Ph.D. THESIS

Improving the welfare of pigs raised in low-input production systems by controlling digestive parasites using aromatic and medicinal plants. (Abstract)

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ABSTRACT

Parasitic diseases have a significant impact on pig production, leading to economic losses attributed to high morbidity and mortality rates. This poses a substantial challenge to efficient and profitable livestock management (LAI et al., 2011; KOCHANOWSKI et al., 2017). Parasites, as disease-causing agents, often overshadow other pathogens such as bacterial and viral infections in extensive production systems, contributing to the deterioration of pig health conditions (POPIOLEK et al., 2009). In Romania, the majority of pigs are raised in low-input farming systems, a trend that has been increasing over the past few decades (ICHIM, 2012). These farms, characterized by minimal technological intervention, aim to promote economic and environmental sustainability (POUX, 2008). Digestive parasites represent a major concern for welfare and health in low-input livestock farming (EIJCK et al., 2005).

Due to increasing resistance to chemical treatments, phytotherapeutic remedies have emerged as alternatives for preventing and treating gastrointestinal parasitosis. However, only a few of these remedies have undergone scientific validation (AL-SNAFI, 2016; ZAJÍČKOVÁ et al., 2020). Low-input swine farming in Romania often relies on traditional phytotherapy for controlling pathogens in livestock (BĂIEȘ et al., 2023). Consequently, smallholders and organic producers face significant challenges in this regard. Integrating traditional phytotherapy and husbandry practices through ethno-veterinary approaches could serve as a sustainable developmental tool. The use of phytotherapeutic remedies has gained popularity over the past decade due to their bioavailability, reduced toxicity, and environmentally friendly nature. While phytotherapeutics are utilized worldwide, evidence of their antiparasitic efficacy remains limited (URBAN et al., 2008; AĆIMOVIĆ et al., 2016; BEIGH and GANAI, 2017; AHMED et al., 2018).

The first part (I. Literature review) of the current thesis consists of four chapters, which provide an up-to-date summary of the information available in scientific publications on the following topics:

- The main digestive parasitoses in pigs raised in low-input production systems (I.1);
- Medicinal plants with antiparasitic potential in swine (*Sus scrofa domesticus*) (I.2);
- Low-input farming systems in Romania (I.3);
- Local swine breeds from Romania (I.4);

The second part (II. Original research) comprises 6 original studies focused on assessing, both *in vitro* and *in vivo*, the antiparasitic potential of the following medicinal plants: *Allium sativum* (garlic), *Artemisia absinthium* (wormwood), *Cucurbita pepo* (pumpkin), *Coriandrum sativum* (coriander), *Calendula officinalis* (marigold), and *Satureja hortensis* (summer savory), on naturally occurring gastrointestinal parasites of swine. This research was conducted in two free-range (low-input) farms located in Transylvania. Additionally, the parasitic prevalence in these farms was also evaluated. These are then followed by the conclusions and recommendations sections, along with the originality of the study itself. The cited references conclude the thesis, with 441 titles.

The first chapter of the second part (II.1) presents the seasonal parasitic profile of swine raised on two free-range farms in Transylvania, categorized into three age groups. Nine hundred sixty samples collected from weaners, fatteners, and sows were investigated by flotation, centrifugal sedimentation, modified Ziehl-Neelsen stained fecal smear, modified Blagg technique, and oocyst/egg cultures. The number of oocysts (OPG), cysts (CPG), and eggs (EPG) were counted per gram of fecal matter. The examination revealed parasitic infections with *Balantioides coli (*syn. *Balantidium coli*), *Eimeria* spp., *Ascaris suum*, *Trichuris suis*, *Oesophagostomum* spp., *Strongyloides ransomi* and *Cryptosporidium* spp. Prevalence (P) and the mean intensity (MI) of the infections varied according to age, swine category, farm, and season. The overall prevalence in both free-range farms according to the age category was 63.2%—*Eimeria* spp., 70.31%—*B. coli*, 9.38%—*Oesophagostomum* spp., 3.75% *S. ransomi*, and 18.12% *Cryptosporidium* spp. in weaners. In fatteners *Eimeria* spp. revealed a prevalence of 50.93%, *B. coli*—72.5 %, *A. suum*—63.13%, *T. suis*—39.06%, and in sows *Eimeria* spp.—39.06%, *B. coli*—62.19%, *A. suum*—34.06%, *Oesophagostomum* spp.—27.19%, *S. ransomi*—1.56% and *Cryptosporidium* spp.—

9.38%. The study revealed statistically significant ($p < 0.05$) differences between age groups, seasons, and farms for all diagnosed parasites. Further research is required to better understand the epidemiology of these infections in swine from Transylvania.

