. However, difficulties in palatability and herb establishment may can be overcome which is described in the self-medication section.

* See appendix for the full interview

Recommendation

In this report, we focused on only two internal parasites, *Haemonchus contortus* and *Fasciola hepatica*. Throughout this report, we concluded that there are large amounts of literature and evidence of effective herbs studied *in vitro* and *in vivo* out there. In addition, we contacted veterinarians, farmers suffering from these specific parasites, biological farmers using herbs to control the parasites, and many experts in the field of parasites and herbs. Moreover, herbs and even mixtures of herbs all have their own specific effect against different parasites. Furthermore, the last years, there is increasing awareness of herbs being effective against parasites and more interest in the search for natural remedies to overcome parasitic resistance in livestock. Thus, there will be enough veterinarians, farmers and experts who are willing to share their experience and knowledge on this topic. Altogether, the search of natural remedies against other internal parasites could be an interesting topic for future researchers or ACT-groups to expand on.

Conclusion

Haemonchus contortus (barber's pole worm)

To conclude, firstly regarding herb application, we advise to increase species richness in pastures by implementing suitable herbs like *Lotus corniculatus* (birdsfoot trefoil), *Cichorium intybus L* (chicory), *Onobrychis viciifolia* (sainfoin). By increasing opportunities of species richness in pasture, self-medication is created. However, to ensure herb establishment, long term persistence and pasture productivity, intraspecific plant species interactions should be investigated. All herbs in this report keep their anthelmintic properties when fed as hay or silage, which increases its availability trough out the year. Furthermore, invasion risk of foreign plants like *Lespedeza cuneata* (Chinese bush-clover) needs consideration. Secondly, oil extracts of herbs, like *Cymbopogon citratus* essential oil (lemongrass) could be added to the feed. Thirdly, dried and fresh herbs could be combined into mixtures and be applied when infection rates are causing a burden to the animals. We have been able to identify promising mixtures however the mechanism is still unclear.

Fasciola hepatica (liver fluke)

All in all, when considering herbs with anthelmintic properties for liver fluke, only *Fumaria parviflora* (fineleaf fumitory) was found to be both promising and applicable to the pasture. This herb could be implemented in a herb-enriched pasture for self-medication. Because *F. parviflor*a keeps its anthelmintic properties when dried, it could also be fed as hay with other plants or herbs. Herbs that could be imported and implemented in the feed for their anthelmintic properties in dried or powdered form are *Moringa oleifera* (drumstick tree) and *Nigella sativa* (black cumin). When infection rate cause a burden to the animals, the Chinese herbal mixture Ganzhisan could be implemented to treat liver fluke. If all ingredients are obtained from Chinese medicine stores to the Netherlands, then expertise is needed to ensure safe application.

Future

For the future, we advise to do more research on the effectiveness of specific herb mixtures and synergy between plant species to treat internal parasites. In addition, we recommend looking internationally, for example at Chinese herb mixtures, especially the Quchongxiehuosan and Ganzhisan recipes, which looked promising against *Haemonchus contortus* and *Fasciola hepatica*, respectively.

References

Abbas, R. Z., Zaman, M. A., Sindhu, D., Sharif, M., Rafique, A., Saeed, Z., . . . Akram, M. S. (2020). Anthelmintic Effects and Toxicity Analysis of Herbal Dewormer against the Infection of Haemonchus contortus and Fasciola hepatica in Goat. *Pakistan Veterinary Journal, 40*(4).

Adeneye, A. A., & Agbaje, E. O. (2007). Hypoglycemic and hypolipidemic effects of fresh leaf aqueous extract of Cymbopogon citratus Stapf. in rats. *Journal of ethnopharmacology, 112*(3), 440-444.

Agatsuma, T., Arakawa, Y., Iwagami, M., Honzako, Y., Cahyaningsih, U., Kang, S.-Y., & Hong, S.-J. (2000). Molecular evidence of natural hybridization between Fasciola hepatica and F. gigantica. *Parasitology International, 49*(3), 231-238. doi:https://doi.org/10.1016/S1383-5769(00)00051-9

Ahmed, A. H., Ejo, M., Feyera, T., Regassa, D., Mummed, B., & Huluka, S. A. (2020). In vitro anthelmintic activity of crude extracts of Artemisia herba-alba and Punica granatum against Haemonchus contortus. *Journal of parasitology research, 2020*.

Akhtar, M., & Aslam, M. (1989). Field trial of Karanjwa seeds against nematodes in sheep. *Pak. J. Agric. Res, 10*, 175-178.

Akkari, H., Ezzine, O., Dhahri, S., B'chir, F., Rekik, M., Hajaji, S., . . . Gharbi, M. (2015). Chemical composition, insecticidal and in vitro anthelmintic activities of Ruta chalepensis (Rutaceae) essential oil. *Industrial Crops and Products, 74*, 745-751. doi:10.1016/j.indcrop.2015.06.008

Akkari, H., Rtibi, K., B'Chir, F., Rekik, M., Darghouth, M. A., & Gharbi, M. (2014). In vitro evidence that the pastoral Artemisia campestris species exerts an anthelmintic effect on Haemonchus contortus from sheep. *Veterinary research communications, 38*(3), 249-255. doi:10.1007/s11259-014-9609-y

Al-Shaibani, I., Phulan, M., & Shiekh, M. (2009). Anthelmintic activity of Fumaria parviflora (Fumariaceae) against gastrointestinal nematodes of sheep. *Int J Agric Biol, 11*, 431-436.

Aliyi Hassen, A., Mebrat, E., Teka, F., Dereje, R., Bahar, M., & Solomon Assefa, H. (2020). In Vitro Anthelmintic Activity of Crude Extracts of Artemisia herba-alba and Punica granatum against Haemonchus contortus. *Journal of parasitology research*. doi:10.1155/2020/4950196

Amudhan, M. S., Begum, V. H., & Hebbar, K. (2012). A review on phytochemical and pharmacological potential of Areca catechu L. seed. *International journal of pharmaceutical sciences and research, 3*(11), 4151.

Anderson, T. J., Blouin, M. S., & Beech, R. N. (1998). Population biology of parasitic nematodes: applications of genetic markers. *Advances in parasitology, 41*, 219-283.

Aquino Mesquita, M., Silva Júnior, J. o., Panassol, A. M., Oliveira, E. F., Vasconcelos, A. L., Paula, H. C., & Bevilaqua, C. M. (2013). Anthelmintic activity of Eucalyptus staigeriana encapsulated oil on sheep gastrointestinal nematodes. *Parasitology research., 112*(9), 3161-3165.

Arun, N., & Singh, D. (2012). Punica granatum: a review on pharmacological and therapeutic properties. *International journal of pharmaceutical sciences and research, 3*(5), 1240.

Asif, M. (2012). Antimicrobial potential of Azadirachta indica against pathogenic bacteria and fungi. *Journal of Pharmacognosy and Phytochemistry, 1*(4), 78-83.

Athanasiadou, S., Gray, D., Younie, D., Tzamaloukas, O., Jackson, F., & Kyriazakis, I. (2007). The

use of chicory for parasite control in organic ewes and their lambs. *Parasitology, 134*(2), 299.

Barros, L., Carvalho, A. M., & Ferreira, I. C. (2010). Leaves, flowers, immature fruits and leafy flowered stems of Malva sylvestris: a comparative study of the nutraceutical potential and composition. *Food and Chemical Toxicology, 48*(6), 1466-1472.

Bassiri-Jahromi, S. (2018). Punica granatum (Pomegranate) activity in health promotion and cancer prevention. *Oncology reviews, 12*(1).

Beesley, N. J., Williams, D. J., Paterson, S., & Hodgkinson, J. (2017a). Fasciola hepatica demonstrates high levels of genetic diversity, a lack of population structure and high gene flow: possible implications for drug resistance. *International Journal for Parasitology, 47*(1), 11-20.

Beesley, N. J., Williams, D. J., Paterson, S., & Hodgkinson, J. (2017b). Fasciola hepatica demonstrates high levels of genetic diversity, a lack of population structure and high gene flow: possible implications for drug resistance. *Int J Parasitol, 47*(1), 11-20. doi:10.1016/j.ijpara.2016.09.007

Besier, R., Kahn, L., Sargison, N., & Van Wyk, J. (2016a). The pathophysiology, ecology and epidemiology of Haemonchus contortus infection in small ruminants. *Advances in parasitology, 93*, 95-143.

Besier, R. B., Kahn, L. P., Sargison, N. D., & Van Wyk, J. A. (2016b). Chapter Four - The Pathophysiology, Ecology and Epidemiology of Haemonchus contortus Infection in Small Ruminants. In R. B. Gasser & G. V. Samson-Himmelstjerna (Eds.), *Advances in parasitology* (Vol. 93, pp. 95-143): Academic Press.

Bhat, R. R., Rehman, M. U., Shabir, A., Mir, M. U. R., Ahmad, A., Khan, R., . . . Ganaie, M. A. (2019). Chemical composition and biological uses of Artemisia absinthium (wormwood). In *Plant and Human Health, Volume 3* (pp. 37-63): Springer.

Blanco, M., Costa, C., Freire, A., Santos Jr, J., & Costa, M. (2009). Neurobehavioral effect of essential oil of Cymbopogon citratus in mice. *Phytomedicine, 16*(2-3), 265-270.

Blouin, M. S., Yowell, C. A., Courtney, C. H., & Dame, J. B. (1995). Host movement and the genetic structure of populations of parasitic nematodes. *Genetics, 141*(3), 1007-1014.

Boonmasawai, S., Sungpradit, S., Jirapattharasate, C., Nakthong, C., & Piasai, L. (2013). Effects of alcoholic extract from pomegranate (Punica granatum L.) peels on gastrointestinal nematode egg counts in doe.

Bowman, D. D. (1999). Georgis' parasitology for veterinarians. 414 pp. *Saunders. London, WB*.

Brooker, J., O'donovan, L., Skene, I., Clarke, K., Blackall, L., & Muslera, P. (1994). Streptococcus caprinus sp. nov., a tannin-resistant ruminal bacterium from feral goats. *Letters in Applied Microbiology, 18*(6), 313-318.

Canadanovic-Brunet, J. M., Djilas, S. M., Cetkovic, G. S., & Tumbas, V. T. (2005). Free-radical scavenging activity of wormwood (Artemisia absinthium L) extracts. *Journal of the Science of Food and Agriculture, 85*(2), 265-272.

Carbonero, C. H., Mueller-Harvey, I., Brown, T. A., & Smith, L. (2011). Sainfoin (Onobrychis viciifolia): a beneficial forage legume. *Plant Genetic Resources, 9*(1), 70.

