Rapport 82

In vivo testing of alternatives for conventional treatment of Ascaris suum in pigs

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Abstract
An experiment was conducted to test the preventive effect of different herbs (*Thymus vulgaris*, *Melissa officinalis, Echinacea purpurea* and/or *Camellia sinensis)* on a mild infection with *Ascaris suum* in growing finishing pigs. Results are discussed in this report.

Keywords: herb, phytotherapy, *Ascaris suum,* growing finishing pigs, antelminthic effect

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Samenvatting
Er is een experiment uitgevoerd om het preventieve effect te testen van een aantal kruiden(*Thymus vulgaris*, *Melissa officinalis, Echinacea purpurea* and/or *Camellia sinensis)* op een milde besmetting van *Ascaris suum* bij vleesvarkens. Resultaten worden in dit rapport besproken.

Trefwoorden: kruiden, fytotherapie, *Ascaris suum,* vleesvarken, antelminthicum

ANIMAL SCIENCES GROUP
WAGENINGEN UR

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In vivo testing of alternatives for conventional treatment of Ascaris suum in pigs

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Summary

The percentage of disapproved livers of growing and finishing pigs has been increased significantly during the last years. In organic pig farms, this percentage is often higher than in conventional pig farms. In most cases, disapproved livers are the result of an infection with *Ascaris suum*. Usually, an infection of *Ascaris suum* is treated or controlled by using conventional synthetic drugs belonging to the benzimidazoles, levamisole and macrocyclic lactones. Organic farmers, however, prefer a non-pharmaceutical approach of worm control. Therefore, phytotherapy could be an perspective alternative.

The objective of this study was to test herb alternatives for the prevention and control of a mild infection of *Ascaris suum* in growing and finishing pigs. Two different herb mixtures were tested. Feed was supplemented with 3% of a herb mixture, thereby adding 1% *Thymus vulgaris*, 1% *Melissa officinalis* and 1% *Echinacea purpurea* to the diet, or with 4% of a herb mixture, thereby adding the mentioned herbs plus 1% *Camellia sinensis* (black tea) to the diet. Pigs were infected by 1000 worm eggs each. Comparatively, a negative control group (no treatment) and a positive control group (treatment with conventional synthetic drug Flubendazole) were included.

An experiment was conducted with 32 young boars (average starter weight was 24 kg) purchased from a SPF-pig farm. The pigs were monitored during 67 days in the period December 2006 until February 2007. In this study, four experimental treatments were compared:
1. negative control: no treatment was applied to prevent or control an infection with Ascaris suum;
2. positive control: pigs were treated with a conventional anthelmintic (Flubendazole) one week before slaughter;
3. herb mixture: pigs were fed a diet supplemented with a herb mixture;
4. herb mixture + tea: pigs were fed a diet supplemented with a herb mixture (as treatment 3) plus black tea.

From this experiment it was concluded that a diet with a herb mixture containing 1% *Thymus vulgaris*, 1% *Melissa officinalis* and 1% *Echinacea purpurea* for growing and finishing pigs did not decrease the number of pigs which are infected with *Ascaris suum*, but did reduce the average number of worms in the gastro intestinal tract. The addition of 1% black tea to this herb mixture did not result in a lower number of infected pigs and also did not reduce the average number of worms in pigs. Flubendazole appeared to be an effective deworming product.

On organic farms with a low worm infection probably a combination of a conventional synthetic drug and a diet with herb mixture containing 1% Thymus vulgaris, 1% Melissa officinalis and 1% Echinacea purpurea is an option. It depends on the level of worm infection whether it is an opportunity to deworm sows, weaners and/or growing finishing pigs with a diet containing the herb mixture to keep the level of Ascaris suum at an acceptable low level. Examination of faeces of sows, weaners and growing and finishing pigs regularly, and also the percentage of disapproved livers of growing and finishing pigs, can support to monitor the level of worm infection on the farm. Based on this monitoring probably a strategy of varying deworming with a synthetic drug and a diet with herb mixture can be developed for the different categories of pigs. Further research on this method, and also the suitable period to supply this herb mixture to sows related to stage of pregnancy and weaners related to age and feed intake, is desirable.

Samenvatting

Aanleiding
Het percentage afgekeurde levers van vleesvarkens vertoont de laatste jaren een duidelijk stijgende lijn. Op biologische varkensbedrijven ligt dit percentage bovendien vaak hoger dan op conventionele bedrijven. De oorzaak hiervan is veelal een spoelworminfectie. Momenteel worden spoelwormen (*Ascaris suum*) op varkensbedrijven bestreden of onder controle gehouden door gebruik te maken van conventionele synthetische medicijnen met onder andere benzimidazoles, levamisol of macrocyclische lactonen als werkzame stoffen. Dergelijke farmaceutische (of allopathische) middelen passen eigenlijk niet bij de filosofie van de biologische varkenshouderij. Ze brengen namelijk chemische stoffen in het milieu die mogelijk ecologische schade veroorzaken. In deze sector geeft men de voorkeur aan het gebruik van natuurlijke stoffen (die het milieu minder schade berokkenen) bij het beheersen van de wormbelasting.

