Effect of some herbs as alternative for conventional treatment of Ascaris suum in pigs

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In Nederland vindt het meeste onderzoek voor biologische landbouw en voeding plaats in voornamelijk door het ministerie van LNV gefinancierde onderzoeksprogramma's. Aansturing hiervan gebeurt door Bioconnect, het kennisnetwerk voor de Biologische Landbouw en Voeding in Nederland (www.bioconnect.nl). Hoofduitvoerders van het onderzoek zijn de instituten van Wageningen UR en het Louis Bolk Instituut. Zij werken in de cluster Biologische Landbouw (LNV gefinancierde onderzoeksprogramma's) nauw samen. Dit rapport is binnen deze context tot stand gekomen. De resultaten van de onderzoeksprogramma's vindt u op de website www.biokennis.nl. Vragen en/of opmerkingen over het onderzoek aan biologische landbouw en voeding kunt u mailen naar: info@biokennis.nl

Abstract
An experiment was conducted to test the preventive effect of different herbs (Papaya, Boldo leaf and Artemisia) 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, anthelmintic effect

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Samenvatting
Er is een experiment uitgevoerd om het preventieve effect van een aantal kruiden (papaja, boldoblad en bijvoet) op een milde besmetting van *Ascaris suum* bij vleesvarkens na te gaan. Resultaten worden in dit rapport besproken.

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

ANIMAL SCIENCES GROUP
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Rapport 169

Effect of some herbs as alternative for conventional treatment of Ascaris suum in pigs

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Samenvatting

Het percentage aan de slachtlijn afgekeurde levers van vleesvarkens vertoont de laatste jaren een duidelijk stijgende lijn. Op biologische varkensbedrijven ligt dit percentage bovendien 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 de biologische sector geeft men de voorkeur aan het gebruik van natuurlijke stoffen (die het milieu minder schade berokkenen) bij het beheersen van de wormbelasting.
Het doel van dit onderzoek was na te gaan wat de preventieve werking is van drie enkelvoudige kruiden, elk met een verschillend vermeend werkingsmechanisme (proteolytische enzymen, alkaloïden, bitterstoffen), op een milde besmetting met spoelwormen (*Ascaris suum*) bij vleesvarkens. Het besmettingsniveau was 1000 wormeieren per vleesvarken. Ter vergelijking is een negatieve controle (geen behandeling) in het onderzoek meegenomen.

Het onderzoek is uitgevoerd met 32 individueel gehuisveste beertjes, afkomstig van een SPF-bedrijf. De dieren zijn opgelegd bij een gewicht van gemiddeld ruim 31 kg en gedurende 67 dagen gevolgd. De volgende vier proefbehandelingen zijn vergeleken:
1) negatieve controle: aan de dieren is géén middel verstrekt om wormbesmetting tegen te gaan. Er is een commercieel biologisch startvoer verstrekt.
2) Papaja: 1% toevoeging aan het commercieel biologische startvoer.
3) Boldoblad: 1% toevoeging aan het commercieel biologische startvoer.
4) Bijvoet: 1% toevoeging aan het commercieel biologische startvoer.

Vanaf opleg is aan de vleesvarkens het betreffende startvoer verstrekt. In de periode van 17 tot en met 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 de kruiden. Alle dieren kregen vanaf die dag hetzelfde commerciële biologische startvoer verstrekt.

De belangrijkste conclusies zijn als volgt:
- Het toevoegen van één van de kruiden papaja, boldoblad of bijvoet aan het voer heeft geen effect op het aantal met wormen besmette varkens; kennelijk werden de wormeieren in aanwezigheid van de kruiden niet direct na de infectie volledig uitgedreven, maar konden ze zich ontwikkelen tot volwassen wormen.
- In vergelijking met de behandeling met bijvoet is er een tendens tot minder wormen in de darmen per besmet varken bij de controlebehandeling en de behandelingen met papaja en boldoblad.
- Er is geen aantoonbaar verschil in groeisnelheid, voeropname en voederconversie tussen de vier proefbehandelingen, ook niet in de verschillende deeltrajecten.