Castilla Gómez de Agüero, V., Luka, J., Gandasegui, J., Valderas-García, E., Ajanusi, O. J., Chiezey, N. P., & Martínez-Valladares, M. (2020). Fasciola hepatica and Fasciola gigantica coexistence in domestic ruminants in Nigeria: application of a PCR-based tool. *Tropical Animal Health and Production, 52*(6), 3893-3897. doi:10.1007/s11250-020-02333-3

Castro-Arnáez, I. C., Montenegro, V. M., Vargas-Leitón, B., Álvarez-Calderón, V., & Soto-Barrientos, N. (2021). Anthelmintic resistance in commercial sheep farms in Costa Rica.

Vet Parasitol Reg Stud Reports, 23, 100506. doi:10.1016/j.vprsr.2020.100506
Chiclana, C. F., Enrique, A., & Consolini, A. E. (2009). Topical antiinflammatory activity of Malva sylvestris L.(Malvaceae) on carragenin-induced edema in rats. *Latin American Journal of Pharmacy, 28*(2), 275-278.
Colvin, A. F., Walkden-Brown, S. W., Knox, M. R., & Scott, J. M. (2008). Intensive rotational grazing assists control of gastrointestinal nematodosis of sheep in a cool temperate environment with summer-dominant rainfall. *Vet Parasitol, 153*(1-2), 108-120. doi:10.1016/j.vetpar.2008.01.014
Costa, C., Bevilaqua, C., Maciel, M., Camurça-Vasconcelos, A., Morais, S., Monteiro, M., . . . Souza, M. (2006). Anthelmintic activity of Azadirachta indica A. Juss against sheep gastrointestinal nematodes. *Veterinary parasitology, 137*(3-4), 306-310.
Coykendall, K. E., & Houseman, G. R. (2014). Lespedeza cuneata invasion alters soils facilitating its own growth. *Biological invasions, 16*(8), 1735-1742.
da Silva, M. A. M. P., Zehetmeyr, F. K., Pereira, K. M., Pacheco, B. S., Freitag, R. A., Pinto, N. B., . . . Berne, M. E. A. (2020). Ovicidal in vitro activity of the fixed oil of Helianthus annus L. and the essential oil of Cuminum cyminum L. against Fasciola hepatica (Linnaeus, 1758). *Experimental Parasitology, 218*, 107984.
Darakhshan, S., Bidmeshki Pour, A., Hosseinzadeh Colagar, A., & Sisakhtnezhad, S. (2015). Thymoquinone and its therapeutic potentials. *Pharmacological Research, 95-96*, 138-158. doi:https://doi.org/10.1016/j.phrs.2015.03.011
Das, B., Srinivas, Y., Sudhakar, C., Mahender, I., Laxminarayana, K., Reddy, P. R., . . . Rao, J. V. (2010). New diterpenoids from Caesalpinia species and their cytotoxic activity. *Bioorganic & medicinal chemistry letters, 20*(9), 2847-2850.
Dausch, J. G., & Nixon, D. W. (1990). Garlic: a review of its relationship to malignant disease. *Preventive Medicine, 19*(3), 346-361.
Debella, A. (2002). Manual for phytochemical screening of medicinal plants. *Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia*, 35-47.
Dery, B., & Otsyina, R. (2000). The 10 priority medicinal trees of Shinyanga, Tanzania. *Agroforestry Today, 12*(1), 5-8.
Desta, B. (1995). Ethiopian traditional herbal drugs. Part I: Studies on the toxicity and therapeutic activity of local taenicidal medications. *Journal of ethnopharmacology, 45*(1), 27-33.
Di Trana, A., Bonanno, A., Cecchini, S., Giorgio, D., Di Grigoli, A., & Claps, S. (2015). Effects of Sulla forage (Sulla coronarium L.) on the oxidative status and milk polyphenol content in goats. *Journal of dairy science, 98*(1), 37-46.
Diao, X., Jensen, J., & Hansen, A. D. (2007). Toxicity of the anthelmintic abamectin to four species of soil invertebrates. *Environmental Pollution, 148*(2), 514-519.
Dingniu, M. (2021 viewed). Quchongsan. Retrieved from http://www.lfdzkj.cn/zhuyao/qiuchong/1319.html
Divakara Sastry, E., & Anandara, J. (2013). Cumin, fennel and fenugreek. *Soils, plant growth and crop production. Encyclopedia of Life Support Systems (EOLSS)*.
Dixon, K. E. (1964). THE RELATIVE SUITABILITY OF SHEEP AND CATTLE AS HOSTS FOR THE LIVER FLUKE, FASCIOLA HEPATICA L. *J Helminthol, 38*, 203-212. doi:10.1017/s0022149x00033782
Eekeren, N. v., & Eysker, M. (2004). Voorkómen van maagdarmworminfecties bij biologische geiten : de mogelijke beheersingsstrategieën. *Ekoland : vakblad voor biologische landbouwmethoden, verwerking, afzet en natuurvoeding 24 (7/8): 26 - 27*. Retrieved

from https://edepot.wur.nl/110983
Eguale, T., Tilahun, G., Debella, A., Feleke, A., & Makonnen, E. (2007). Haemonchus contortus: In vitro and in vivo anthelmintic activity of aqueous and hydro-alcoholic extracts of Hedera helix. *Experimental Parasitology, 116*(4), 340-345. doi:10.1016/j.exppara.2007.01.019
El Shanawany, E. E., Fouad, E. A., Keshta, H. G., Hassan, S. E., Hegazi, A. G., & Abdel-Rahman, E. H. (2019). Immunomodulatory effects of Moringa oleifera leaves aqueous extract in sheep naturally co-infected with Fasciola gigantica and Clostridium novyi. *Journal of Parasitic Diseases, 43*(4), 583-591.
Erika, A. v. Z., Francien, S. B., Kobus, J. N. E., Phumzile, P. M., Peter, A. O., & Cathy, S. (2017). The use of Lespedeza cuneata for natural control of gastrointestinal nematodes in Merino sheep. *Onderstepoort Journal of Veterinary Research, 84*(1), 1-7. doi:10.4102/ojvr.v84i1.1259
Fairweather, I. (2011). Reducing the future threat from (liver) fluke: realistic prospect or quixotic fantasy? *Veterinary parasitology, 180*(1-2), 133-143.
Fairweather, I., Brennan, G. P., Hanna, R. E. B., Robinson, M. W., & Skuce, P. J. (2020). Drug resistance in liver flukes. *Int J Parasitol Drugs Drug Resist, 12*, 39-59. doi:10.1016/j.ijpddr.2019.11.003
Floron, N. (2021). *Fumaria parviflora* verspreidingsatlas. Retrieved from https://www.verspreidingsatlas.nl/5635
Forbes, A. B. (2020). *Parasites of Cattle and Sheep: A Practical Guide to Their Biology and Control*: CABI.
Fort, F., Cruz, P., Catrice, O., Delbrut, A., Luzarreta, M., Stroia, C., & Jouany, C. (2015). Root functional trait syndromes and plasticity drive the ability of grassland Fabaceae to tolerate water and phosphorus shortage. *Environmental and Experimental Botany, 110*, 62-72.
Githiori, J. B. (2004). *Evaluation of anthelmintic properties of ethnoveterinary plant preparations used as livestock dewormers by pastoralists and small holder farmers in Kenya* (Vol. 173).
Githiori, J. B., Höglund, J., Waller, P. J., & Baker, R. L. (2003). The anthelmintic efficacy of the plant, Albizia anthelmintica, against the nematode parasites Haemonchus contortus of sheep and Heligmosomoides polygyrus of mice. *Vet Parasitol, 116*(1), 23-34. doi:10.1016/s0304-4017(03)00218-8
Goldstein, J. L., & Swain, T. (1963). Changes in tannins in ripening fruits. *Phytochemistry, 2*(4), 371-383.
Goodenough, A. E., Webb, J. C., & Yardley, J. (2019). Environmentally-realistic concentrations of anthelmintic drugs affect survival and motility in the cosmopolitan earthworm Lumbricus terrestris (Linnaeus, 1758). *Applied Soil Ecology, 137*, 87-95.
Gradé, J., Tabuti, J. R., & Van Damme, P. (2009). Four footed pharmacists: indications of self-medicating livestock in Karamoja, Uganda. *Economic Botany, 63*(1), 29-42.
Gradé, J. T., & Longok, A. (2000). Karamojong scientists: participatory field trial of a local dewormer.
Gradé, J. T., Tabuti, J. R., Van Damme, P., & Arble, B. L. (2007). Deworming efficacy of Albizia anthelmintica in Uganda: preliminary findings. *African Journal of Ecology, 45*, 18-20.
Hall, J. B. (1992). Ecology of a key African multipurpose tree species, Balanites aegyptiaca (Balanitaceae): the state-of-knowledge. *Forest Ecology and Management, 50*(1-2), 1-30.

Haque, A. N. M. A., Remadevi, R., & Naebe, M. (2018). Lemongrass (Cymbopogon): a review on its structure, properties, applications and recent developments. *Cellulose, 25*(10), 5455-5477.

Häring, D., Scharenberg, A., Heckendorn, F., Dohme, F., Lüscher, A., Maurer, V., . . . Hertzberg, H. (2008). Tanniferous forage plants: agronomic performance, palatability and efficacy against parasitic nematodes in sheep. *Renewable Agriculture and Food Systems*, 19-29.

Hayashi, K., Ichikawa-Seki, M., Mohanta, U. K., Shoriki, T., Chaichanasak, P., & Itagaki, T. (2017). Hybrid origin of Asian aspermic Fasciola flukes is confirmed by analyzing two single-copy genes, pepck and pold. *Journal of Veterinary Medical Science, advpub*. doi:10.1292/jvms.17-0406

Heckendorn, F., Häring, D. A., Maurer, V., Senn, M., & Hertzberg, H. (2007). Individual administration of three tanniferous forage plants to lambs artificially infected with Haemonchus contortus and Cooperia curticei. *Veterinary parasitology, 146*(1-2), 123-134.

Heckendorn, F., Häring, D. A., Maurer, V., Senn, M., & Hertzberg, H. (2007). Individual administration of three tanniferous forage plants to lambs artificially infected with Haemonchus contortus and Cooperia curticei. *Veterinary parasitology, 146*(1), 123-134. doi:10.1016/j.vetpar.2007.01.009

Heckendorn, F., Häring, D. A., Maurer, V., Zinsstag, J., Langhans, W., & Hertzberg, H. (2006). Effect of sainfoin (Onobrychis viciifolia) silage and hay on established populations of Haemonchus contortus and Cooperia curticei in lambs. *Veterinary parasitology, 142*(3-4), 293-300.

