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# IFRO Report



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*Morten Gylling*

**IFRO Report 224**

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## Preface

This is one of two final reports of work package 5 of the research project “Production and use of *Artemisia annua* against parasites and bacterial diseases in poultry stocks”, referred to as the Artemisia Project in this report. The aim of the Artemisia Project is to develop a biological production of *Artemisia annua* for use as a feed additive for broilers against the coccidia protozoa and pathological bacteria. Following four work packages that focus on infection models in poultry, antimicrobial properties of *A. annua*, the cultivation of the plant, and characterization of active compounds for use against infections, work package 5 i) describes a complete production chain for an *A. annua* feed additive concept, ii) assesses the economic potential of the feed additives using a quantitative microeconomic model, and iii) describes and clarifies the legal regulations governing the production and marketing of a coccidiostat feed additive.

This report describes the different phases of the production of two different feed additives based on *A. annua*; ground dried leaves and an extract powder made from microencapsulation through spray drying of the plant extract. Results, data and information from other work packages in the Artemisia Project are used to develop the production chains and form the basis for the microeconomic assessment. Furthermore, a number of private companies in Denmark and abroad and agricultural institutions have contributed with information and ideas during the development of the feed additive production chains.

In a second final report of work package 5, F. Lauritzen, NutriDoc, describes and completes a dossier for the approval by the European Feed Safety Authority of the imaginary *A. annua* based feed additive ArtCox botanical<sup>®</sup>. The two reports supplement each other, but they also stand alone and can be read separately.

Throughout the report references are made to some of the many scientific reports and articles published in the Artemisia Project. These are prefixed with an asterisk in the list of references. Further references to scientific reports and articles can be found at the Artemisia Project’s website:

<https://djfextranet.agrsci.dk/sites/artemisia/offentligt/Sider/Front.aspx>

The Artemisia Project was financed by the Danish Council for Strategic Research (grant number 09-065168) and carried out during the period January 2009 to June 2013.

## Summary

Parasitic and bacterial infections are causing large production losses and economic costs in the European broiler industry, which in 2011 counted more than 6 billion broilers. The parasitic infection coccidiosis is estimated to cost € 285 million annually at EU level and around € 4,8 million in Denmark. Almost similar costs are inflicted by the bacteria causing necrotic enteritis.

The main treatment of coccidiosis, necrotic enteritis and the parasite infection histomoniasis is a prophylactic use of in-feed antibiotic and synthetic coccidiostats and histomonostats. Due to the risk of development of resistance to these therapeutic agents as well as drug residues in food products, the EU is considering phasing them out, as it was done for antibiotics as growth promoters in 2006. However, with no good alternatives the decision to implement the ban is postponed.

The plant *Artemisia annua* L. has been shown to have positive effects on the various broiler diseases, not least coccidiosis, due to the antimicrobial activity of the active compound artemisinin and the essential oils produced in the leaves of the plant. As part of a research project on the use of *A. annua* against the coccidia protozoa and pathological bacteria, two concepts for coccidiostat feed additives based on a Danish cultivation *A. annua* are described and their economic potential is assessed.

A simple feed additive, consisting of the ground, dried *A. annua* leaves, is produced in a mainly agricultural production model. The same dried leaves are used as input in an extended production model for the second feed additive consisting of a spray dried plant extract. Using a microeconomic quantitative model, the production costs of the two additives per broiler are estimated at DKK 0,661 for a dosage of 20 g/kg feed of dried leaves and DKK 0,213 for 3 g/kg feed of extract powder. Compared to costs of DKK 0,070 – 0,208 for two conventional, widely used coccidiostats, only the extract powder is economically competitive.

The cost estimates rely on several parameters that may change. Reduced infection symptoms have been found with very small dosages of *A. annua*, indicating that dosages, and thus the cost, could be reduced substantially once a suitable level of active substances is identified. Reduced artemisinin content, also illustrating inefficiencies in the production, can be expected in a commercial production, leading to increased costs of 33 % when the content decreases 25 %. Crop yields may vary, affecting the cost of dried leaves relatively more than the extract powder. Another major cost component is the production of plantlets. Efficiencies in this component will reduce costs substantially.

The market potential for *A. annua* feed additives, theoretically reaching DKK 1,5 billion at EU level for the extract powder, is limited by the existing coccidiostats and challenged by ongoing developments of vaccines for coccidiosis. However, an EU ban on conventional coccidiostats would change the market. Without an EU ban, the *A. annua* feed additives may target the organic poultry production, albeit a small market (DKK 7 million at EU level for the extract powder) but increasing, allowing for first mover advantages once the demand expands.