Er zijn aanwijzingen dat bepaalde kruidenmengsels in staat zijn om de wormbelasting laag te houden. Uit een eerder door ASG uitgevoerde studie (Gaasenbeek et al., 2004) bleek dat een mengsel van tijm, citroenmelisse en zonnehoed in een dosering van 5% door het voer bij individueel gehuisveste vleesvarkens een goede preventieve bescherming gaf tegen *Ascaris suum*. Deze varkens waren individueel besmet met 1000 wormeieren. Ook de resultaten bij een dosering van 1% van het kruidenmengsel waren hoopvol. Een opvolgend experiment (Van der aag et al., 2004), waarin hetzelfde kruidenmengsel werd ingezet (dosering 3%) liet deze preventieve werking echter niet zien. De varkens in het tweede experiment waren gehuisvest in groepen van 6 varkens per hok. Om een natuurlijke besmetting na te bootsen werden 60.000 wormeieren per hok geplaatst. Het achterwege blijven van een positief resultaat kan mogelijk toegeschreven worden aan een te hoge wormbelasting.

Het werkingsmechanisme van dit kruidenmengsel is vermoedelijk vooral gebaseerd op antibacteriële eigenschappen. De wormen zouden mogelijk worden verdoofd en/of beschadigd en via de faeces worden uitgescheiden. Volgens deskundigen kan bovengenoemd kruidenmengsel, aangevuld met een looistof, wellicht een nog breder werkingsmechanisme hebben. Looistoffen verminderen namelijk niet alleen het aantal geproduceerde eieren maar lijken ook de verschillende groeistadia van *Ascaris suum* te verstoren. Daarnaast verstoren looistoffen mogelijk de aanhechting van spoelwormen aan het darmepitheel. Een voorbeeld van een kruid dat rijk is aan looistoffen is zwarte thee.

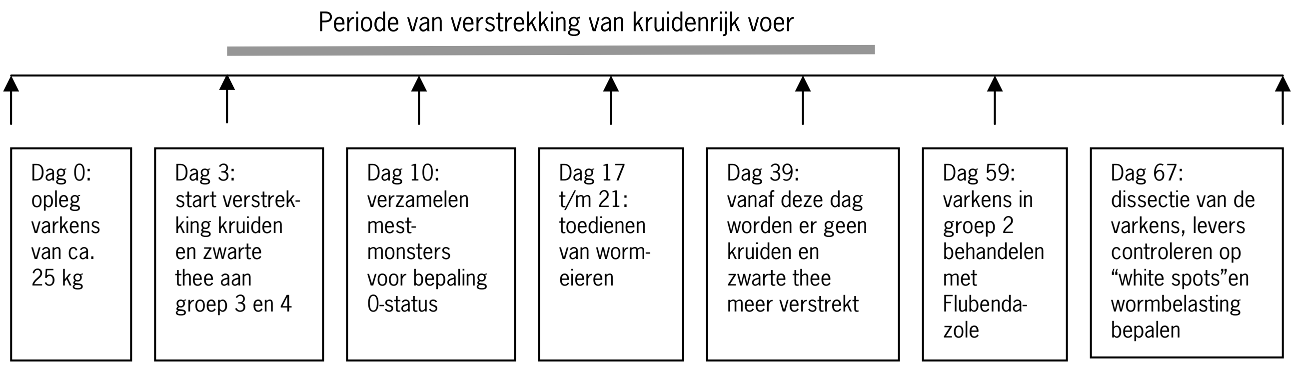
Het doel van dit onderzoek was na te gaan wat de preventieve werking is van het genoemde kruidenmengsel (1/3 deel tijm, 1/3 deel citroenmelisse en 1/3 deel zonnehoed) in een dosering van 3%, als ook van dit kruidenmengsel aangevuld met 1% zwarte thee, op een milde besmetting met spoelwormen (*Ascaris suum*) bij vleesvarkens. Het besmettingsniveau was 1000 wormeieren per vleesvarkens. Ter vergelijking zijn een negatieve controle (geen behandeling) en een positieve controle (behandeling met chemisch ontwormmiddel Flubendazole) in het onderzoek meegenomen.

Uitvoering van het onderzoek
Het onderzoek is uitgevoerd met 32 individueel gehuisveste beertjes, afkomstig van een SPF-bedrijf. De dieren zijn opgelegd bij een gewicht van gemiddeld 24 kg en gedurende 67 dagen gevolgd. Voorafgaand aan de wormbesmetting is gecontroleerd dat de dieren vrij waren van *Ascaris suum*. De volgende vier proefbehandelingen zijn vergeleken:
1) negatieve controle: aan de dieren is géén middel verstrekt om wormbesmetting tegen te gaan;
2) positieve controle: de dieren zijn 1 week voor slachten behandeld met Flubendazole;
3) kruidenmengsel: aan de dieren is gedurende 36 dagen voer met een kruidenmengsel verstrekt;
4) kruidenmengsel + zwarte thee: de dieren kregen gedurende 36 dagen voer met het kruidenmengsel als bij proefbehandeling 3 verstrekt, plus zwarte thee.

Alle vleesvarkens kregen vanaf opleg gedurende de eerste drie dagen een commercieel biologisch startvoer. Vanaf dag 3 na opleg werd aan de dieren in de proefbehandelingen 3 en 4 een vergelijkbaar startvoer met respectievelijk het kruidenmengsel (met 1% tijm (*Thymus vulgaris*), 1% citroenmelisse (*Melissa officinalis*) en 1% zonnehoed (*Echinacea purpurea*)) en het kruidenmengsel plus 1% zwarte thee (*Camellia sinensis*) verstrekt. In de periode van 17 tot 21 dagen na opleg zijn aan ieder dier dagelijks 200 wormeieren oraal toegediend. Op dag 39 is gestopt met het verstrekken van de voeders met kruidenmengsel en zwarte thee. Alle dieren kregen vanaf die dag hetzelfde commerciële startvoer verstrekt. Op dag 59 zijn de varkens in proefbehandeling 2 behandeld met Flubendazole. Omdat vleesvarkens aan het einde van de vleesvarkensfase in staat zijn spoelwormen af te drijven, zijn de dieren al op dag 67 van het experiment gedissecteerd.