Op basis van deze resultaten wordt niet geadviseerd om de onderzochte kruiden te gaan gebruiken als alternatief voor conventionele behandeling van Ascaris suum bij varkens. Een combinatie van grondig reinigen van de stal plus regelmatig ontwormen met een gangbaar ontwormingsmiddel lijkt de beste strategie om worminfecties bij varkens te voorkomen (Vermeer et al., 2008).

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 current study describes the effects of three individual herbs for the prevention and control of a mild infection of *Ascaris suum* in growing and finishing pigs. In our study, feed (a commercial organic starter diet) was supplemented with 1% of one of the three herbs (Papaya, Boldo leaf and Artemisia). Comparatively, a negative control group (no treatment) was included.
A trial was conducted with 32 young boars (average starter weight was 31 kg) purchased from a SPF-pig farm. All pigs were housed individually and were monitored during 67 days. Pigs of treatment 1 were fed a commercial organic diet during the whole experimental period. Pigs in treatments 2 to 4 received a comparable starter diet supplemented with 1% of one of the herbs Papaya, Boldo leaf and Artemisia , 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 2 to 4 received the same diet as the pigs in treatment 1.

From this experiment it can be concluded that:

- the tested herbs did not affect the number of worm infected pigs; apparently, worm eggs were not completely excreted in the presence of herbs, but they were able to develop to adult worms.
- the number of worms per infected pig was slightly reduced (P<0.10) in the control, papaya and boldo leaf treatments compared to the Artemisia treatment.
- the tested herbs did not affect performance of the pigs.

Based on the results of this experiment we do not advise to use the tested herbs as appropriate alternatives for conventional treatment of *Ascaris suum* in pigs. A combination of thoroughly cleaning the pig house and regularly deworming with a conventional drug seems to be the most effective strategy to prevent infections of *Ascaris suum* in pigs (Vermeer et al., 2008).

Content

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. To validate the former experiment, the similar mixture (1% *Thymus vulgaris*, 1% *Melissa officinalis* and 1% *Echinacea purpurea),* with or without addition of 1% black tea, was tested in individual housed growing and finishing pigs, that were infected with 1000 worm eggs (Van Krimpen et al., 2007). From this experiment it was concluded that the herb mixture did not decrease the number of pigs which are infected with *Ascaris suum*, but reduced 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. Based on these three experiments, we concluded that the tested herb mixture did not seem to be a perspective alternative for conventional treatment of *Ascaris suum* in pigs. Therefore, based on their possible anthelmintic properties against *Ascaris suum* as described in literature, other herbs were selected. Positive anthelmintic effects against *Ascaris suum* in pigs were found with the use of Papaya latex (Satrija et al., 1994). It was suggested that the strong anthelmintic effect of Papaya was due to proteolytic enzymes papain, chymopapain and lysozyme. Another perspective full herb seemed to be Boldo leaf (*Peumus boldus*), because of it's high contents of alkaloids, e.g. boldine, and asaridone (Raintree Nutrition, 2007). Finally, we selected Artemisia (*Artemisia brevifolia)* because of it's powerful anthelmintic effects in sheep (Iqbal et al., 2004) due to a high content of bitter content. The current study describes the effects of these three individual herbs for the prevention and control of a mild infection of *Ascaris suum* in growing and finishing pigs. In our study, feed was supplemented with 1% of one of the three herbs (Papaya, Boldo leaf and Artemisia). Pigs were infected by 1000 worm eggs each. Comparatively a negative control group (no treatment) was included.

2 Material and methods

2.1 Animals

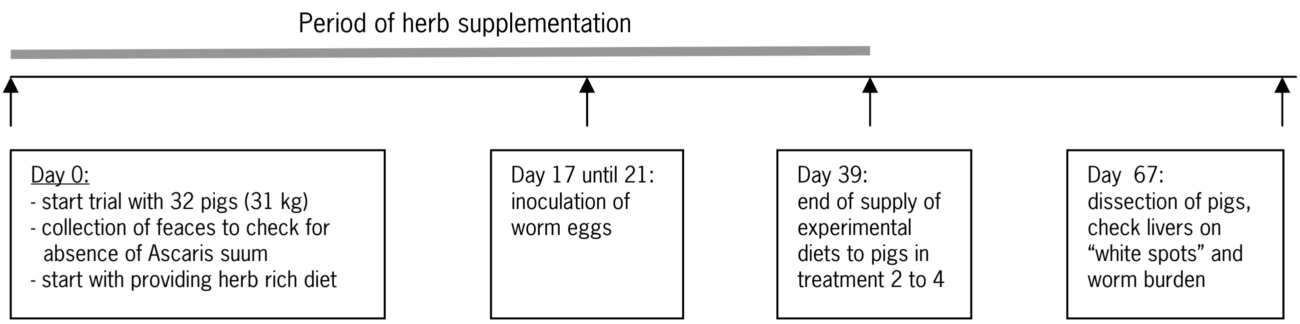
At the experimental facility of Wageningen University and Research Centre in Wageningen, The Netherlands, a trial was conducted with 32 young boars (average starter weight was 31 kg) purchased from a SPF-pig farm. The pigs were monitored during 67 days in the period December 2007 until February 2008.