Hegazi, A. G., Megeed, K. N. A., Hassan, S. E., Abdelaziz, M. M., Toaleb, N. I., Shanawany, E. E. E., & Aboelsoued, D. (2018). Comparative ovicidal activity of Moringa oleifera leaf extracts on Fasciola gigantica eggs. *Vet World, 11*(2), 215-220. doi:10.14202/vetworld.2018.215-220

Hodžić, A., Zuko, A., Avdić, R., Alić, A., Omeragić, J., & Jažić, A. (2013). Influence of Fasciola hepatica on serum biochemical parameters and vascular and biliary system of sheep liver. *Iranian journal of parasitology, 8*(1), 92.

Hördegen, P., Hertzberg, H., Heilmann, J., Langhans, W., & Maurer, V. (2003). The anthelmintic efficacy of five plant products against gastrointestinal trichostrongylids in artificially infected lambs. *Veterinary parasitology, 117*(1-2), 51-60.

Hoskin, S., Barry, T., Wilson, P., Charleston, W., & Hodgson, J. (1999). Effects of reducing anthelmintic input upon growth and faecal egg and larval counts in young farmed deer grazing chicory (Cichorium intybus) and perennial ryegrass (Lolium perenne)/white clover (Trifolium repens) pasture. *The Journal of Agricultural Science, 132*(3), 335-345.

Hoste, H., Martinez-Ortiz-De-Montellano, C., Manolaraki, F., Brunet, S., Ojeda-Robertos, N., Fourquaux, I., . . . Sandoval-Castro, C. A. (2012). Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections. *Veterinary parasitology, 186*(1), 18-27. doi:https://doi.org/10.1016/j.vetpar.2011.11.042

Hoste, H., Torres-Acosta, J. F. J., Sandoval-Castro, C. A., Mueller-Harvey, I., Sotiraki, S., Louvandini, H., . . . Terrill, T. H. (2015). Tannin containing legumes as a model for nutraceuticals against digestive parasites in livestock. *Veterinary parasitology, 212*(1), 5-17. doi:https://doi.org/10.1016/j.vetpar.2015.06.026

HPRA. (2013). Use of Flukicides in Dairy Cows. Retrieved from https://www.hpra.ie/homepage/veterinary/safety-information/safety-notices/item?t=/use-of-flukicides-in-dairy-cows&id=a5d4f825-9782-6eee-9b55-

ff00008c97d0
Hsueh, Y. P., Mahanti, P., Schroeder, F. C., & Sternberg, P. W. (2013). Nematode-trapping fungi eavesdrop on nematode pheromones. *Current biology : CB, 23*(1), 83-86. doi:10.1016/j.cub.2012.11.035
Humphries, D. J., & Reynolds, C. K. (2014). The effect of adding stinging nettle (Urtica dioica) haylage to a total mixed ration on performance and rumen function of lactating dairy cows. *Animal Feed Science and Technology, 189*, 72-81.
Hutchings, M. R., Athanasiadou, S., Kyriazakis, I., & Gordon, I. J. (2003). Can animals use foraging behaviour to combat parasites? *The Proceedings of the Nutrition Society, 62*(2), 361-370.
Ibekwe, H. (2019). In vitro anthelmintic activities of aqueous crude extract of Azadirachta indica on Paramphistomum cervi and Fasciola hepatica. *International Journal of Veterinary Sciences and Animal Husbandry, 4*(1), 14-18.
Ichikawa, M., Bawn, S., Maw, N. N., Htun, L. L., Thein, M., Gyi, A., . . . Itagaki, T. (2011). Characterization of Fasciola spp. in Myanmar on the basis of spermatogenesis status and nuclear and mitochondrial DNA markers. *Parasitology International, 60*(4), 474-479. doi:https://doi.org/10.1016/j.parint.2011.08.007
Ijaz, M., Khan, M., Avais, M., Ashraf, K., Ali, M., & Khan, M. (2009). Infection rate and chemotherapy of various helminthes in diarrhoeic sheep in and around Lahore. *J Anim Plant Sci, 19*(1), 13-16.
Jabeen, N., Anwar, S., Mahmood, Q., Zia, M. A., & Murtaza, G. (2015). In vitro anthelmintic efficacy of native plants against haemonchus contortus. *Acta poloniae pharmaceutica, 72*(5), 1051-1055.
Jackson, J. A., Friberg, I. M., Little, S., & Bradley, J. E. (2009). Review series on helminths, immune modulation and the hygiene hypothesis: immunity against helminths and immunological phenomena in modern human populations: coevolutionary legacies? *Immunology, 126*(1), 18-27. doi:10.1111/j.1365-2567.2008.03010.x
Jaiswal, P., Kumar, P., Singh, V., & Singh, D. (2011). Areca catechu L.: A Valuable Herbal Medicine Against Different. *Research Journal of Medicinal Plant, 5*(2), 145-152.
Jaiswal, P., & Singh, D. (2008). Molluscicidal activity of Carica papaya and Areca catechu against the freshwater snail Lymnaea acuminata. *Veterinary parasitology, 152*(3-4), 264-270.
Jameel, M., Ali, A., & Ali, M. (2014). New phytoconstituents from the aerial parts of Fumaria parviflora Lam. *Journal of advanced pharmaceutical technology & research, 5*(2), 64.
Jayaprakash, A. (2017). Punica granatum: A Review on Phytoche. *Journal of Academia and Industrial Research, 5*(9).
Jensen, J., Diao, X., & Hansen, A. D. (2009). Single-and two-species tests to study effects of the anthelmintics ivermectin and morantel and the coccidiostatic monensin on soil invertebrates. *Environmental Toxicology and Chemistry: An International Journal, 28*(2), 316-323.
Jeyathilakan, N., Murali, K., Anandaraj, A., & Abdul Basith, S. (2010). In vitro evaluation of anthelmintic property of herbal plants against Fasciola gigantica. *Indian Journal of Animal Sciences, 80*(11), 1070.
Junkuszew, A., Milerski, M., Bojar, W., Szczepaniak, K., Le Scouarnec, J., Tomczuk, K., . . . Bracik, K. (2015). Effect of various antiparasitic treatments on lamb growth and mortality. *Small ruminant research, 123*(2), 306-313. doi:https://doi.org/10.1016/j.smallrumres.2014.11.019
Kailani, S., Akhtar, M., & Ashraf, M. (1995). Antifasciolic efficacy of indigenous plant drugs:

Kalonji, Shahterah and Karanjwa in buffaloes. *Pakistan journal of pharmaceutical sciences, 8*, 17-27.

Kandil, O. M., Hassan, N. M., Sedky, D., Ata, E. B., Nassar, S. A., Shalaby, H. A., . . . Gabrashanska, M. (2018). Anthelmintic efficacy of Moringa oleifera seed methanolic extract against Fasciola hepatica. *Journal of Parasitic Diseases, 42*(3), 391-401.

Kaplan, R. M. (2001). Fasciola hepatica: a review of the economic impact in cattle and considerations for control. *Vet Ther, 2*(1), 40-50.

Kaplan, R. M. (2004). Drug resistance in nematodes of veterinary importance: a status report. *Trends in parasitology, 20*(10), 477-481.

Kaplan, R. M., & Vidyashankar, A. N. (2012). An inconvenient truth: global worming and anthelmintic resistance. *Veterinary parasitology, 186*(1-2), 70-78.

Kaufman, P. B., Duke, J. A., Brielmann, H., Boik, J., & Hoyt, J. E. (1997). A comparative survey of leguminous plants as sources of the isoflavones, genistein and daidzein: implications for human nutrition and health. *J Altern Complement Med, 3*(1), 7-12. doi:10.1089/acm.1997.3.7

Kaur, A., Kaur, P. K., Singh, S., & Singh, I. P. (2014). Antileishmanial compounds from Moringa oleifera Lam. *Zeitschrift für Naturforschung C, 69*(3-4), 110-116.

Keshava Bhat, S., Mythri, S., & Ashwin, D. (2016). Anthelmintic property of arecanut (Areca catechu Linn.)-A review.

Kholif, A. E., Matloup, O. H., Hadhoud, F. I., Kassab, A. Y., Adegbeye, M. J., & Hamdon, H. A. (2020). Lemongrass supplementation to Farafra ewes improved feed utilization, lactational performance and milk nutritive value in the subtropics. *Animal Biotechnology*, 1-10.

Kirwan, L., Lüscher, A., Sebastià, M. T., Finn, J. A., Collins, R. P., Porqueddu, C., . . . Coran, C. (2007). Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology, 95*(3), 530-539.

Knubben-Schweizer, G., Rüegg, S., Torgerson, P. R., Rapsch, C., Grimm, F., Hässig, M., . . . Braun, U. (2010). Control of bovine fasciolosis in dairy cattle in Switzerland with emphasis on pasture management. *The Veterinary Journal, 186*(2), 188-191. doi:https://doi.org/10.1016/j.tvjl.2009.08.003

Knubben-Schweizer, G., & Torgerson, P. R. (2015). Bovine fasciolosis: control strategies based on the location of Galba truncatula habitats on farms. *Vet Parasitol, 208*(1-2), 77-83. doi:10.1016/j.vetpar.2014.12.019

Köhler, P. (2001). The biochemical basis of anthelmintic action and resistance. *International Journal for Parasitology, 31*(4), 336-345.

Koko, W., Galal, M., & Khalid, H. (2000). Fasciolicidal efficacy of Albizia anthelmintica and Balanites aegyptiaca compared with albendazole. *Journal of ethnopharmacology, 71*(1-2), 247-252.

Kothari, Lee, Niu, K., & Kim. (2019). The Genus Allium as Poultry Feed Additive: A Review. *Animals, 9*, 1032. doi:10.3390/ani9121032

Kuniyal, C. P., Rawat, Y. S., Oinam, S. S., Kuniyal, J. C., & Vishvakarma, S. C. (2005). Kuth (Saussurea lappa) cultivation in the cold desert environment of the Lahaul valley, northwestern Himalaya, India: arising threats and need to revive socio-economic values. *Biodiversity & Conservation, 14*(5), 1035-1045.

Lakshmi, T., Krishnan, V., Rajendran, R., & Madhusudhanan, N. (2015). Azadirachta indica: A herbal panacea in dentistry–An update. *Pharmacognosy reviews, 9*(17), 41.

Lange, K. C., Olcott, D. D., Miller, J. E., Mosjidis, J. A., Terrill, T. H., Burke, J. M., & Kearney, M.