De levers zijn beoordeeld op het vóórkomen van white spots en de wormbelasting in de dunne darm is bepaald. In figuur 1 staat de uitvoering van het onderzoek schematisch weergegeven. Bij opleg, op dag 17 (start toedienen wormeieren), dag 39 (einde verstrekking proefvoeders) en dag 67 (einde van het onderzoek) zijn de dieren gewogen. Daarbij is de verstrekte hoeveelheid voer vastgelegd. Hieruit zijn groei, voeropname en voederconversie berekend.

Figuur 1 Schematische weergave van de proef



Resultaten
In tabel 1 staan de resultaten van de wormbesmetting vermeld.

Tabel 1 Resultaten spoelwormbesmetting van vleesvarkens bij verschillende ontwormstrategieën om besmetting met *Ascaris suum* (spoelworm) tegen te gaan

	Geen behandeling	Fluben-dazole	Kruiden-mengsel	Kruidenmengsel + zwarte thee	Signifi-cantie[1]
Aantal dieren	8	8	8	8	
Levers met white spots (%)	100	100	100	100	
Aantal dieren met wormbesmetting	6[a]	0[b]	5[a]	5[a]	*
Gem. aantal wormen	48[a]	0[b]	23[ab]	41[a]	*
Spreiding (sd) aantal wormen	65	0	29	43	
Wormeieren volwassen (%)	4,8	0,0	2,3	4,1	n.s.

[1] n.s. = niet significant; * = (p< 0,05)

Uit het feit dat bij alle levers in meer of mindere mate white spots zijn waargenomen blijkt dat de worminfectie goed is aangeslagen. Uit het aantal dieren waarin bij dissectie spoelwormen in de dunne darm zijn gevonden bleek dat het kruidenmengsel in het voer er niet toe heeft geleid dat de wormeieren, direct na de infectie, volledig zijn uitgedreven. Echter, het gemiddelde aantal wormen in dieren die het kruidenmengsel kregen verschilde niet significant van dieren die met Flubendazole zijn behandeld. Omdat alle acht dieren die met Flubendazole zijn behandeld geheel vrij waren van spoelwormen blijkt Flubendazole een effectief ontwormmiddel te zijn. Het gemiddelde aantal wormen in dieren die het kruidenmengsel plus zwarte thee kregen verschilde niet significant van het gemiddelde aantal wormen bij dieren die alleen het kruidenmengsel kregen, maar het lag numeriek gezien op een vergelijkbaar niveau als bij de dieren die in het geheel niet behandeld waren tegen wormen.

In tabel 2 staan de technische resultaten van de vleesvarkens vermeld.

Tabel 2 Technische resultaten van vleesvarkens bij verschillende ontwormstrategieën om besmetting met *Ascaris suum* (spoelworm) tegen te gaan

	Geen behandeling	Fluben-dazole	Kruiden-mengsel	Kruidenmengsel + zwarte thee	SEM[1]	Signifi-cantie[2]
Aantal dieren	8	8	8	8		
Van opleg tot start wormbesmetting (D0 – D17):						
Gewicht D0 (kg)	23,9	23,9	23,9	23,9		
Gewicht D17 (kg)	36,2	37,8	36,3	35,6		
Groei (g/d)	722	818	730	686	35,9	#
Voeropname (kg/d)	1,46	1,61	1,51	1,41	0,068	n.s.
Voederconversie	2,02	2,00	2,06	2,06	0,063	n.s.
Van start wormbesmetting tot einde verstrekking proefvoeders (D17 – D39):						
Gewicht D17 (kg)	36,2	37,8	36,3	35,6		
Gewicht D39 (kg)	58,0	59,6	57,4	55,8		
Groei (g/d)	993	989	961	915	27,3	n.s.
Voeropname (kg/d)	1,99	2,01	1,95	1,84	0,063	n.s.
Voederconversie	2,00	2,04	2,03	2,01	0,039	n.s.
Van einde verstrekking proefvoeders tot einde proef (D39 – D67):						
Gewicht D39 (kg)	58,0	59,6	57,4	55,8		
Gewicht D67 (kg)	87,2	89,5	85,4	85,9		
Groei (g/d)	1043	1069	1000	1078	36,4	n.s.
Voeropname (kg/d)	2,38	2,56	2,34	2,36	0,095	n.s.
Voederconversie	2,29	2,40	2,34	2,20	0,067	n.s.
Van opleg tot einde verstrekking proefvoeders (D0 – D39):						
Gewicht D0 (kg)	23,9	23,9	23,9	23,9		
Gewicht D39 (kg)	58,0	59,6	57,4	55,8		
Groei (g/d)	875	914	860	815	27,1	n.s.
Voeropname (kg/d)	1,76	1,84	1,75	1,65	0,059	n.s.
Voederconversie	2,00	2,02	2,04	2,03	0,034	n.s.
Van start wormbesmetting tot einde proef (D17 – D67):						
Gewicht D17 (kg)	36,2	37,8	36,3	35,6		
Gewicht D67 (kg)	87,2	89,5	85,4	85,9		
Groei (g/d)	1021	1034	983	1006	28,1	n.s.
Voeropname (kg/d)	2,21	2,32	2,16	2,13	0,075	n.s.
Voederconversie	2,16	2,25	2,21	2,12	0,045	n.s.