The second chapter on of the second part (II.2) contains two studies and provides the *in vitro* antiparasitic potential of six alcoholic plant extracts against *Eimeria* spp. oocysts and *Ascaris suum* eggs. The first study (II.2.1) comparatively evaluated the *in vitro* antiparasitic effects of *A. sativum*, *A. absinthium*, *C. pepo*, *C. sativum*, *S. hortensis,* and *C. officinalis* against *A. suum* egg hatching and larval development. *A. suum* eggs were sampled from randomized fecal specimens collected from traditionally raised swine. The egg suspension (ES, 12×10^3/mL) was divided into two controls (C) (1C—1 mL ES + 1 mL distilled water, 2C—five plates of 1 mL ES + 1 mL ethanol of 70%, 35%, 17.5%, 8.75%, and 4.375%, respectively) and six experimental groups, and placed in 3 mL cell plates. The experimental groups (EG, 1–6) included ES + each alcoholic plant extract (10%, 5%, 2.5%, 1.25%, 0.625%). Both C and EG were performed in quintuplicate. All variants were incubated at 27 °C for a total of 21 days. *A. suum* eggs were examined after 2, 14 (L1), and 21 (L2/L3) days of incubation. The efficacy of all tested plant extracts increased with concentration. Anti-embryogenic effects on *A. suum* eggs were expressed by all plants. A superior influence was observed in *A. sativum, A. absinthium*, *C. pepo,* and *S. hortensis* extracts, at all concentrations tested. *A. sativum* and *A. absinthium* extracts showed the strongest antihelminthic activity, while *C. sativum* and *C. officinalis* were the weakest ascaricids. Future in-depth phytochemical studies are required to identify the compounds responsible for the anthelminthic properties of these plant species.

The second study of this chapter (II.2.2) aimed to investigate the effects of the alcoholic extracts from garlic, wormwood, coriander, pumpkin, summer savory, and marigold on the sporulation of *Eimeria suis* and *Eimeria debliecki* oocysts, isolated from piglets. The stock solution of oocysts (58% *E. suis* + 42% *E. debliecki*) was incubated for three days, before adding the tested solutions. The unsporulated *Eimeria* spp. oocysts were then placed in a 3 mL well plate and incubated for 96 h at 27 °C, in a suspension containing serial dilutions of alcoholic plant extracts (5%, 2.5%, 1.25%, 0.625%, and 0.312%). The percentage of sporulated and destroyed oocysts was recorded every 24 h for 96 h. All extracts had a good *in vitro* anticoccidial effect against oocysts of *Eimeria* spp. compared with the control groups. Oocyst sporulation was significantly ($p = 0.05$) inhibited by the 5% marigold extract (3.6% sporulated oocysts). The same extract had the highest lytic effect on oocysts (65.2% destroyed oocysts). Our results prove that the most effective alcoholic plant extract is the marigold extract, followed, in order of efficiency, by the wormwood, coriander, garlic, pumpkin, and summer savory extracts. This study represents a preliminary contribution for establishing a new generation of natural disinfectants aimed at destroying *Eimeria* oocysts in the context of swine contamination.

The third chapter of the second part (II.3) contains three manuscripts regarding the *in vivo* antiparasitic effects of six Romanian plants against digestive parasites of pigs. The first study (II.3.1) aimed at assessing the antiparasitic potential of *C. pepo* and *C. sativum* against protozoa and nematodes found in swine. The samples were collected from weaners, fatteners, and sows and examined via flotation (Willis and McMaster), active sedimentation, Ziehl-Neelsen staining as modified by Henricksen, a modified Blagg method, and eggs/oocyst culture. The parasite species detected were *Ascaris suum*, *Trichuris suis*, *Oesophagostomum* spp., *Balantioides coli*, *Eimeria* spp., and *Cryptosporidium* spp., depending on age category. A dose of 500 mg/kg bw/day of *C. pepo* and 170 mg/kg bw/day of *C. sativum* powders, administered for ten consecutive days, demonstrated a pronounced anthelmintic (pumpkin) and antiprotozoal (coriander) effect against the aforementioned parasites. Future studies are required to ascertain the optimal dose that maximizes their antiparasitic effectiveness. The current study represents the first Romanian report on the in vivo antiparasitic activity of these two plants tested on digestive parasites in swine.

The second study of this chapter (II.3.2) aimed to evaluate the *in vivo* antiparasitic activity of *C. officinalis* and *S. hortensis* powders against digestive parasites in swine, in two low-input farms from the Transylvania area. The fecal samples were collected from sows, fatteners, and weaners, and were tested using the following coproparasitological methods: centrifugal sedimentation, flotation (Willis, McMaster egg counting technique), Ziehl–Neelsen stain modified by Henricksen, modified Blagg method, and in vitro nematode larvae/protozoan oocyst cultures. Six species of digestive parasites were diagnosed, namely *Ascaris suum, Trichuris suis, Oesophagostomum* spp., *Balantioides coli, Eimeria* spp., and *Cryptosporidium* spp., in various combinations, dependent on the swine category. A dose of 140 mg/kg bw/day of *C. officinalis* and 100

mg/kg bw/day of *S. hortensis* powders administered for 10 consecutive days revealed a strong antiprotozoal and anthelmintic activity on the aforementioned parasites. The curative efficacy can be attributed to the presence of polyphenols, sterols, tocopherols, and methoxylated flavones. In conclusion, our results indicate that *S. hortensis* and *C. officinalis* are promising alternatives to the commercially available antiparasitics, enabling their use as natural antiparasitic products against gastrointestinal parasites in pigs.