T. (2006). Effect of sericea lespedeza (Lespedeza cuneata) fed as hay, on natural and experimental Haemonchus contortus infections in lambs. *Veterinary parasitology, 141*(3-4), 273-278. doi:10.1016/j.vetpar.2006.06.001

Le Jambre, L. F. (1995). Relationship of blood loss to worm numbers, biomass and egg production in Haemonchus infected sheep. *International Journal for Parasitology, 25*(3), 269-273. doi:https://doi.org/10.1016/0020-7519(94)00118-8

Li, F., Pei, L., Yang, B., Lu, Y., Hou, Y., Wang, H., & Zhang, Z. (2016). A Clinical Trial of Effect of Micron-sized Traditional Mongolian Veterinary Medicine " Quchongxiehuosan " on Parasitic Infection of Sheep. *Animal Husbandry and Feed Science, 37*(1), 42-42.

Li, G., & Kemp, P. D. (2005). Forage chicory (Cichorium intybus L.): a review of its agronomy and animal production. *Advances in agronomy, 88*, 187-222.

Li, S.-J., Zhang, D.-X., Li, L., & Chen, Z.-Y. (2004). Pollination ecology of Caesalpinia crista (Leguminosae: Caesalpinioideae). *Acta botanica sinica, 26*, 271-278.

Lindberg, A., & Vatta, A. (2006). Managing anthelmintic resistance in small ruminant livestock of resource-poor farmers in South Africa. *Journal of the South African Veterinary Association, 77*(1), 2-8.

Liu, J., Fan, G., Chen, J., & Zhong, H. (2011). Trial of Chinese Medicine Quchongjingsan on Repelling Parasites in the Digestive Tract of Sheep. *Heilongjiang Animal Husbandry and Veterinary, 6*.

Londhe, V. (2011). Role of garlic (Allium sativum) in various diseases: An overview. *angiogenesis, 12*, 13.

Lonngren, K. J., Barone, C. D., Zajac, A. M., Brown, R. N., Reed, J. D., Krueger, C. G., & Petersson, K. H. (2020). Effect of birdsfoot trefoil cultivars on exsheathment of Haemonchus contortus in fistulated sheep. *Veterinary parasitology, 287*, 109271. doi:10.1016/j.vetpar.2020.109271

Macedo, I. T. F., Oliveira, L. M. B. d., André, W. P. P., Araújo Filho, J. V. d., Santos, J. M. L. d., Rondon, F. C. M., . . . Paula, H. C. B. d. (2019). Anthelmintic effect of Cymbopogon citratus essential oil and its nanoemulsion on sheep gastrointestinal nematodes. *Revista Brasileira de Parasitologia Veterinária, 28*(3), 522-527.

Machado Pereira da Silva, M. A., Zehetmeyr, F. K., Pereira, K. M., Pacheco, B. S., Freitag, R. A., Pinto, N. B., . . . da Silva Nascente, P. (2020). Ovicidal in vitro activity of the fixed oil of Helianthus annus L. and the essential oil of Cuminum cyminum L. against Fasciola hepatica (Linnaeus, 1758). *Experimental Parasitology, 218*, 107984. doi:https://doi.org/10.1016/j.exppara.2020.107984

Machen, R. V., Craddock, F., Craig, T., & Fuchs, T. W. (1998). A Haemonchus contortus management plan for sheep and goats in Texas. *Texas FARMER Collection*.

Manvitha, K., & Bidya, B. (2014). Review on pharmacological activity of Cymbopogon citratus. *International Journal of Herbal Medicine, 1*(6), 07-07.

Maqbool, A., Hayat, C., & Tanveer, A. (2004). Comparative efficacy of various indigenous and allopathic drugs against fasciolosis in buffaloes. *Veterinarski Arhiv, 74*.

Marley, C. L., Cook, R., Keatinge, R., Barrett, J., & Lampkin, N. H. (2003). The effect of birdsfoot trefoil (Lotus corniculatus) and chicory (Cichorium intybus) on parasite intensities and performance of lambs naturally infected with helminth parasites. *Veterinary parasitology, 112*(1), 147-155. doi:https://doi.org/10.1016/S0304-4017(02)00412-0

Martin, R., Robertson, A., & Bjorn, H. (1997). Target sites of anthelmintics. *Parasitology, 114*(7), 111-124.

Mata-Padrino, D. J., Belesky, D. P., Crawford, C. D., Walsh, B., MacAdam, J. W., & Bowdridge, S.

A. (2019). Effects of grazing birdsfoot trefoil-enriched pasture on managing Haemonchus contortus infection in Suffolk crossbred lambs. *Journal of Animal Science, 97*(1), 172-183. doi:10.1093/jas/sky405

McLeod, R. (2004). Economic impact of worm infections in small ruminants in South East Asia, India and Australia. *Worm control for small ruminants in tropical Asia, 23*.

Mehlhorn, H. (2016). *Animal parasites : diagnosis, treatment, prevention* [1 online resource (xvii, 719 pages) : illustrations (some color)]. doi:10.1007/978-3-319-46403-9

Meijer, F., & Visch, J. (1995). Praktische ervaring met gras/klaver weiden. *Praktijkonderzoek/Praktijkonderzoek Rundvee, Schapen en Paarden (PR), Waiboerhoeve, 8*(5), 20-21.

Min, B., & Hart, S. (2003). Tannins for suppression of internal parasites. *Journal of Animal Science, 81*(14_suppl_2), E102-E109.

Min, B. R., Pomroy, W. E., Hart, S. P., & Sahlu, T. (2004). The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing wether goats. *Small ruminant research, 51*(3), 279-283.

Mirghani, M., Liyana, Y., & Parveen, J. (2012). Bioactivity analysis of lemongrass (Cymbopogan citratus) essential oil. *International Food Research Journal, 19*(2), 569.

Molan, A. L., Duncan, A. J., Barry, T. N., & McNabb, W. C. (2003). Effects of condensed tannins and crude sesquiterpene lactones extracted from chicory on the motility of larvae of deer lungworm and gastrointestinal nematodes. *Parasitology International, 52*(3), 209-218.

Morley, F. H. W., & Donald, A. D. (1980). Farm management and systems of helminth control. *Veterinary parasitology, 6*(1), 105-134. doi:https://doi.org/10.1016/0304-4017(80)90040-0

Mravčáková, D., Komáromyová, M., Babják, M., Urda Dolinská, M., Königová, A., Petrič, D., . . . Váradyová, Z. (2020). Anthelmintic Activity of Wormwood (Artemisia absinthium L.) and Mallow (Malva sylvestris L.) against Haemonchus contortus in Sheep. *Animals : an open access journal from MDPI, 10*(2). doi:10.3390/ani10020219

Mravčáková, D., Sobczak-Filipiak, M., Váradyová, Z., Kucková, K., Čobanová, K., Maršík, P., . . . Várady, M. (2021). Effect of Artemisia absinthium and Malva sylvestris on Antioxidant Parameters and Abomasal Histopathology in Lambs Experimentally Infected with Haemonchus contortus. *Animals : an open access journal from MDPI, 11*(2). doi:10.3390/ani11020462

Mravčáková, D., Váradyová, Z., Kopčáková, A., Čobanová, K., Grešáková, Ľ. r., Kišidayová, S., . . . Várady, M. n. (2019). Natural chemotherapeutic alternatives for controlling of haemonchosis in sheep. *BMC Veterinary Research, 15*(1), 1-13. doi:10.1186/s12917-019-2050-2

Mulcahy, G., O'Connor, F., McGonigle, S., Dowd, A., Clery, D. G., Andrews, S. J., & Dalton, J. P. (1998). Correlation of specific antibody titre and avidity with protection in cattle immunized against Fasciola hepatica. *Vaccine, 16*(9-10), 932-939. doi:10.1016/s0264-410x(97)00289-2

Murdiati, T. B., McSweeney, C. S., & Lowry, J. (1991). Complexing of toxic hydrolysable tannins of yellow-wood (Terminalia oblongata) and harendong (Clidemia hirta) with reactive substances: An approach to preventing toxicity. *Journal of Applied Toxicology, 11*(5), 333-338.

Mushtaq, A., Ahmad, N., Akbar, H., Durrani, A. Z., Waqas, M., Nawaz, M., . . . Hussain, A. (2015). Therapeutic Efficacies of Fumaria parviflora, Caesalpinia crista and Oxyclozanide

against Fasciolosis in Naturally Infected Sheep in Rawalakot-Azad Jammu and Kashmir, Pakistan. *World Journal of Zoology, 10*(2), 118-123.

Nassef, N., El-Kersh, W., El Sobky, M., Harba, N., & El Refai Khalil, S. (2014). In-vitro and in-vivo assessment of the effect of soybean extract on Fasciola gigantica infection in comparison with triclabendazole. *Menoufia Medical Journal, 27*(1), 93-102. doi:10.4103/1110-2098.132768

Negrelle, R., & Gomes, E. (2007). Cymbopogon citratus (DC.) Stapf: chemical composition and biological activities. *Revista Brasileira de Plantas Medicinais, 9*(1), 80-92.

Niezen, J. H., Robertson, H. A., Waghorn, G. C., & Charleston, W. A. G. (1998). Production, faecal egg counts and worm burdens of ewe lambs which grazed six contrasting forages. *Veterinary parasitology, 80*(1), 15-27. doi:10.1016/S0304-4017(98)00202-7

Nin, S., & Bennici, A. (2001). Transgenic Artemisia (Wormwood). In *Transgenic Crops III* (pp. 27-42): Springer.

Nirala, R. K. (2019). Medicinal plants and its activity against helminth: A review. *Journal of Pharmacognosy and Phytochemistry, 8*(5), 2348-2355.

Nkunya, M., Weenen, H., & Bray, D. (1990). *Chemical Evaluation of Tanzanian medicinal plants for the active constituents as a basis for the medicinal usefulness of the plants.* Paper presented at the Proceedings of International Conference on Traditional Medicinal Plants. Arusha.

Ohlenbusch, P. D., & Bidwell, T. G. (2007). *Sericea lespedeza: history, characteristics, and identification*: Agricultural Experiment Station and Cooperative Extension Service, Kansas ….

Olorunnisola, S., Asiyanbi, H., Hammed, A., & Simsek, S. (2014). Biological properties of lemongrass: An overview. *International Food Research Journal, 21*(2), 455.

Pena-Espinoza, M., Thamsborg, S. M., Desrues, O., Hansen, T. V., & Enemark, H. L. (2016). Anthelmintic effects of forage chicory (Cichorium intybus) against gastrointestinal nematode parasites in experimentally infected cattle. *Parasitology, 143*(10), 1279-1293.

Perry, B. D. (2002). *Investing in animal health research to alleviate poverty*: ILRI (aka ILCA and ILRAD).

Pirhofer-Walzl, K., Rasmussen, J., Høgh-Jensen, H., Eriksen, J., Søegaard, K., & Rasmussen, J. (2012). Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. *Plant and soil, 350*(1), 71-84.