[1] SEM = gepoolde standaard error van het gemiddelde (geeft een indicatie van de nauwkeurigheid van de gemeten variabele)
[2] n.s. = niet significant; # = ($p < 0{,}10$)

Er is geen aantoonbaar verschil in groeisnelheid tussen de vier proefbehandelingen. Wel is er in de periode van opleg tot start van de wormbesmetting (D0 – D17) een tendens (p=0,09) tot lagere groeisnelheid van de dieren die voer met kruidenmengsel plus zwarte thee verstrekt kregen dan bij de Flubendazole-groep. Ook over de gehele periode dat de proefvoeders zijn verstrekt (D0 – D39) is er sprake van deze tendens (p=0,10). Dit is opvallend omdat de dieren die tijdens de proef op dag 59 Flubendazole kregen toegediend tot dat moment hetzelfde zijn behandeld en gevoerd als de dieren in de groep ‘geen behandeling’. De verschillen in technische resultaten tussen de dieren in de groep ‘geen behandeling’ en de dieren die voer met het kruidenmengsel plus zwarte thee verstrekt kregen zijn, absoluut gezien, kleiner.

Ook is er geen aantoonbaar verschil in voeropname en voederconversie tussen de vier proefbehandelingen. Absoluut gezien lijkt de opname van het voer waaraan de combinatie van kruidenmengsel en zwarte thee is toegevoegd wat lager (D0 – D39). Dit geldt niet voor het voer met alleen het kruidenmengsel: de voeropname ligt hier op een vergelijkbaar niveau als bij negatieve controlegroep (groep ‘geen behandeling’). Mogelijk beïnvloedt de zwarte thee, door de smaak, de voeropname enigszins negatief.

Conclusies
Toepassing van 3% kruidenmengsel (met 1% tijm, 1% citroenmelisse en 1% zonnehoed) in het voer leidt bij een besmetting met 1000 wormeieren niet tot een vermindering van het aantal met spoelwormen besmette vleesvarkens maar wel tot een iets lager aantal wormen in het dier. De toevoeging van 1% zwarte thee aan dit kruidenmengsel geeft geen vermindering van het aantal met spoelwormen besmette dieren en ook niet van het aantal wormen in het dier. Flubendazole is een effectief ontwormmiddel.

Praktijktoepassing
In dit onderzoek is geen verschil aangetoond in het aantal geïnfecteerde vleesvarkens tussen het niet behandelen tegen spoelwormen en het behandelen middels 3% kruidenmengsel (met 1% tijm, 1% citroenmelisse en 1% zonnehoed) in het voer, dan wel dit kruidenmengsel plus 1% zwarte thee. De mate van wormbesmetting (uitgedrukt in het aantal wormen in het dier) is bij het verstrekken van het kruidenmengsel wel gunstiger. Het hangt af van het niveau van wormbesmetting op het bedrijf of voer met het kruidenmengsel, naast ontwormen met een chemisch middel, een mogelijkheid kan zijn om de infectiedruk van spoelwormen op biologische bedrijven op een voldoende laag niveau te houden. Te denken valt aan mestonderzoek bij zeugen, gespeende biggen en vleesvarkens om het niveau van wormbesmetting te monitoren. Ook geeft het percentage afgekeurde levers van vleesvarkens een goede indruk. Op basis daarvan kan mogelijk een strategie van het afwisselend ontwormen van dieren met een chemisch middel en met een kruidenmengsel voor de verschillende diercategorieën ontwikkeld worden. Nader onderzoek naar deze methode, als ook de geschikte periode voor het verstrekken van deze kruiden aan zeugen in relatie tot drachtstadium en aan gespeende biggen in relatie tot leeftijd en voeropname, is gewenst.

Contents

1 Introduction

The percentage of disapproved livers of growing and finishing pigs has been increased significantly during the last years. In organic pig farms, this percentage is often higher than in conventional pig farms. In most cases, disapproved livers are the result of an infection with *Ascaris suum*. Usually, an infection of *Ascaris suum* is treated or controlled by using conventional synthetic drugs belonging to the benzimidazoles, levamisole and macrocyclic lactones. Organic farmers, however, prefer a non-pharmaceutical approach of worm control. Therefore, phytotherapy could be an perspective alternative. Earlier studies (Gaasenbeek *et al.*, 2004) have shown promising results for the prevention of *Ascaris suum*-infections in pigs when they were fed concentrates supplemented with 5% of a mixture of three herbs. Each pig was infected individually with 1000 worm eggs. Improved results were also found with a dosage of 1% of the herb mixture. A following experiment (Van der Gaag *et al.*, 2005) in which the same herb mixture (dosage 3%) was tested under practical conditions, however, did not show any positive effect. The growing and finishing pigs in the second trial were housed in groups of 6 pigs. To simulate a natural infection, 60.000 worm eggs per pen were put on the floor. The lack of a positive result can probably be explained by a too high infection level.

The mode of action of the herb mixture is probably based on antibacterial qualities. It was assumed that the worms are stunned and/or killed and removed via the faeces. Experts were suggesting that this herb mixture, supplemented with tannins, could even have a larger effect (Van Asseldonk *et al.*, 2006). Tannins were supposed to reduce the number of produced eggs, to reduce the rate of larval development, and to decrease the mobility of larvae of gastrointestinal nematodes (Min en Hart, 2003). Black tea (*Camellia sinensis)* is an example of a tannin rich herb (Van Asseldonk *et al.*, 2006).