In conclusion, the assessment of a Danish production of *A. annua* based feed additives for broilers shows a promising economic potential even before an EU ban on conventional coccidiostats is implemented. The results call for further investigations into the best formulation and production of the additives.

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## 1. Introduction

The production of broilers, which in 2011 counted more than 6 billion in the EU and 106 million in Denmark alone, is challenged by a high prevalence of loss causing diseases such as coccidiosis, necrotic enteritis and blackhead disease (histomoniasis). Coccidiosis, a parasitic disease, alone has been estimated to have a cost of € 0.023 per kg live weight of chickens produced in Sweden, owing to a less efficient feed conversion ratio and cost of prophylactic treatment (Waldenstadt, 2004). Assuming a similar cost level in Denmark and two kg live weight per chicken, the total costs of coccidiosis is around € 4,8 million annually. Extrapolating to the EU level, the total costs amount to € 285 million every year. Almost similar costs may be inflicted on the broiler industry by the bacteria causing necrotic enteritis, which an US study estimated at € 0,035 per chicken, corresponding to € 3,6 million in Denmark and € 217 million in EU (Van der Sluis, 2000). The main treatment of coccidiosis and necrotic enteritis is a prophylactic use of in-feed coccidiostats, such as narasin and salinomycin, two ionophores, and the synthetic product nicarbazin<sup>2</sup>. Due to the risk of development of bacterial and parasitic resistance to the therapeutic agents as well as drug residues in food products (Abbas et al., 2011; Danaher et al. 2008; Mortier et al. 2005), the EU is contemplating phasing out the use of the existing antibiotic and synthetic coccidiostats (EC, 2003), as it was done for antibiotics as growth promoters in 2006. However, as long as there are no alternatives that “... offer the same advantages as the use of coccidiostats as feed additives” the phasing out will not be implemented (EC, 2008, p. 10).

In the search for alternative control of coccidiosis and the replacement of antibiotics in general in animal production, feed additive companies and researchers have turned their focus to plant derived substances (Hashemi et al., 2010; Feed Business Worldwide, May, 2012). The plant *Artemisia annua* L., also known as sweet wormwood, has been suggested as a natural therapeutic agent against the various broiler diseases due to the antimicrobial activity of compounds produced especially in the leaves of the plant (Abbas et al., 2012). The active compound artemisinin, a sesquiterpene lactone, has anti-protozoan properties and is the most widely used drug against malaria in so called artemisinin based combination therapies (ACT). Plant extracts of *A. annua* also possess antibacterial properties due to a high content of essential oils, most notably camphor, 1,8-cineole and Artemisia ketone (Juteau et al., 2002; Ivarsen et al., 2013). Therefore, it is suggested that the plant extract may constitute a novel therapeutic agent against coccidiosis, necrotic enteritis and blackhead due to the combination of the antibacterial and anti-protozoan properties of the essential oils and artemisinin.

*A. annua* is an annual shrub indigenous to China, but today it is also grown and cropped in Africa, India, Pakistan and South East Asia, almost exclusively for the production of artemisinin for antimalarial purposes. The plant is able to grow in a range of climatic zones and environmental regimes, also in the temperate climate found in Denmark. As such, the potential exists for a Danish cultivation of *A. annua* and local

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<sup>2</sup> In the definitions given in EC Regulation No 1831/2003, the often used narasin, salinomycin, monensin and lasalocid are categorized as coccidiostats and antibiotics (as they are derived from *Streptomyces* spp. microorganisms) and all have ionophore properties, while nicarbazin is a synthetically derived coccidiostat with a different mode of action.



processing of the plant for use as a feed additive with coccidiostat and histomonostat properties. The aim of this report is to assess the economic potential of *A. annua* based feed additives produced in Denmark and in competition with conventional coccidiostat feed additives.