Ploeger, H. W., Antonis, A. F. G., Verkaik, J. C., Vellema, P., & Bokma-Bakker, M. H. (2016). Perceptions and actions of Dutch sheep farmers concerning worm infections. *Veterinary parasitology, 229*.

Ploeger, H. W., Verkaik, J. C., Bokma-Bakker, M. H., & Antonis, A. F. G. (2021 in review). The effect of two phytotherapy products or feeding sainfoin in lambed ewes heavily infected with Haemonchus contortus.

Preston, J. M., & Allonby, E. W. (1979). The influence of haemoglobin phenotype on the susceptibility of sheep to Haemonchus contortus infection in Kenya. *Research in veterinary science, 26*(2), 140-144.

Prichard, R. (2001). Genetic variability following selection of Haemonchus contortus with anthelmintics. *Trends Parasitol, 17*(9), 445-453. doi:10.1016/s1471-4922(01)01983-3

Qamar, M. F., Maqbool, A., & Ahmad, N. (2011). Economic losses due to haemonchosis in sheep and goats. *Sci Intern, 23*(4), 321-324.

Radovich, T. (2011). Farm and forestry production and marketing profile for Moringa (Moringa

oleifera). In: Permanent Agriculture Resources (PAR): Holualoa, HI, USA.
Ramírez-Restrepo, C., Kemp, P., Barry, T., & López-Villalobos, N. (2006). Production of Lotus corniculatus L. under grazing in a dryland farming environment. *New Zealand Journal of Agricultural Research, 49*(1), 89-100.
RANA, M. A. A. (2015). *EPIDEMIOLOGY, SEROLOGY AND THERAPEUTIC TRIALS OF FASCIOLOSIS IN CROSSBRED COWS.* UNIVERISTY OF PUNJAB, LAHORE,
Rehman, A., Ullah, R., Gupta, D., Khan, M. A. H., Rehman, L., Beg, M. A., . . . Abidi, S. M. A. (2020). Generation of oxidative stress and induction of apoptotic like events in curcumin and thymoquinone treated adult Fasciola gigantica worms. *Experimental Parasitology, 209*, 107810. doi:https://doi.org/10.1016/j.exppara.2019.107810
Relf, V., Good, B., McCarthy, E., & de Waal, T. (2009). Evidence of Fasciola hepatica infection in Radix peregra and a mollusc of the family Succineidae in Ireland. *Veterinary parasitology, 163*(1), 152-155. doi:https://doi.org/10.1016/j.vetpar.2009.04.003
Ringold, P. L., Magee, T. K., & Peck, D. V. (2008). Twelve invasive plant taxa in US western riparian ecosystems. *Journal of the North American Benthological Society, 27*(4), 949-966.
Rizvi, W., Fayazuddin, M., Singh, O., Syed, S. N., Moin, S., Akhtar, K., & Kumar, A. (2017). Anti-inflammatory effect of Fumaria parviflora leaves based on TNF-α, IL-1, IL-6 and antioxidant potential. *Avicenna journal of phytomedicine, 7*(1), 37.
Saijuntha, W., Tantrawatpan, C., Agatsuma, T., Wang, C., Intapan, P. M., Maleewong, W., & Petney, T. N. (2018). Revealing genetic hybridization and DNA recombination of Fasciola hepatica and Fasciola gigantica in nuclear introns of the hybrid Fasciola flukes. *Molecular and Biochemical Parasitology, 223*, 31-36. doi:https://doi.org/10.1016/j.molbiopara.2018.06.004
Santos, F. O., Cerqueira, A. P. M., Branco, A., Batatinha, M. J. M., & Botura, M. B. (2019). Anthelmintic activity of plants against gastrointestinal nematodes of goats: a review. *Parasitology, 146*(10), 1233-1246.
Scales, G., Knight, T., & Saville, D. (1995). Effect of herbage species and feeding level on internal parasites and production performance of grazing lambs. *New Zealand Journal of Agricultural Research, 38*(2), 237-247.
Schabussova, I. U. L. P., & Wiedermann, U. (2014). Allergy and worms: let's bring back old friends? *Wiener Medizinische Wochenschrift, 164*(19-20), 382-391. doi:10.1007/s10354-014-0308-7
Scharenberg, A., Arrigo, Y., Gutzwiller, A., Soliva, C. R., Wyss, U., Kreuzer, M., & Dohme, F. (2007). Palatability in sheep and in vitro nutritional value of dried and ensiled sainfoin (Onobrychis viciifolia) birdsfoot trefoil (Lotus corniculatus), and chicory (Cichorium intybus). *Archives of Animal Nutrition, 61*(6), 481-496.
Sengupta, M. E., Keraita, B., Olsen, A., Boateng, O. K., Thamsborg, S. M., Pálsdóttir, G. R., & Dalsgaard, A. (2012). Use of Moringa oleifera seed extracts to reduce helminth egg numbers and turbidity in irrigation water. *Water research, 46*(11), 3646-3656.
Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., & Mann, A. (2011). Scientific basis for the therapeutic use of Cymbopogon citratus, stapf (Lemon grass). *Journal of advanced pharmaceutical technology & research, 2*(1), 3.
Shah, R. (2006). Nature's medicinal plants of Uttaranchal: Herbs. *Grasses and Ferns, Nanital, Uttarakhand, India*.
Shaik, S., Terrill, T., Miller, J., Kouakou, B., Kannan, G., Kaplan, R., . . . Mosjidis, J. (2006). Sericea lespedeza hay as a natural deworming agent against gastrointestinal nematode

infection in goats. *Veterinary parasitology, 139*(1-3), 150-157.
Skuce, P., & Zadoks, R. (2013). Liver fluke–a growing threat to UK livestock production. *Cattle Pract, 21*(part 2), 138-149.
Sousa, R. M. O. F., Rosa, J. S., Cunha, A. C., & Fernandes-Ferreira, M. (2017). Molluscicidal activity of four Apiaceae essential oils against the freshwater snail Radix peregra. *Journal of Pest Science, 90*(3), 971-984. doi:10.1007/s10340-017-0842-3
Southcott, W. H., & Barger, I. A. (1975). Control of nematode parasites by grazing management—II. Decontamination of sheep and cattle pastures by varying periods of grazing with the alternate host. *International Journal for Parasitology, 5*(1), 45-48. doi:https://doi.org/10.1016/0020-7519(75)90096-X
Sunita, K., Kumar, P., & Singh, D. (2013). Fasciolosis Control: Phytotherapy of host snail Lymnaea acuminata by allicin to kill Fasciola gigantica larvae. *Annual Research & Review in Biology*, 694-704.
Sunita, K., & Singh, D. (2011). Fascioliasis control: in vivo and in vitro phytotherapy of vector snail to kill Fasciola larva. *Journal of parasitology research, 2011*.
Tattelman, E. (2005). Health effects of garlic. *American family physician, 72*(1), 103-106.
Taylor, M. A., Coop, R. L., & Wall, R. (2016). *Veterinary parasitology* [1 online resource](4th edition. ed.).
Thulin, M. (1993). Flora of Somalia, Volume 1-Pteridophyta; Gymnospamae; angiospamae (Annonacae-Fabaceae). *Flora of Somalia*.
Todd Jr, K., Levine, N., & Boatman, P. (1976). Effect of temperature on survival of free-living stages of Haemonchus contortus. *American Journal of Veterinary Research, 37*(8), 991-992.
Todd Jr, K. S., Levine, N. D., & Whiteside, C. C. (1970). Moisture stress effects on survival of infective Haemonchus contortus larvae. *Journal of Nematology, 2*(4), 330.
Toner, E., Brennan, G., Wells, K., McGeown, J., & Fairweather, I. (2008). Physiological and morphological effects of genistein against the liver fluke, Fasciola hepatica. *Parasitology, 135*(10), 1189.
Tsarong, T. (1994). Tibetan Medicinal Plants Tibetan Medical Publications. In: India ISBN.
Tzamaloukas, O., Athanasiadou, S., Kyriazakis, I., Jackson, F., & Coop, R. (2005). The consequences of short-term grazing of bioactive forages on established adult and incoming larvae populations of Teladorsagia circumcincta in lambs. *International Journal for Parasitology, 35*(3), 329-335.
Ullah, R., Rehman, A., Zafeer, M. F., Rehman, L., Khan, Y. A., Khan, M. A. H., . . . Abidi, S. M. A. (2017). Anthelmintic Potential of Thymoquinone and Curcumin on Fasciola gigantica. *PloS one, 12*(2), e0171267-e0171267. doi:10.1371/journal.pone.0171267
Urban, J., Kokoska, L., Langrova, I., & Matejkova, J. (2008). In Vitro Anthelmintic Effects of Medicinal Plants Used in Czech Republic. *Pharmaceutical Biology, 46*(10-11), 808-813. doi:10.1080/13880200802315618
Valderrábano, J., Calvete, C., & Uriarte, J. (2010). Effect of feeding bioactive forages on infection and subsequent development of Haemonchus contortus in lamb faeces. *Veterinary parasitology, 172*(1-2), 89-94. doi:10.1016/j.vetpar.2010.04.018
van Andel, J. M., & Ploeger, H. W. (2014). *Leverbot bij sachapen en geiten : behandeling, preventie, herkennen leverbotslak*. [S.l.]: Provinos Schapenadviesgroep.
Van der Nat, J., Klerx, J., Van Dijk, H., De Silva, K., & Labadie, R. (1987). Immunomodulatory activity of an aqueous extract of Azadirachta indica stem bark. *Journal of ethnopharmacology, 19*(2), 125-131.

Váradyová, Z., Kišidayová, S., Čobanová, K., Grešáková, Ľ., Babják, M., Königová, A., . . . Várady, M. (2017). The impact of a mixture of medicinal herbs on ruminal fermentation, parasitological status and hematological parameters of the lambs experimentally infected with Haemonchus contortus. *Small ruminant research, 151*, 124-132. doi:10.1016/j.smallrumres.2017.04.023

Váradyová, Z., Mravčáková, D., Babják, M., Bryszak, M., Grešáková, Ľ. r., Čobanová, K., . . . Várady, M. n. (2018). Effects of herbal nutraceuticals and/or zinc against Haemonchus contortus in lambs experimentally infected. *BMC Veterinary Research, 14*(1). doi:10.1186/s12917-018-1405-4

Veldhuis, A. M. B. (2009). The effect of chicory as bioactive forage on parasitism in lambs. *Master thesis, Adaptation Physiology group, Wageningen university*.

Villalba, J. J., Miller, J., Ungar, E. D., Landau, S. Y., & Glendinning, J. (2014). Ruminant self-medication against gastrointestinal nematodes: evidence, mechanism, and origins. *Parasite, 21*.

Waghorn, G. (2008). Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. *Animal Feed Science and Technology, 147*(1-3), 116-139.