The objective of this study was to test herb alternatives for the prevention and control of a mild infection of *Ascaris suum* in growing and finishing pigs. Two different herb mixtures were tested. Feed was supplemented with 3% of a herb mixture, thereby adding 1% *Thymus vulgaris*, 1% *Melissa officinalis* and 1% *Echinacea purpurea* to the diet, or with 4% of a herb mixture, thereby adding the mentioned herbs plus 1% *Camellia sinensis* to the diet. Pigs were infected by 1000 worm eggs each. Comparatively a negative control group (no treatment) and a positive control group (treatment with conventional synthetic drug Flubendazole) were included.

2 Material and methods

2.1 Animals

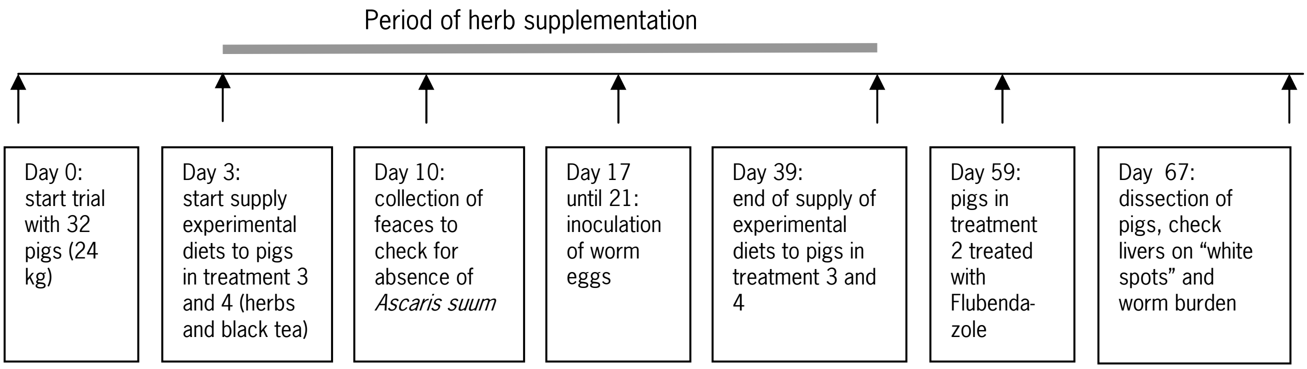
At the facility in Lelystad, The Netherlands, a trial was conducted with 32 young boars (average starter weight was 24 kg) purchased from a SPF-pig farm. The pigs were monitored during 67 days in the period December 2006 until February 2007.

2.2 Experimental treatments

In this study four experimental treatments were compared:
1. negative control: no treatment was applied to prevent or control an infection with Ascaris suum;
2. positive control: pigs were treated with a conventional anthelmintic (Flubendazole) one week before slaughter;
3. herb mixture: pigs were fed a diet supplemented with herb mixture;
4. herb mixture + tea: pigs were fed a diet supplemented with herb mixture (as treatment 3) plus black tea.

All pigs were housed individually and fed a commercial organic starter diet during the first three days. Pigs of treatment 1 and 2 were fed this diet during the whole experimental period. From day 3 onwards, pigs in treatments 3 and 4 received a comparable starter diet supplemented with herbs (1% *Thymus vulgaris*, 1% *Melissa officinalis* and 1% *Echinacea purpurea*), or herbs plus tea (also including 1% *Camellia sinensis*) respectively. From day 17 until day 21, all pigs were infected daily by oral inoculation of 200 *Ascaris suum* eggs. In total 1000 eggs per pig were inoculated. From day 39 onwards, supply of the herb diets was stopped and pigs in treatments 3 and 4 received the same diet as the pigs in treatments 1 and 2. On day 59, pigs in treatment 2 were treated with Flubendazole. On day 67, all pigs were dissected to determine the worm burden in the small intestine. In figure 1 the experiment is described schematically.

Figure 1 Schematic description of the experiment



2.3 Experimental design

The experiment was conducted with 32 growing and finishing pigs (8 pigs per treatment, average starter weight 24 kg). At the start of the experiment, worm status of pigs was checked by counting the number of worms in the manure. All pigs were free from *Ascaris suum*. Pigs were housed individually and assigned to one of the four treatments based on initial weight and origin (mother). The four experimental treatments were allotted to the pens at random.

2.4 Feeding and water supply

Pigs were fed ad libitum by single-space dry feeders. The supplied diets are described in paragraph 2.2. The control diet was a commercial organic starter diet (NE 9.6 MJ/kg; dig. Lysine 8.9 g/kg). The nutrient contents of this diet are presented in Appendix 1. All the diets were pelletised by steam addition; pellets had a diameter of 3.2 mm. Water was provided ad libitum.

2.5 Housing and climate

Pigs were housed in one room with 32 pens (1.5 x 1.5m) with fully slatted plastic floors. The room was equipped with a computer-controlled heating system and a mechanical ventilation system. Room temperature was set up to 20.5 °C.

2.6 Analysis of active components

Samples of the three individual herbs and the four experimental diets were analyzed on free and total phenolic contents. Samples of black tea and the four experimental diets were also analyzed on tannin content. These analysis were performed by PhytoGeniX BV, Department Medicinal Chemistry, Faculty Pharmaceutical Sciences, University Utrecht, PO Box NL-80082, 3508 TB Utrecht, the Netherlands.

Upon receipt, samples were stored in the dark and at room temperature in original packaging. Prior to experiments, samples were ground using a cutting mill (MF10-basic IKA, equipped with a 1,5 mm sieve) and stored in screw-capped glass containers in the dark and at room temperature.

To determine the free and total phenolic content, samples were accurately weighed and extracted in methanol/water (1:1; free phenolic content) or diluted hydrochloric acid in methanol/water (1:12:12; total phenolic content) for 3 hrs at 90˚C according to the protocol described by Vinson *et al.* (1998). After cooling, samples were centrifuged (IEC Centri 8R) for 10 min at 3000 rpm to remove any solids. Supernatants were tested in the Folin-Ciocalteu assay according to a modified version of the method described by Singleton *et al.* (1999).