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; pigs were fed a commercial organic starter diet;
2. Papaya: pigs were fed a commercial organic starter diet supplemented with 1% papaya;
3. Boldo leaf: pigs were fed a commercial organic starter diet supplemented with 1% boldo leaf;
4. Artemisia: pigs were fed a commercial organic starter diet supplemented with 1% artemisia.

All pigs were housed individually. Pigs of treatment 1 were fed the commercial organic diet during the whole experimental period. Pigs in treatments 2 to 4 received a comparable starter diet supplemented with 1% of one of the herbs, 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 2 to 4 received the same diet as the pigs in treatment 1. 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 31 kg). At the start of the experiment, worm status of the pigs was checked by counting the number of worm eggs 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 (ME 13.58 MJ/kg; dig. Lysine 8.8 g/kg). The nutrient contents of this diet are presented in Appendix 1. All the diets were cold pelletised to prevent negative impact of high pelleting temperatures on effectiveness of the herbs. Meal temperature during pelleting varied between 49 (treatment 2) and 53 ^{0}C (treatment 1). Pellets had a diameter of 3.2 mm. Pigs had ad libitum access to water.

2.5 Housing and climate

Pigs were housed in one room with 32 pens (3.2 x 1.0 m) with fully slatted concrete floors. Direct pig to pig contact was not possible in these pens. 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 active components. These analyses were performed by PhytoGeniX BV, Department Medicinal Chemistry, Faculty Pharmaceutical Sciences, University Utrecht, PO Box NL-80082, 3508 TB Utrecht, The Netherlands.

To confirm the identity of the herbal ingredients, appropriate extracts of the dried and powdered plant materials were analyzed for the presence of alkaloids and flavonoids (boldo), flavonoids (Artemisia), or volatile oil components, flavonoids, and free sugars (papaya). Supplemented feeds and the negative control diet were also extracted and analyzed for the presence of alkaloids (boldo-supplemented feed), flavonoids (Artemisia-supplemented), or free sugars (papaya-supplemented feed). Thin-layer chromatography (TLC) was used to detect the characteristic plant constituents. For this purpose, extracts and reference solutions were applied using an automated application device (CAMAG-Linomat IV). After development and spraying with the appropriate reagents, TLC-plates were digitally recorded and analyzed (CAMAG videoscan TLC/HPTLC software, version 1.01.00). Since no validated protocol to confirm the identity of papaya fruit was found in literature, it was decided to characterize this plant material on basis of the presence of several classes of commonly found plant constituents.

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:

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

where φ = difference in sensitivity for worm infection and 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 were analyzed in the tested herbs and in the complete diets. TLC-analysis of boldo confirmed the identity of the plant material since finger-prints of characteristic alkaloids (amongst which boldine) and flavonoids in boldo corresponded to the chemical profiles as previously published in literature (Wagner and Bladt, 1996; European Directorate for the Quality of Medicines - Council of Europe (COE), 2008). Boldo-alkaloids were also detected in the boldo-supplemented feed but not in the negative control diet. On basis of the areas under the curves of the densitograms, it was estimated that boldo was added to the feed in a concentration of 0,5-1,0% (w/w). Since other components in the feed samples interfered in the TLC-analysis, the boldo-content in the supplemented feed sample could not be determined with more accuracy. Nevertheless, the estimated concentration is in line with the specifications of ASG (1% (w/w)).
TLC-analysis of Artemisia confirmed the identity of the plant material since the finger-print of flavonoids and phenylpropanoids in Artemisia corresponded to the chemical profile as previously reported (Wetton *et al.*, 1986). In both Artemisia-supplemented feed and in the negative control diet, spots were detected which were similar in comparison to the phenylpropanoids of Artemisia in color as well as in location in the chromatogram, indicating the presence of these constituents. However, in the negative control diet, these spots were of low intensity, only. The spots in the Artemisia-supplemented feed were of higher intensity and the fluorescence-pattern combined characteristics of both the Artemisia plant material and the negative control diet. Based on the areas under the curves of the most prominent spots in the densitograms of Artemisia and Artemisia-supplemented feed (in the upper part of the chromatogram), it was estimated that Artemisia was added to the feed in a concentration of approximately 0,8% (w/w). Since other constituents in the feed samples significantly interfered in the fluorescence pattern and hampered quantitative analysis, the Artemisia-content in the supplemented feed sample could however not be determined accurately. Nevertheless, the estimated concentration is in line with the specifications of ASG (1% (w/w)).
Papaya extracts were found to contain no volatile oil components or flavonoids, at least not in the dosage-range tested (data not shown). However, papaya-extract was found to produce spots in a TLC-system generally used for the detection of free sugars. Spots with similar color are found in the chromatogram of the negative control diet, indicating the presence of these constituents in the negative control diet, as well. However, due to the high concentrations of free sugars in the negative control diet, the location of the spots in this finger-print slightly differed from that of the papaya reference. The finger-print of papaya-supplemented feed was found to combine characteristics of both papaya and negative control diet, in particular since the intensity of the spots corresponding to the papaya-constituents was selectively increased in comparison to the control feed. Since free sugars are not characteristic for papaya and are also found in large quantities in the feed samples, it was considered to be unfeasible to use these constituents for quantitative determination of the papaya content in papaya-supplemented feed.

3.2 Worm parameters

In table 1, the effect of dietary treatments on worm parameters after infection of the pigs with *Ascaris suum* are presented.

Table 1 Effect of dietary treatments on worm parameters in growing and finishing pigs after infection with *Ascaris suum*

	No treatment	Papaya	Boldo leaf	Artemisia	Signifi-cance[1]
Number of pigs	8	8	6	8	
Livers with white spots (nr.)	6	3	3	6	
Number of pigs with worm infection	3	5	4	5	n.s.
Number of worms per infected pig	21	13	4	58	#
Variation (sd) in number of worms	40	20	5	76	

[1] n.s. = not significant; # = $p<0.10$

White spots were observed in about 60% of the infected animals. White sports were expected at least in all worm infected pigs. During the development of egg to (adult) worm, larvae are migrating through the liver, thereby creating white spots in this organ. In treatment 2 (papaya) and 3 (boldo), however, the incidence of white spots was lower than the incidence of worm infections. About 62 to 66% of the pigs was infected by worms. Moreover, the number of worms infected pigs differed not significantly from the negative control. The number of worms per infected pig was slightly lower in the papaya ($p=0.06$) and boldo leaf ($p=0.08$) treatments compared to the Artemisia treatment.
It can be concluded that none of the tested herbs was effective in completely excreting worm eggs directly after inoculation and in preventing the development of eggs to adult worms in the pigs.

3.3 Performance

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 2. The performance of the pigs that died before the end of the experiment is not included in the calculation of these results. In none of the different periods, significant differences between treatments were observed in daily gain, feed intake and feed conversion ratio. Therefore, it can be concluded that the tested herbs did not affect performance of the pigs.

3.4 Health status

Health status of the pigs was very good, as was shown by a high level of daily gain and a very efficient feed conversion ratio. Some health problems, however, were observed in the boldo leaf treatment. One pig was medically treated for leg problems (Finiclyne/Albipen during three days). Two pigs in the boldo leaf group died; one pig died due to a prolaps, and one pig died due to an unknown reason. It's not known if these health problems were related to the addition of boldo leaf into the diet.

3.5 Cost of herbs

The cost of papaya, boldo leaf and artemisia were € 8.20/kg, € 4.50/kg and € 3.60/kg, respectively. Based on a dosage of 1% and a feed price of € 47,-/100kg, feed cost will increase with 17.4%, 9.6% and 7.7%, respectively.