Waller, P., & Chandrawathani, P. (2005). Haemonchus contortus: parasite problem No. 1 from tropics-Polar Circle. Problems and prospects for control based on epidemiology. *Trop Biomed, 22*(2), 131-137.

Waller, P. J., Rudby-Martin, L., Ljungström, B. L., & Rydzik, A. (2004). The epidemiology of abomasal nematodes of sheep in Sweden, with particular reference to over-winter survival strategies. *Vet Parasitol, 122*(3), 207-220. doi:10.1016/j.vetpar.2004.04.007

Weewer, W. (2021). How acquisitive and conservative traits within fertilized grass-legume pastures affect N-cycling. *Master thesis, Soil Biology group, Wageningen university*.

Williams, D. J., Howell, A., Graham-Brown, J., Kamaludeen, J., & Smith, D. (2014). Liver fluke–an overview for practitioners. *Cattle Pract, 22*, 238-244.

Wolstenholme, A., & Rogers, A. (2005). Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. *Parasitology, 131*(S1), S85.

Yamson, E. C., Tubalinal, G. A. S., Viloria, V. V., & Mingala, C. N. (2019). Anthelmintic effect of betel nut (Areca catechu) and neem (Azadirachta indica) extract against liver fluke (Fasciola spp.). *Journal of advanced veterinary and animal research, 6*(1), 44.

Zahara, K., Tabassum, S., Sabir, S., Arshad, M., Qureshi, R., Amjad, M. S., & Chaudhari, S. K. (2014). A review of therapeutic potential of Saussurea lappa-An endangered plant from Himalaya. *Asian Pacific journal of tropical medicine, 7*, S60-S69.

Zhong, L. (2012). *Observation on the effect of 4 kinds of drugs on the treatment of Fasciola hepatica in Qinghai semi-wool sheep.*

Zhou, D., & Han, Z. (2004). Traditional Chinese Medicine "Gan Zhi San" for the treatment of Fasciolasis in Bovine Liver. *Guizhou Animal Husbandry and Veterinary, 28*(5), 18-18.

Appendix

Adriaan Antonis - Researcher Teams meeting time: **9:00-10:00 April 7, 2021**

Adriaan Antonis is a researcher who focus on gastro-intestinal parasites in sheep and also have an experience on synthetic medicine.
For the sheep industry, there are less data available on anthelmintic using, but the result of sheep farmers interviews shows there are huge problems of drug abuse, including antibiotics and anthelmintics. To reduce the medicine abuse, A. Antonis tries to only treat individual animals. and less susceptibility of the whole group for worm infection by excluding infected animals in breeding.
But animals need to get infected with parasites to build natural resistance against these worms, and the infection needs be under control and not too much (old friend hypothesis). Due to the fact that worm infection mostly appears in young animals, when these young animals haven't seen worms in their first year of life, they get ill more often.
Haemonchus contortus is only a problem in June, July and August, but certain condition every year is also related to season/temperature. During these months, extra prevention management is needed. Unlike *Haemonchus contortus*, liver fluke is only problematic in wet areas, but if animals get infected, they can suffer a lot and even die from it. A. Antonis believes multidisciplinary approach is the best natural way to solve the problem (biological control, microbiome, immunization/breeding, feeding and grassland management), more tips is on www.wormenwijzer.nl

Table 15. Management options suggested by A. Antonis.

Factors	Management	Mechanism
Pasture management	Move the sheep every 2-3 weeks to another field before the larvae reached the infectious state (L3), then mowing the field or farming horses on the field during the period.	Larvae only become infectious in state L3, as it normally takes 2-12 weeks for parasites grow to L3. Mowing cleans the parasites left on the land. Horse and sheep are susceptible on different intestinal parasite species, horses 'clean' the field before the youngest sheep graze on that field, also the other way around. And also soil worms play a role in lower the infection rate by eating infectious worms away.
Grassland/ Animal density	Always keep enough grass for the animals	If sheep don't feel they have enough grass to eat, they start to eat the places where infectious larvae are.
Herbs	Add herbs as a part of the feeding Adding minerals	Finding the exact mechanisms for herbs are difficult, Amount of sun hours or manures can affect the result.
Biological control	Keep a good circumstance of dung beetles and fungi in the ground	They kill nematodes
Microbiome	Need to be stable	

Table 16. Herbs suggested by A. Antonis.

Herbs	How to use	Mechanism
Sainfoin	Feed it as hay or silage	Sainfoin contains lots of tannins
Plantain		Studied showed that plantain was not palatable to sheep and therefore sheep performed poorly on this (Niezen et al., 1998).

A. Antonis agrees that a farm with multiversity plants would benefit, but it's difficult to get a more herbs-rich farms. Another limit on herb using is that farmers are not willing to feed useful herbs to cows, since most herbs effective are wooden but cows fancy tender food. Apart from that, A. Antonis thinks it is logical to believe that there is an effect of anthelmintics on soil health, but the story is not clear yet.

Tedje van Asseldonk - Biologist Teams meeting time: **10:00-11:00 April 8, 2021**

Tedje van Asseldonk is a biologist focused on medicinal herbs who works with the Dutch association of phytotherapy, in a study group vets on herbs, and she owns an herb farm in Italy.
According to T. van Asseldonk, coccidiosis is a big problem in calves and chickens. But she believes that the parasite itself is not the problem, the overgrowth of parasite is, therefore the coexistence of parasites and animals is important. Considering the health of animals and the controlling of the parasites, she prefers less toxic herbs than herbs with strong toxicity.
T. van Asseldonk also believes it is organic secondary compounds that function effectively against parasites, for example tannins, but at the same time tannins are also toxic. In addition, the balance of plants is maintained by a lot of organic secondary compounds in plants, they don't have nutritional value, but do have a physiological effect. And speaking of resistance in internal parasites against plant compounds, resistance occurs quickly if there is only one compound, but with more compounds the resistance could come slower. Therefore, she suggests using several herbs in a row, by combining several plant species, toxicity may be reduced. Another option is to make strong essential oil out of useful herbs because it is easy to restore. For more plant compounds she will send Liza prize book, which is a syllabus about plant compounds.
In terms of feasibility of planting herbs in the Netherlands, tanasetum and artemisia species can grow in the Netherlands, while papaya couldn't. Besides, planting more trees around the meadows may also help, since the animals can eat from them themselves. Combining the elements of the landscape helps as well, for example if you have a canal, you can probably have a steady supply of mint there, and nearly every farm has specific nettle spots, so that animals can be organized and eat there. And clover grass fields are popular in the Netherlands recently, but she did not speak about the reason of it.

Overall, she thinks in order to realistically implement the herbs in the livestock industry, the future should be focusing on permaculture farms with a rich biodiversity.

Table 17. Herbs suggested by T. van Asseldonk.

Herbs	How to use	Mechanism
Tanacetum vulgare	Can be used as a part of the formula or only use a certain part of the herbs. Can also be made into essential oils.	They can diminish the number of parasites. But they are quite bitter and toxic.
Tagetes minuta		
Unripe papaya		
Artemisia annua		
Herba alba-alba		
Sambucus nigra		Good against viruses
Sainfoin		Much higher antioxidant value
Digitalis (heart problems)	Medicine has been made from it where the concentration is well-known	Really toxic, and the dosage should be very precise

T. van Asseldonk pointed out several obstacles of using natural remedies. First of all, there are too many animals in the Netherlands thus it is not possible to supply herbs on all of them. Secondly, legislation could be another limit in the development of herbal remedies. In the end, she thinks highly of traditional Chinese medicine (TCM) remedies, but there is the different definition of illness of western and China.

Harm Ploeger - Parasitologist Teams meeting time: **10:00-11:00 April 9, 2021**

Harm Ploeger is a parasitologist from Utrecht University
Speaking about problematic parasites, H. Ploeger thinks *Haemonchus contortus* more problematic than *Nematodirus battus*, because in the Netherlands, animal density is way higher than in most other (neighbouring) countries. For cattle, the most problematic parasite is *Ostertagia ostertagi*. As for liver fluke, multiple hosts can be infected, including sheep, cows, rabbits, dears and horses. But the issue in sheep is more problematic, because sheep don't develop immunity against liver fluke, while cattle can contain the infection in one liver lobe while the other remains intact. And this parasite can live to be as old as the host, up to 13 years old.

Table 18. Possible parasites to look into suggested by H. Ploeger.

Parasites	Species	Infection spot	Problem statement
Teladorsagia circumcincta		Stomach	Diarrhea and anaemia
Complex of species called *otostrongylus* sp.		Intestine	Diarrhea and anaemia
Nematodirus battus			Depends on the harshness of the winters, not a big issue for now. The *Nematodirus battus* may quickly become a problem if it quickly warms up after a cold period. In a mild winter, lambs have time to develop immunity due to the mild hatching process. *Nematodirus battus* risk can be predicted using weather models.

Coccidiosis			Condition depends on the first explode.
Haemonchus contortus	Sheep & Cattle		*Haemonchus contortus* population stay in animals through the whole winter, therefore have a high anthelmintic resistance.
Ostertagia ostertagi	Very young cows		
Teladorsagia circumcincta	Sheep		
Dictyocaulus viviparus	Cow	Lungworm	5-10% farms would be serious infection.
Liver fluke	Sheep, cow rabbits, dears, horses		Sheep do not develop immunity against liverfluke Have long longevity and multiple hosts, so hard to get rid of

Talking about controlling and preventing parasites, H. Ploeger thinks is not about parasite extinction, but is about controlling the infection to let the animals develop immunity without the infection getting out of hand. He made some comments on several possible measures and herbs against parasites.
On the aspect of resistance, H. Ploeger thinks resistance will eventually be built by parasites, but it will take a very long time to establish resistance of anthelmintic for now, and in a long term, herb-rich, species-rich pastures will develop resistance much slower than only using one herb/plant.

Table 19. Several comments H. Ploeger gives on possible measures and herbs against parasites.

Possible measure	Comments
Select genetic resistance	Done by a Dutch company
Haemonchus contortus vaccine in Australia	purified proteins collected from *Haemonchus* 'soup', which is effective for about 50-70% after injected 5 times
Pasture movement	Not to be too strict is good for immunity development, but maximum 2 weeks flexibility
Permaculture farms with a rich biodiversity	Because of the thin type of plants on the pasture, it's good to culture and harvest herbs separately. Not grow sainfoin in pasture because animals graze and walk through it and all goes lost.
Sainfoin	Better in silage of fine diet because sheep have more difficulty to eat the long sainfoin in hay which has high amount of fibres
Garlic	No evidence found in scientific article

To summarize H. Ploeger's opinion on parasitical management strategy, first of all, he thinks raising management is the more important than feeding natural remedies. Smaller pastures but more labour on switching the animals would shorten the time of sheep eating at the same grass, therefore lower the chance of parasite infection; secondly, enrich pastures is also an option since sheep can self-medicate.