To determine the tannin content, samples were accurately weighed and extracted in demineralized water for 30 min at 100˚C. After cooling, samples were centrifuged (IEC Centri 8R) for 10 min at 3000 rpm to remove any solids. Supernatants –as such or after preincubation with hidepowder for 30 min at room temperature– were tested in the Folin-Ciocalteu assay according to a modified version of the method described by Singleton *et al.* (1999).

2.7 Data collection

At the start of the experiment, on day 17 (start inoculating worm eggs), day 39 (end of feeding experimental diets) and day 67 (end of the trial) all pigs were weighed individually, and the supplied amount of feed was recorded. These data were used to calculate growth rate per day, feed intake per day and feed conversion ratio for each period. After dissection, livers were checked on the presence of white spots. Also the number of worms in the small intestine were counted.

2.8 Statistical analysis

Daily growth rate, feed intake and feed conversion ratio were analysed using regression analysis (SAS, 1990). The model used was:

$$y = \mu + \text{treatment} + \text{error}$$

The X^2-test was used to determine whether number of pigs without worms in the gastro intestinal tract differed between treatments.

Worm burden in the gastro intestinal tract showed a binomial distribution. The number of worms in the small intestine is expressed as percentage (p) of the number of inoculated eggs (1000). The effect of treatments on p is tested by using the following binomial regression model, after transformation of the data to a logic scale:

$$\text{Logit}(p) = \ln(p/1-p)) = \mu + \text{treatment} + \text{error}$$

where p = number of worms in the small intestine, expressed as percentage of the number of inoculated eggs, μ = overall mean, and treatment is fixed effect of dietary treatment.

Distribution, however, is not pure binomial, due to differences in sensitivity for worm infection in individual pigs. Variation in sensitivity per pig (φ) is estimated as residual term in the regression procedure:

$$\text{variance}(Y \mid p) = \varphi/n\,p(1-p)$$

where φ = difference in sensitivity for worm infection, n = number of inoculated eggs. This variance is used in the statistical model to calculate deviance ratio.

3 Results

3.1 Diets

The active components free and total phenolic content and tannin content were analysed in the tested herbs and in the complete diets. The results of these analyses are presented in table 1.

Table 1 Analysis of the diets for free and total phenolic and tannin content (µm/mg Gallic Acid Equivalent, GAE)

Diet/herb	Free Phenolic content	Total Phenolic content	Phenolics bound (%)	Phenolic content without hide powder	Phenolic content with hide powder	Phenolics as tannins (%)
Echinacea purpurea	39.7	53.7	26.8			
Melissa officinalis	93.1	143.3	35.1			
Thymus vulgaris	95.8	140.7	32.2			
Camellia sinensis	192.1	224.6	14.7	149.9	14.7	90.0
Blanc feed	1.5	7.8	80.0	1.3	0.6	55.2
Feed + herb mixture	3.6	10.8	67.2	2.6	1.0	60.6
Feed + herb mixture + Camellia sinensis	3.4	11.3	69.7	3.1	0.9	72.9

The analysed free phenolic content and total phenolic content in Melissa officinalis and Thymus vulgaris were about 2.5 times higher compared to Echinacea purpurea. Percentage phenolic bounds was more or less similar for these herbs. In Camellia sinensis, however, the concentration of free and total phenols were 100% and 60% higher, respectively, compared to Thyme, whereas the percentage phenolic bounds was only 46%. Based on these results, we expected an increase in free phenolic concentration in feed plus herb mixture, and this feed plus Camellia sinensis of 2.3 and 4.2 µm/mg GAE respectively, while the analysed increase was 2.1 and 1.9 µm/mg GAE. Especially in the feed plus Camellia sinensis, a higher free phenols content was expected. For the total phenolic content, we expected an increase in feed plus herb mixture, and this feed plus Camellia sinensis of 3.4 and 5.6 µm/mg GAE respectively, while the analysed increase was 3.0 and 3.5 µm/mg GAE. Especially in the feed plus Camellia sinensis, a higher total phenols content was expected. Percentage phenolic bounds in feed plus herb mixture, and this feed plus Camellia sinensis showed a decrease of 13 and 10%, respectively. Addition of 1% Camellia sinensis to the diet should increase phenolic content without hide powder by 1.5 µm/mg GAE, while the analysed increase was 0.5 µm/mg GAE. Addition of 1% Camellia sinensis to the diet should increase phenolic content with hide powder by 0.15 µm/mg GAE, but the analyses showed a small decrease in this content. As expected, addition of Camellia sinensis to the diet clearly increased percentage phenols as tannins.

3.2 Worm parameters

Table 2 Effect of dietary treatments on worm parameters in growing and finishing pigs after infection with *Ascaris suum*

	No treatment	Flubendazole	Herb mixture	Herb mixture + black tea	Significance[1]
Number of pigs	8	8	8	8	
Livers with white spots (%)	100	100	100	100	
Number of pigs with worm infection	6[a]	0[b]	5[a]	5[a]	*
Number of worms per infected pig	48[a]	0[b]	23[ab]	41[a]	*
Variation (sd) in number of worms	65	0	29	43	
Adult worms (% of inoculated eggs)	4,8	0,0	2,3	4,1	n.s.