### **1.1 Scope and structure of the report**

Based on the results of the Artemisia Project, literature reviews, and information provided by a number of private companies, this report introduces production chains of two feed additives based on *Artemisia annua*, and assesses the economic potential of a Danish production as well as the market potential for the feed additives characterized as plant derived coccidiostats. The report is divided into three parts. First, a set of generic phases in the development process of new feed additives is presented alongside a decision tree that gives an overview of the steps of decisions to either proceed or opt out of a development project. Second, a production chain for a Danish cultivation of *A. annua* and the processing of a plant extract is developed and presented with costs estimates for each phase of the chain. The economic analysis encompass both a final end product consisting of a powder made from spray dried plant extract and an intermediate product based on the dried *Artemisia* leaves. A simple sensitivity analysis is carried out, identifying important assumptions made in the production chain and the related uncertainties in the cost estimation. Third, the market potential for the feed additive is evaluated based on the existing market conditions and assuming a phasing out of antibiotic and synthetic coccidiostats, supplemented with comments on the market for organic chicken meat.

This report is one of two reports from Work Package 5 of the Artemisia Project. In the second report, Lauritzen (2013) develops a dossier for the documentation requirements and associated tests for the approval of the imaginary *A. annua* feed additive ArtCox botanical® by the European Feed Safety Authority (EFSA). Lauritzen (2013) describes the costs of testing and documentation of a feed additive's characteristics, efficacy etc. and is therefore highly informative in regards to these phases of developing and marketing a new feed additive. The two reports supplement each other, but may also stand alone.

## **2 Development of a phytogenic feed additive**

There are no former studies on the costs of developing a new plant derived feed additive. However, for the development of any new veterinary pharmaceutical, there is a number of phases from the first research and development of an idea until the market launch of the final product. In a description of the development of a campylobacter vaccine for broilers, Lund et al. (forthcoming) identify six generic phases in the product development, production and marketing: 1) research and development (R&D), 2) patenting, 3) laboratory experiments and field tests, 4) documentation and approval of the product, its active substance and its efficacy, 5) production and distribution of the end product, and 6) continuous test and improvement of the product. The six phases are also relevant for the development of a plant derived, or phytogenic, feed additive.

The development process of a coccidiostat feed additive based on a Danish cultivation of *Artemisia annua* differs from most other veterinary pharmaceuticals as it includes the development of an agricultural system that produces the base material of the feed additive. Thus, the R&D phase includes reviews of previous research on *A. annua*, both in relation to its cultivation in the northern hemisphere and the antimicrobial and anticoccidial effects of the plant, with the aim of describing a production chain of the feed additive. The R&D phase also includes agricultural field trials for optimal crop yields and content of active substances, followed by analyses of the content of artemisinin and the essential oil components known to have antibacterial effects. The R&D phase develops into the testing phase, where the plant material is tested in different experimental setups, from *in vitro* test of the active substances to field trials using the feed additive, i.e. the dried leaves or a plant extract administered to broilers with the feed or drinking water. During the R&D and testing phases it is possible to apply for patents, e.g. of new formulations of active substances or novel processing methods. This is a protective measure for safeguarding the investments made in the R&D and testing phases.

Knowledge from the R&D phase and results from the testing phase feeds into the documentation and approval phase. Before the *A. annua* feed additive can be marketed as a coccidiostat feed additive, the European Feed Safety Authority (EFSA) has to approve the documentation of the anticoccidial efficacy of the product, as well as a number of other characteristics of the feed additive and the active substances. Lauritzen (2013) describes and completes the formal dossier required for EFSA approval for an imaginary feed additive based on *A. annua*. A commercial production of the feed additive can start once the product has been approved, and provided there is a market for the product. In the case of the *A. annua* feed additive the market potential is heavily influenced by a likely future ban on antibiotic and synthetic coccidiostats. Even after a successful market launch of the feed additive, the product has to be improved and the market value maintained through continuous test and research.

Each phase may last several years and entail risks for the company developing the new product, e.g. realizing increased costs and possibly ultimately a loss if tests prove unsuccessful. In the following section a decision tree depicts how these risks may influence decision making in the development process, from R&D to the production of the feed additive.

## 2.1 Decision tree

The decision tree in Fig. 1 contains the different phases of a product development and outlines the steps of decisions that have to be made, based on project results, towards the market launch of the final product. Each rectangular box in Fig. 1 represents a phase or a process in the project development, associated with certain activities, and/or a decision to carry on or stop the project. Outcomes of each phase are represented by circular boxes.