Klaartje van Wijk – Biological dairy farmer Telephone call **April 14, 2021**

K. van Wijk has a Dutch dairy farm in the north of the Netherlands on a sandy soil, she therefore does not experience infections with liverfluke in her cows. For years she did not experience infections with internal parasites, however, last year she bought some new livestock which carried some internal parasites with them. She has the experience that when cows suffer from internal parasites this is in coexistence with external parasites. K. van Wijk asked the feed business **(Jan Bruins & Zn)**, which delivers the concentrates to her for years, for some feed to strengthen the immune response. He added 0.5% of oregano to the concentrates K. van Wijk normally fed to the cows. She fed one group of young calves this concentrate and another group of older cows no concentrates. By accident she came to the discovery that this feed was also very effective against parasite infections. Namely, the young calves, which were fed the concentrates with oregano, did not have any parasite infection. Whereas the older group which did not receive the concentrates with oregano, had parasite infections. After this discovery, she also started to feed the group with parasite infections the concentrates with oregano and that helped to control the infections. Next to that, she makes use of classical homeopathy: spraying high dilutions on the nose and vulva of the cows in order to prevent both internal and external parasite infections, for example, tuberculinum, drosera and spongia). In addition, she is advised by a German company from **Hubert Cremer**[1], who is specialised in making herbal mixtures for biological farmers. These herbal mixtures are composed of 30-40 different herbs, for example, garlic, cinnamon, mariadistel, rosemary, oregano). According to K. van Wijk it is not about treating or preventing specific parasite infections, but more about getting the system in balance again.

Jiaguo Liu - Chinese Veterinarian Email **April 14, 2021**

Professor Liu is a Chinese veterinarian who has a lot of experience study the use of traditional Chinese medicine on animals. He also published several textbooks on the specialization of traditional Chinese veterinarian medicine.
In China, herbs are mainly used as natural remedies against parasite. Some traditional Chinese medicine are feed itself, so they are feed to prevent parasite, for example *Lappula myosotis*. As for practice management, farms will use herb mixtures to deworm preventively, 驱虫散 quchongsan(a herb mixture mainly against Nematode) was used in this case.
Haemonchus contortus and liver fluke are quarantine disease in China, and liver fluke is more observed than *Haemonchus contortus*, drugs mixture 肝蛭散 ganzhisan is used for it. For sheep, coccidia is more common in China.
Compared to normally used Chinese medicine, anthelmintics used drugs have more toxicity, and they need to be used in a specific manner, some need to be eaten before the meal, others need to be eaten after the meal. And for different kinds of worms, there are specific drugs against them. But still, the toxicity of herbal medicines is milder than the correspondent synthetic drugs.

Harmen van der Sluis - International technical specialist roughage Barenbrug
Telephone call **April 15, 2021**

International technical specialist roughage Barenbrug.
The NutriHerb mixture does contain sainfoin and birdsfoot trefoil and has been on the market for multiple years. How long the herbs consist into the mixtures depends highly on management. How often and high it is mown fertilized, fertilization rates etc. As sainfoin and birdsfoot trefoil are nitrogen fixers fertilization highly influences persistence. A combination of mowing and grazing is advised to ensure flowering. Flowering is important to store energy and thereby persistence for the next season. At least for two years all the herbs in the mixture so also sainfoin and birdsfoot trefoil persist into the pasture. However, the first herbs to go are often sainfoin and birdsfoot trefoil. After losing herb

species it is difficult to get them back by seeding trough the existing pasture due to the higher competitive grass species.

Jan Bruins – Owner feeding company Jan Bruins & Zn Telephone Call **April 16, 2021**

J. Bruins suggested that indeed adding oregano to the feed will help against gastrointestinal parasites. Not only at the farm from K. van Wijk, but J. Bruins experienced this at other farms as well. He buys the oregano from Trouw Nutrition. Furthermore, he said that adding monensin-natrium (Coxidin) to the feed was an excellent medium against coccidiosis. In the past, this was used a lot by young calves and bulls. However, nowadays the use of monensin-natirum is forbidden by law.

Dr. S. K. Kumar - Researcher Teams meeting time: **11:30-12:30 April 16, 2021**

Dr. S. K. Kumar studied at the University of Trans-Disciplinary Health Sciences and Technology, working at the Centre for Ethnoveterinary Sciences and Practices. He has 15 years of experience in authentic Ayurveda practice. He works on the documentation, implementation and spreading awareness on traditional ethnoveterinary practices. He aims to reduce the use of synthetic drugs, and empower farmers to use natural remedies to keep their animals healthy.
He advises the use of a traditional Ayurveda formula that consists of 12 different ingredients, 10 of which are medicinal herbs.

Table 20. Herb ingredients suggested by S. K. Kumar.

Herbs/ingredients	**Function**
Salt	Palatability
Jaggery sugar	Palatability

The exact Ayurveda formula used depends on the availability of the resources. Based on Ayurveda practice, fresh herbs have the highest efficacy. By crushing the herbs into a pasta and applying the paste on the animal's tongue, the animal's saliva and slower transit to the digestive tract can improve digestion and absorption compared to drenching methods.
Besides, combinations of herbs or herb mix can be more effective and stronger than a single herb. In the formula, balance of herbs is also important. For example, garlic has a hot character and should be combined with a cool herb. Some compounds in the formula are acting, others are working synergistically or maintaining homeostasis in the formula/counteracting the negative effects of other compounds. Because of the balance it has, herb mix not only cure diseases, but also maintain the general health of the animal. Ideally, standardization of formula dosages should be established for different species and breeds of livestock. And usually, the formula that are tested are more effective than the synthetic drugs because it against more than 10 clinical conditions (work for any parasite) and improves the immunity of the animal.
It is believed that not much side-effect is in Ayurveda formulas because most of ingredients are also people use in the kitchen, but dose need to be obeyed correctly. Furthermore, dosing the remedy can be done more precisely with extracts, and the active compound of herbs can be identified when extracts are made. However, the use of extracts has a higher risk of resistance development. It may be possible for resistance to occur against fresh herbal remedies. However, there is currently not enough data to confirm this. Once herbal remedies are implemented on a larger scale, this can be investigated. And when the use of fresh herbs is not feasible, herb extracts may be easier to transport and incorporate into commercially available products.

Jos Eldering - **Dairy farmer** Telephone call time: **10:30 – 10:50 April 19, 2021**

Jos Eldering is a dairy farmer in Twente with 140 cows and 125 hectares. K. van Wijk advised us to talk to him. He has been using herbs mixtures for 10 years provided **Hubert Cremer**[1]. Jos does not deworm with chemical drugs and does not use any antibiotics since 2003. The herb mixture is mostly to prevent infection and increase overall animal health. In his experiences it has been working well. When calves are coughing, he feeds them some more herbs are askes his adviser to adjust the herb mixture. The herbs are provided dried and grinded. He feeds them together with minerals in the feeding bins in the pasture, 2/3 minerals 1/3 herbs. The herbs are expensive, 2 to 3 euro per kg product but worthy the investment. No chemical drugs are needed, and the animals are health and reaching old age with less problems. In his pasture he recently seeded chicory and narrowleaf plantain. These adjustments were mostly made to create a greater drought tolerance. Future, timothy white and red clover, cuckoo flowers and dandelions are found in the pastures,

Karin Dijkstra – Dairy farmer Telephone call time **16:00 –16:30 April 19, 2021**

Dairy farmer from Friesland. Since 3 years K. Dijkstra has been using product from AHV (https://ahvint.com/). In her experiences they work however they can be expensive. She does not experience major issues with liver fluke. However, after slaughter some minor damage can be seen in the liver probably caused by liver fluke. She does apply a tea for the calves who are having diarrhea, the recipe can be found below. Further she heard that thyme rosemary, garlic and onion is a healthy plant/herb combination also for ruminants. She decreased the applying artificial fertilizer to the pastures over the last 10 years ago and in the last 3 years also less manure has been applied. Therefore, the soil is less nitrogen rich resulting in more herbs to develop within the pasture. She has seen that the cows eat Bitter Dock common reed and dandelions this could possibly be form of self-medication.

Recipe for 7 L:
First take one big hand of hay, 1 thee spoon of thyme 1 thee spoon of rosemary. Pour some cooking hot water of it and let it sit for 15 minutes. Add some could water so the tea reaches about 40 °C. Next, 1 tablespoon biological crystal sugar and 1 tablespoon Celtic salt is added. Also, a small hand of soil could be added, as calves are seen to eat soil by self-medication if they are sick. Further, some natrium bicarbonate could be added to increase the pH when needed.

Application:
Don't feed the calves milk for a day but feed the tea unlimited through a teat bucket. When the calve does not want to drink and is badly sick a sonde can be used to feed the calve 1 l thee every two hours.

Michael Walkenhorst - Veterinarian Teams meeting time: **11:30-12:30 April 19, 2021**

Michael Walkenhorst is a veterinarian that has worked at the Research Institute of Organic Agriculture (FIBL) in Switzerland for over 20 years. The organisation works closely with farmers, and regularly performs clinical research. FIBL does a lot of research on both clinical veterinary medicine and ethnoveterinary medicine.
Parasites with different life cycle and parasitism styles require different management strategies. For example, *H. contortus* attaches to the abomasum wall and can thus be affected by compounds that remain in the gastrointestinal tract. For gastrointestinal parasites, the microbiome must be considered. Using herbs to influence the microbiome may cause textural changes of the gastrointestinal tract, making the gut a more hostile environment for parasites.

F. hepatica on the other hand is found in the liver and can thus only be acted upon by compounds that are absorbed from the gut and transported to the liver. This requires the use of a compound that is toxic to the parasite, but not to the host. To reduce the *F. hepatica* parasite load, herbs that elevate the overall immune response may be more useful. Liquorice may be useful for this, as it influences the liver positively and enhances the immune system. It may be necessary to tolerate liver flukes to a certain degree, as they normally do not kill their host. However, if the animal is infected, the liver cannot be used after slaughtering the animal.

Animals have been known to self-medicate. However, most animals do not have a wide variety of herbs to choose from in the pasture nowadays. Herbs like *Plantago lanceolata* and sainfoin are useful, but the latter one is difficult to establish in the pasture. Furthermore, the addition of tannin-rich plants can provide the animals with more variety.
As herbs contain many different active compounds, it is unlikely that resistance against them will be generated in parasites, especially when mixing different herbs. By using different botanical families, the diversity of the mixture can be increased further.
Mixing herbs also increases the diversity of active compounds. Adding herbs that are known to have an immunoboosting effect can help to control parasitic infections both directly and indirectly. Furthermore, these compounds may complement each other, or reduce each other's toxicity. For example, tannin rich plants and chamomile can be combined to treat diarrhoea, as the tannins reduce binding of e. coli to the gastrointestinal wall, while chamomile reduces inflammation.