[1] n.s. = not significant; * = (p< 0,05)

In all treatments, 100% of the livers showed white spots, indicating that inoculated worm eggs developed into adult worms in all pigs. The number of worm-infected pigs were similar for both the herb supplemented and the unsupplemented treatments (5 to 6 from 8), while the treatment with flubendazole resulted in 0 infected pigs. Addition of the herb mixture to the diet almost significantly reduced the number of worms per infected pig (48 vs

23) compared to unsupplemented diet. Addition of herb mixture plus black tea did not affect the number of worms per infected pig compared to unsupplemented diet. Percentage of adult worms did not differ between treatments.
Worm parameters per individual pig are presented In appendix 2, showing that worm parameters varied enormously per pig within treatments, except for the Flubendazole-group.

3.3 Performance

The performance of the growing and finishing pigs from start of the experiment to day 17 (start inoculating worm eggs), from day 17 to day 39 (end of feeding experimental diets), from day 39 to day 67 (end of the trial), from day 0 to day 39 and from day 17 to day 67 are presented in table 3.

Table 3 Performance of growing and finishing pigs after different treatments to control worm infection with *Ascaris suum*

	No treatment	Fluben-dazole	Herb mixture	Herb mixture + black tea	SEM[1]	Signifi-cance[2]
Number of pigs	8	8	8	8		
From start trial to start worm infection (D0 – D17)						
Body weight D0 (kg)	23.9	23.9	23.9	23.9		
Body weight D17 (kg)	36.2	37.8	36.3	35.6		
Growth (g/d)	722	818	730	686	35.9	#
Feed intake (kg/d)	1.46	1.61	1.51	1.41	0.068	n.s.
Feed conversion ratio	2.02	2.00	2.06	2.06	0.063	n.s.
From start worm infection to end of supplying herb diets (D17 – D39)						
Body weight D17 (kg)	36.2	37.8	36.3	35.6		
Body weight D39 (kg)	58.0	59.6	57.4	55.8		
Growth (g/d)	993	989	961	915	27.3	n.s.
Feed intake (kg/d)	1.99	2.01	1.95	1.84	0.063	n.s.
Feed conversion ratio	2.00	2.04	2.03	2.01	0.039	n.s.
From end of supplying herb diets to end of the trial (D39 – D67)						
Body weight D39 (kg)	58.0	59.6	57.4	55.8		
Body weight D67 (kg)	87.2	89.5	85.4	85.9		
Growth (g/d)	1043	1069	1000	1078	36.4	n.s.
Feed intake (kg/d)	2.38	2.56	2.34	2.36	0.095	n.s.
Feed conversion ratio	2.29	2.40	2.34	2.20	0.067	n.s.
From start of the trial to end of supplying herb diets (D0 – D39)						
Body weight D0 (kg)	23.9	23.9	23.9	23.9		
Body weight D39 (kg)	58.0	59.6	57.4	55.8		
Growth (g/d)	875	914	860	815	27.1	n.s.
Feed intake (kg/d)	1.76	1.84	1.75	1.65	0.059	n.s.
Feed conversion ratio	2.00	2.02	2.04	2.03	0.034	n.s.
From start worm infection to end of the trial (D17 – D67)						
Body weight D17 (kg)	36.2	37.8	36.3	35.6		
Body weight D67 (kg)	87.2	89.5	85.4	85.9		
Growth (g/d)	1021	1034	983	1006	28.1	n.s.
Feed intake (kg/d)	2.21	2.32	2.16	2.13	0.075	n.s.
Feed conversion ratio	2.16	2.25	2.21	2.12	0.045	n.s.

[1] SEM = pooled standaard error of the means (gives an indication of the accuracy of the estimate of the variable measured)
[2] n.s. = not significant; # = (p < 0,10)

Over the period of day 0 to day 17, daily gain of the flubendazole group seemed to be higher compared to the both herb groups ($P < 0.10$). Daily gain of the flubendazole group over that period seemed also to be higher compared to the control group, although until day 17 treatment of pigs was fully similar for both groups.
Performance of pigs in the other periods or during the whole experimental period was not significantly different between treatments.
No clinical health problems were noticed and no pigs were veterinary treated during the trial.

4 Discussion and conclusions

4.1 Contents of active components

Based on the measured phenolic contents of the individual herbs, in the diet with herb mixture a theoretical increase in free and total phenolic content of 2,3 and 3,4 µg/mg GAE, respectively, can be calculated. Measured values are 88,6% and 89,0% of these theoretical values. Likewise, in the diet with herb mixture and black tea a theoretical increase in free and total phenolic content of 4,2 and 5,6 µg/mg GAE, respectively, can be calculated in the diet . Measured values are only 45,3% and 61,6% of these theoretical values. These differences in theoretical and experimental values of free and total phenolic content, in particular in case of the data concerning herbal mixture plus black tea, might be explained by the presence of tannins. Due to their strong protein-binding capacity (Haslam, 1996), it is likely that tannins have formed insoluble complexes with these feed components resulting in their removal during the extraction procedure and, consequently, an attenuated reactivity in the Folin-Ciocalteu assay.

4.2 Worm infection

Because in all livers more or less white spots were found, it is concluded that the used method to infect pigs with worms (inoculation of 200 worm eggs during 5 consecutive days) was highly effective. This also means that part of the worm eggs developed to mature worms in all pigs. At the end of the experiment, on average 48 worms per worm positive pig were found in the negative control group. Similar number of worms (70) were found in the untreated pigs in the experiment of Van der Gaag *et al.* (2004), who also infected individually housed growing-finishing pigs with 1000 *Ascaris suum* eggs.