The third study of this chapter (II.3.3) focused on exploring the antiparasitic potential of garlic (*A. sativum*) and wormwood (*A. absinthium*) plants native to Romanian's flora and renown for their manifold beneficial properties against naturally occurring gastrointestinal parasites of pigs on two low-input (free-range) farms from NW Romania. The examination confirmed the presence of infections with *Eimeria* spp., *Cryptosporidium* spp., *Balantioides coli, Ascaris suum*, *Oesophagostomum* spp., *Strongyloides ransomi*, and *Trichuris suis*, distributed based on age category. A dose of 180 mg/kg bw/day of *A. sativum* and 90 mg/kg bw/day of *A. absinthium* powders, administered for 10 consecutive days, revealed a strong, taxonomy-based antiprotozoal and anthelmintic activity. The results highlighted the therapeutic potential of both *A. sativum* and *A. absinthium* against gastrointestinal parasites in pigs. Their therapeutic effectiveness may be attributed to the content in polyphenols, tocopherols, flavonoids, sterols, sesquiterpene lactones, and sulfoxide. Further research is required to establish the minimal effective dose of both plants against digestive parasites in pigs.

References:

1. AĆIMOVIĆ, M.G., KOSTADINOVIĆ, L.M., PUVAČA, N.M., POPOVIĆ, S.J., UROŠEVIĆ, M.I., 2016. Phytochemical constituents of selected plants from Apiaceae family and their biological effects in poultry. *Food & Feed Research* 43, 35–41.
2. AHMED E.H., ABADI R.S., MOHAMMED A.M., 2018. Phytochemical screening, chemical composition and antioxidant activity of seeds essential oil of *Coriandrum sativum* L. from the Sudan. *International Journal of Herbal Medicine* 6, 1-4.
3. AL-SNAFI, A.E., 2016. A review on chemical constituents and pharmacological activities of *Coriandrum sativum*. *IOSR Journal of Pharmacy* 6, 17–42.
4. BĂIEŞ, M.H., COTUŢIU, V.D., SPÎNU, M., MATHE, A., COZMA-PETRUŢ, A., MIERE, D., BOLBOACĂ, S.D., COZMA, V., 2023. The effects of *Coriandrum sativum* L. and *Cucurbita pepo* L. against gastrointestinal parasites in swine: An *in vivo* study. *Microorganisms* 11, 1230.
5. BEIGH, Y.A., GANAI, A.M., 2017. Potential of wormwood (*Artemisia absinthium* Linn.) herb for use as additive in livestock feeding: A review. *The Pharma Innovation* 6, 176–187.
6. EIJCK, I.A.J.M., BORGSTEEDE, F.H.M., 2005. A survey of gastrointestinal pig parasites on free-range, organic and conventional pig farms in The Netherlands. *Veterinary Research Communications* 29, 407–414.
7. ICHIM, O., 2012. An overview of organic pig farming in Romania. *Porcine Research* 2, 50–65.
8. KOCHANOWSKI, M., KARAMON, J., DĄBROWSKA, J., DORS, A., CZYŻEWSKA-DORS, E., CENCEK, T., 2017. Occurrence of intestinal parasites in pigs in Poland-the influence of factors related to the production system. *Journal of Veterinary Research* 61, 459–466.
9. LAI, M., ZHOU, R.Q., HUANG, H.C., HU, S.J., 2011. Prevalence and risk factors associated with intestinal parasites in pigs in Chongqing, China. *Research in Veterinary Science* 91, 121–124.
10. POPIOLEK, M., KNECHT, D., BORUTA, O., KOT, M., 2009. Effect of breeding conditions, phenology, and age on the occurrence of helminths in pigs. A preliminary study. *Bulletin of the Veterinary Institute in Pulawy* 53, 213–220.
11. POUX, X., 2008. Low input farming systems in Europe: What is at stake. In *Low Input Farming Systems: An Opportunity to Develop Sustainable Agriculture, Proceedings of the JRC Summer University Ranco, 2–5 July 2007*; Office for Official Publications of the European Communities: Luxembourg, pp. 1–11.
12. URBAN, J., KOKOSKA, L., LANGROVA, I., MATEJKOVA, J., 2008. *In vitro* anthelmintic effects of medicinal plants used in Czech Republic. *Pharmaceutical Biology* 46, 808–813.
13. ZAJÍČKOVÁ, M., NGUYEN, L.T., SKÁLOVÁ, L., STUCHLÍKOVÁ, L.R., MATOUŠKOVÁ, P., 2020. Anthelmintics in the future: Current trends in the discovery and development of new drugs against gastrointestinal nematodes. *Drug Discovery Today* 25, 430–437.