Tannins are a very broad category of phytomolecules. They might play a role in controlling parasites, but they only act in the gastrointestinal tract. Most tannins are not toxic, but this depends on the chemical structure. For example, tannins derived from oak bark can be absorbed from the gut and thus have more toxic effects on the body. In contrast, tannins found in *Potentilla erecta* are more gastro-intestinal tract related and will not be absorbed, thus reducing their toxicity.

Current synthetic anthelmintic drugs preferably work after only one or a few administrations, and then last as long as possible. However, this can cause them to persist in the manure of the treated animals. Thus, organisms in the soil and environment can be negatively affected, which upsets the balance of the ecosystem. In contrast, herbal medicine is less potent and works for a shorter time, which decreases the negative effect on the soil and environment around the animal. However, administration of herbal medicine needs to be repeated more often and thus, this is more labour intensive.

Deyun Wang - Chinese Veterinarian Questionnaire via e-mail **April 20, 2021**

- Which parasites are problematic or most common in china (in ruminants)?

Nematode **:**
Toxocara vitulorum, Haemonchus contortus, Oesophagostomum columbianum, Oesophagostomum r adiatum, Oesophagostomum venulosum, Oesophagostomum asperum, Oesophagostomum kansuen sis, Bunostomun trigonocephalun, Bunostomun phlebotomun, Dictyocaulidae viviparus, Dictyocaulid ae filaria.
Trematode: *Fasciola hepatica, Fasciola gigantica, Dicrocoelium dendriticum, Dicrocoelium chinensis, Orientobilhariza turkestazicu, Schistosoma japanica.*
Tapeworm: *Moniezia expansa, Moniezia benedeni, Coenurus cerebralis, Cysticercus bovis*
Protozoan: Babesia, Theileria, Cryptosporidium, Toxoplasma gondii.
Coccidia: *Eimeriidae Eimeria, Isospora, Tritrichomonas foetus.*
Ectozoa: *Hypoderma lineatum, Hypoderma bovis, Oestrus ovis.*

- How commonly are natural remedies used in china?

Originally it takes 1/3 of market, now it is increasing every year.

- These natural remedies are for prevention or treatment?

Both, for prevention traditional Chinese medicine are used as additives to replace antibiotics; for treatment it has specific therapy on each disease, for example it has applications in respiratory diseases, intestinal diseases, reproductive system diseases and improving production performance.

- Which herbs are useful against common parasites appear in practical?

Commonly used compounds: Artemisinin, Halofuginone

Table 21. Commonly used herbs in China according to D. Wang.

Herb	Characteristic	Works against
Rangooncreeper Fruit (使君子)	Sweet, warm Enter the spleen and stomach	Roundworms, pinworms
Chinaberry - Tree Bark (苦楝皮)	Bitter, cold, toxic. Enter liver, spleen, stomach	Roundworms, pinworms
Betelnutpalm Seed (槟榔)	Bitter, pungent, and warm. Enter the stomach and large intestine.	Tapeworm, ascariasis, fasciolopsis buski, malaria
Stone-like omphalia (雷丸)	Slightly bitter, cold, slightly poisonous. Enter the stomach, large intestine.	Tapeworms, hookworms, roundworms
Grand Torreya (榧子)	Sweet, calm. Enter lung, stomach and large intestine.	Hookworms, roundworms, tapeworms.
Common Carpesium Fruit (鹤虱)	Bitter, pungent, calm. Enter the liver.	Roundworms, pinworms, tapeworms
Pumpkin Seed(南瓜子)	Sweet, warm. Enter the stomach, large intestine meridian	Tapeworms, schistosomiasis, and enterobius vermicularis
Others: Gemma Agrimoniae(鹤 草 芽) 、 Szechwan Chinaberry Fruit （ 川楝 ） 、 Common Cnidium Fruit （ 蛇 床 子 ） 、 Java Brucea Fruit （ 鸦 胆 子 ） 、 Red Orpiment（雄黄）		

Commonly used formula: Qanyingsan(万应散)、Quchongsan(驱虫散)、Guanzhongsan(贯众散)、Huachongtang(化虫汤), also these formulas can be adjusted base on personal understanding.

- Do you know any herbs against *Haemonchus contortus* & liverfluke?

Common Carpesium Fruit, Szechwan Chinaberry Fruit, Betelnutpalm Seed etc. But they always need to make the formula.

- Do you know the mechanism of herbs that are useful?

A guide under the theory of Chinese veterinary medicine is needed in order to get the best effectiveness. For instance, to differentiate the symptoms and signs of clinical diseases and choose the prescriptions, and for animals relatively cheap material is preferred.

- What else TCM might be useful for treating the parasites? (Acupuncture management practices?)

There's a lot of use on anthelmintic function, for example make vaccine using Artemisinin which is an extract compound against malaria. In addition, traditional Chinese medicine will choose a prescription based on the clinical manifestations of different parasites.

- Is there any side effect when treating ruminants or other things needs to be cautious?

The dose needs to be obeyed because medicine can kill worms, in other words it has some toxicity to animals as well.

- Are there any side-effect on soil health of using anthelmintic?

No, because most of these herbs are made by plants, and plants is not considered harmful against soil health, as plants are grown on soil.

- Is there knowledges known for resistance on herbs or compounds(anthelmintic)?

No, because traditional Chinese medicine are always using formula contains multiple herbs to kill worms, it's not easily to build up resistance because its complex compound.

Frank Wennekers - Dairy farmer Telephone call **April 21, 2021**

Frank Wennekers is a farmer who owns Boerderij Wennekers, an organic farm in Hobrede, which is located in Noord-Holland. The pastures in this area are notoriously suitable for liver fluke, due to high water levels. At the moment, the farm is not using any herbal remedies against liver fluke, but they are interested in controlling the parasite by using medicinal herbs. Recent test results from the vet indicated that some animals on the farm have been infected with liver fluke. The animals on Boerderij Wennekers are usually treated with Iver Plus, which contains ivermectin and albendazole. However, the problem persists, which points to resistance development in these liver flukes. On the farm, sheep appear to be more vulnerable to liver fluke infections than cows. This may also be due to the pasture rotation scheme, where the sheep graze on the pastures shortly after the cows.

Mara van den Berg - Dairy farmer Telephone call **April 21, 2021**

Mara van den Berg is a farmer living in a peat area who has had many problems with liver fluke over the years. Because her pasture falls under regulations of 'Natuurmonumenten', her pasture gets flooded every summer, and is kept wet throughout the year. This causes a lot of liver fluke infections in her cattle. From veterinarians, mostly tribex and zenyl are recommended to treat liver fluke infections. Because she only has limited space available for her cattle to graze on, managing options such as pasture rotation are not available.
Apart from her cattle, she often uses homeopathic remedies to treat herself and her pets. She thinks it would be advantageous if natural remedies could be used on livestock as well and is open to the idea of implementing more natural remedies into her management.

Hubert Cremer – Herb specialist Meeting in Lunteren **April 28, 2021**

Hubert Cremer is a herb specialist who has worked on providing herb mixtures to improve cows' health for decades. He works closely together with several farms, including Remeker, a biological farm in Lunteren. He provides several herbal supplements which are used prophylactically to strengthen the immune system, such as Booster, Power Vital and Kraft Vital. When animals are ill, the normal dose is doubled for specific plants. H. Cremer changes the herbal composition depending on the season and the milk values of the animals. When an animal has health problems, milk production is reduced for several days. Sick animals have been observed to consume large amounts of Booster, which is an indication that the animal is ill.
By taking milk samples and measuring fatty acid ratios, cell counts, urea and lactate levels, H. Cremer can assess the cow's health. Furthermore, the omega 3, 6 and 9 fatty acid levels are important indicators of health. For example, linoleic and alpha-linolenic acid are considered to be 'good' omega 6 variants, while arachidonic acid is considered a 'bad' variant. Arachidonic acid levels are increased by feeding soy and corn, while this increase is lower for grains. Increasing omega 9 levels is beneficial, as it helps the cow withstand stress from heat, cold or bad feed.

Cows have a 'depot' of fat and nutrients that they absorb back when they are experiencing stress. When this occurs, the animals rapidly lose weight. This can be seen in the fatty acid composition of the milk, as the animal uses proteins from the body rather than the feed to produce milk. By feeding the right nutrients and herbs, the depot can be preserved, and the cow's weight remains stable.

When cows are fed with grass, silage or hay that has been treated with pesticides, their ruminal bacteria can be negatively impacted. For example, feed from floodplains can be contaminated with heavy metals or other pollutants from the river. Hubert Cremer mentioned a case where cows were fed with contaminated feed, and subsequently produced milk that caused diarrhoea in some consumers. In order to remove toxins from the body, the cow's liver needs large amounts of energy. By supplying high quality feed, the natural detoxification process of the liver can be supported.

Soy, a commonly used animal feed, has a high protein content, but low omega 3 saturated fatty acids. Due to this, the protein in soy is mainly used for milk production, and not for maintenance of the cow's health and body weight. Corn and sunflower seeds have a similarly low omega 3 content. Other protein sources such as nuts, hemp and grains provide a more balanced protein profile. Furthermore, methionine is a very important amino acid that can be found in these alternative protein sources, while there are only very low amounts of methionine in soy.

Bad feed can severely impact ruminal bacteria families. Especially in summer, when grass has a high protein but low energy content, H. Cremer advises to feed 0.5 Kg crushed grains to provide nutrients for the ruminal bacteria. Microbiota composition is highly variable, which means that some cows are more resistant to bad feed than others. By providing high quality feed, high diversity of ruminal bacteria families and species can be maintained.

Different breeds and types of cows react differently to stress, feed quality and herbal treatment. Cows that are purely used for milk production are less stable and 'burn out' much faster than cows kept for both milk and meat. These animals have a higher body weight and thus a larger depot to sustain themselves in times of stress. Heavier cows that eat large amounts of grass show very good fatty acid ratios in their milk.

Pastures should contain plants that have a high nutritional value. However, herbs mostly have high levels of active compounds, but low nutritional value. If herbs are fed in excess without sufficient nutritional plants, cows stop giving milk and high toxicity can occur. Thus, there should be balance when implementing herbs in the field. Fields with wild garlic, thistle and nettles around the edges allow for cows to consume sufficient nutrients, while still being able to self-medicate with herbs when needed. Additionally, whole herbs have a high diversity of active compounds which causes synergy. Thus, whole herbs in or from the pasture may have higher efficacy than extracts.