In the current experiment no effect of the tested herb mixture (without black tea) on the number of worm positive pigs was found, whereas the number of worms per infected pig was only non-significantly reduced. These findings did not accord with the results of Van der Gaag *et al.* (2004), while herb mixture and number of inoculated worm eggs were similar in both experiments. In the experiment of Van der Gaag *et al.* (2004) a significant effect of herb mixture, tested in two concentrations (1% and 5%), on reduction of percentage worm positive pigs and number of worms in the small intestine of worm positive pigs was found. The reason for the differences in both findings are not fully clear. In the experiment of Van der Gaag *et al.* (2004), herb supplemented diet was fed until the end of the experimental period (day 77), while we stopped feeding herb-rich diet at day 39. We stopped earlier, because we expected only a positive effect of herbs on worm burden during the first two weeks after egg inoculation, during the developing phase from egg to adult worm. Probably, the tested herbs could have an anthelmintic effect on adult worms, or the immune system of the pig was more triggered by long term feeding of these herbs. The positive results of Van der Gaag *et al.* (2004), however, could not be confirmed in a study under practical conditions. In that study, the effect of supplementation of 3% of the same herb mixture to the diet was tested in group housed pigs that were infected with 60,000 worm eggs spread on the floor of each group (Van der Gaag *et al.*, 2005).

Based on findings in literature, we expected that the tested herbs plus the tannin-rich black tea, would have additional effects on worm burden, compared to the herb mixture without black tea. Tannins have shown to reduce the number of worm eggs hatching, to reduce the rate of larval development and to decrease the mobility of larvae in sheep (Min en Hart, 2003). Addition of 3% of Quebracho extract, containing 73% of condensed tannins, to a sheep diet reduced worm burden (*Trichostrongylus colubriformis)* during a 10-week experiment (Athanasiadou *et al.*, 2000). Fertility and performance, however, were also reduced in that experiment.
In the current experiment, however, no additional effect of herb mixture with black tea on the number of worm infected pigs was found. Surprisingly, the number of adult worms found in the small intestine of infected pigs was even numerically lower in pigs fed the herbal mixture without black tea compared to pigs fed the mixture plus black tea. Tannin concentration, but also free and total phenolic content, was higher in the diet with black tea compared to the herb diet without black tea. In sheep and goats, a positive dose – response ratio was found for tannin concentration and reduction in faecal worm egg count (Min en Hart, 2003). Probably, tannins have stronger anthelmintic effects in sheep than in pigs, or anthelmintic effect against *Trichostrongylus colubriformis* in sheep and goats can not be translated to *Ascaris suum* in pigs.

Because all eight pigs in de the Flubendazole-group were free of worms, Flubendazole appears to be an effective deworming product.

4.3 Performance

Marked is the absolute difference in growth and feed intake between the two groups, no treatment and Flubendazole, in the period from start of the experiment up to start of the worm infection, although they were treated the same. Pigs were placed in the room randomly and next assigned to one of the treatments based on initial weight and origin (mother). The impression is that part of the pens were influenced by way of ventilation negatively, due to the location in the room related to air supply and removal.

4.4 Conclusions

A diet with a herb mixture containing 1% *Thymus vulgaris*, 1% *Melissa officinalis* and 1% *Echinacea purpurea* for growing and finishing pigs does not decrease the number of pigs which are infected with *Ascaris suum*, but does reduce the average number of worms in the gastro intestinal tract. The addition of 1% black tea to this herb mixture does not result in a lower number of infected pigs and also does not reduce the average number of worms in pigs. Flubendazole appears to be an effective deworming product.

4.5 Practical implementation

On organic farms with a low worm infection rate, probably a combination of conventional synthetic drug and a diet with herb mixture containing 1% *Thymus vulgaris*, 1% *Melissa officinalis* and 1% *Echinacea purpurea* is an option. It depends on the level of worm infection whether it is an opportunity to deworm breeding sows, weaners and/or growing finishing pigs with a diet containing the herb mixture to keep the level of *Ascaris suum* at a sufficient low level. Examination of faeces of breeding sows, weaners and growing and finishing pigs regularly, and also the percentage of disapproved livers of growing and finishing pigs, can support to monitor the level of worm infection on the farm. Based on this monitoring probably a strategy of varying deworming with a synthetic drug and a diet with herb mixture can be developed for the different categories of pigs. Further research on this method, and also the suitable period to supply this herb mixture to breeding sows related tot stage of pregnancy and weaners related to age and feed intake, is desirable.

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Appendices

Appendix 1 Nutrient contents of the control diet (g/kg as-fed base)

Nutrient contents (g/kg)	Control diet
Dry matter	876.7
Crude protein	187.2
Fat	50.3
Crude Fibre	51.6
Crude ash	51.3
Starch	356.1
Sugars	42.5
Calcium	6.5
Phosphorus	5.3
Digestible Phosphorus	2.7
Sodium	2.1
Potassium	8.3
Nett Energy (MJ)	9.6
Digestible Lysine	8.9
Digestible Meth.+Cyst.	5.2
Digestible Threonine	6.0
Digestible Tryptophan	1.7

Appendix 2 Number of worms on day 67 in the gastro intestinal tract in growing and finishing pigs after different treatments to control worm infection with *Ascaris suum*

	No treatment	Flubendazole	Herb mixture	Herb mixture + black tea
Pig 1	0	0	71	0
Pig 2	198	0	16	82
Pig 3	37	0	0	0
Pig 4	47	0	3	0
Pig 5	0	0	0	40
Pig 6	18	0	29	28
Pig 7	72	0	64	65
Pig 8	12	0	0	115
Average number of worms	48^{a}	0^{b}	23^{ab}	41^{b}
Standard deviation	65.5	0.0	29.4	43.0