Table 2 Performance of growing and finishing pigs after different treatments to control worm infection with *Ascaris suum*

	No treatment	*Papaya*	*Boldo leaf*	*Artemisia*	*SEM*[1]	*Significance*[2]
Number of pigs	8	8	6	8		
From start trial to start worm infection (D0 – D17)						
Body weight D0 (kg)	31.5	31.5	31.3	31.5		
Body weight D17 (kg)	45.3	46.9	46.6	45.9		
Daily gain (g/d)	810	906	893	848	51.3	n.s.
Feed intake (kg/d)	1.70	1.82	1.91	1.80	0.102	n.s.
Feed conversion ratio	2.12	2.02	2.15	2.12	0.060	n.s.
From start worm infection to end of supplying herb diets (D17 – D39)						
Body weight D17 (kg)	45.3	46.9	46.6	45.9		
Body weight D39 (kg)	68.1	69.8	68.2	69.2		
Daily gain (g/d)	1040	1040	1002	1057	30.2	n.s.
Feed intake (kg/d)	2.39	2.40	2.38	2.50	0.071	n.s.
Feed conversion ratio	2.30	2.33	2.40	2.36	0.058	n.s.
From end of supplying herb diets to end of the trial (D39 – D67)						
Body weight D39 (kg)	68.1	69.8	68.2	69.2		
Body weight D67 (kg)	99.5	101.0	99.2	101.0		
Daily gain (g/d)	1162	1156	1169	1179	38.0	n.s.
Feed intake (kg/d)	3.00	2.96	3.02	3.02	0.088	n.s.
Feed conversion ratio	2.59	2.55	2.60	2.55	0.071	n.s.
From start of the trial to end of supplying herb diets (D0 – D39)						
Body weight D0 (kg)	31.5	31.5	31.3	31.5		
Body weight D39 (kg)	68.1	69.8	68.2	69.2		
Daily gain (g/d)	939	981	954	966	30.9	n.s.
Feed intake (kg/d)	2.09	2.15	2.18	2.20	0.066	n.s.
Feed conversion ratio	2.22	2.20	2.29	2.27	0.039	n.s.
From start worm infection to end of the trial (D17 – D67)						
Body weight D17 (kg)	45.3	46.9	46.6	45.9		
Body weight D67 (kg)	99.5	101.0	99.2	101.0		
Daily gain (g/d)	1107	1104	1094	1124	29.7	n.s.
Feed intake (kg/d)	2.73	2.71	2.74	2.79	0.060	n.s.
Feed conversion ratio	2.46	2.45	2.51	2.47	0.053	n.s.

[1] SEM = pooled standard error of the mean (gives an indication of the accuracy of the measured parameter)
[2] n.s. = not significant

4 Conclusions

From this experiment it can be concluded that:

- the tested herbs did not affect the number of worm infected pigs; apparently, worm eggs were not completely excreted in the presence of herbs, but they were able to develop to adult worms.
- the number of worms per infected pig was slightly reduced ($P<0.10$) in the control, papaya and boldo leaf treatments compared to the Artemisia treatment.
- the tested herbs did not affect performance of the pigs.

Based on the results of this experiment we do not advise to use the tested herbs as appropriate alternatives for conventional treatment of *Ascaris suum* in pigs. A combination of thoroughly cleaning the pig house and regularly deworming with a conventional drug seems to be the most effective strategy to prevent infections of *Ascaris suum* in pigs (Vermeer et al., 2008).

Appendix 1 Dietary ingredients and calculated contents of the diet (g/kg)

Dietary ingredients (g/kg)	
Barley	287
Maize	167
Peas	106
Oat	100
Potato protein	78
Wheat middling's	75
Soya beans heat treated	68
Molasses	60
Vitamins and minerals	24
Rape seed expeller	24
Acid	7
Vegetable oil	4
Calculated contents	
ME (MJ/kg)	13.58
NE (MJ/kg)	9.67
Crude protein	185
Crude fat	43
Crude fiber	44
Crude ash	56
Dig. Lysine	8.8
Dig. Meth.+Cyst.	5.2
Calcium	7.7
Phosphorus	5.5

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