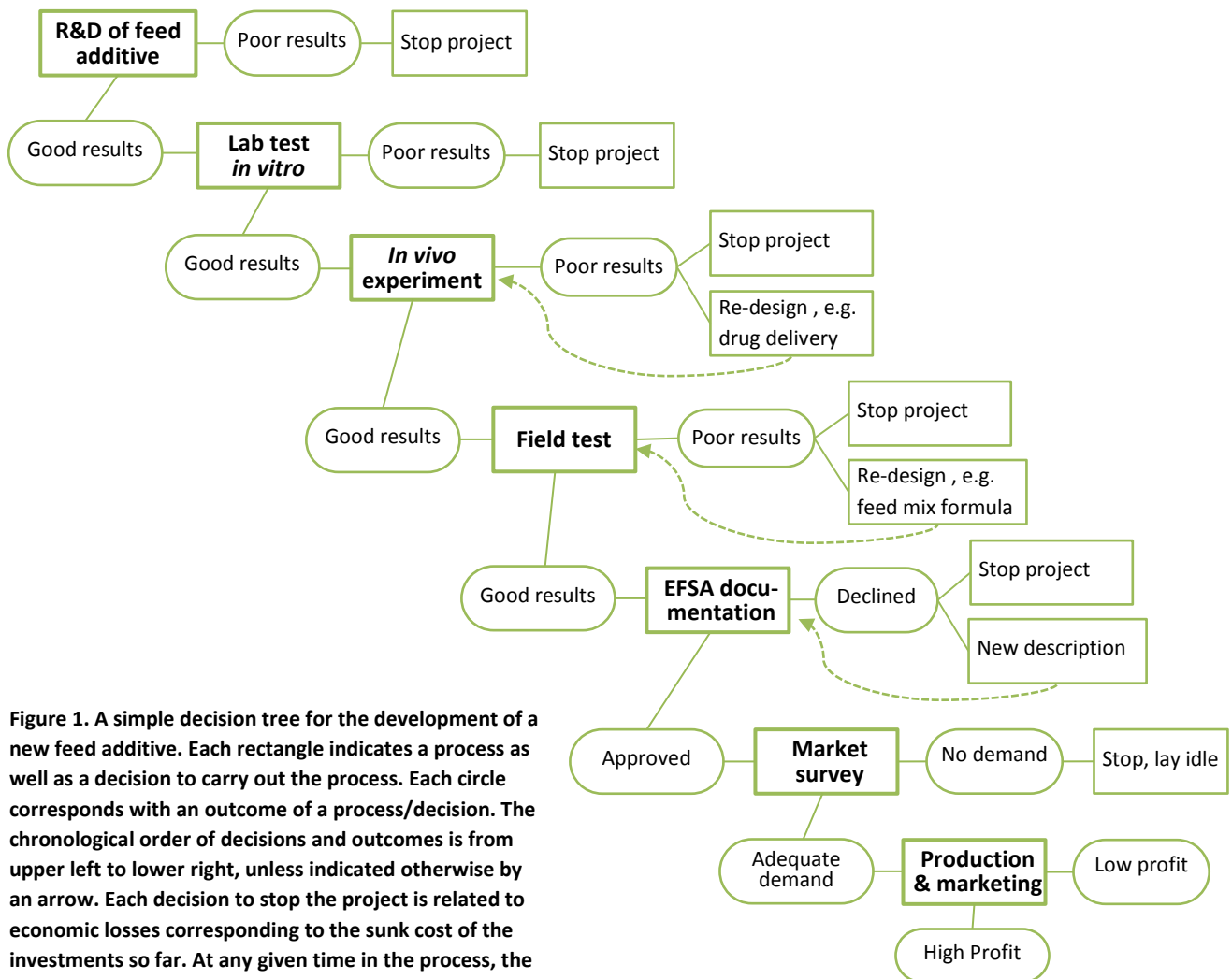


Figure 1. A simple decision tree for the development of a new feed additive. Each rectangle indicates a process as well as a decision to carry out the process. Each circle corresponds with an outcome of a process/decision. The chronological order of decisions and outcomes is from upper left to lower right, unless indicated otherwise by an arrow. Each decision to stop the project is related to economic losses corresponding to the sunk cost of the investments so far. At any given time in the process, the project may be sold and profits realized corresponding to the sale price less investments.

If the R&D phase does not yield promising results the project may be stopped at this time at a loss corresponding to the costs incurred so far. Without a proper yield and content of artemisinin and essential oils in the plant material, the local production of the plant may be substituted with imported dried plant material. However, this would entail leaving the development of a Danish high-value niche crop. On the other hand, if the results are promising, it is decided to carry on with the *in vitro* test in the laboratory in order to test the antibacterial and antiparasitic properties of different formulation of *A. annua* derived substances, whether it is dried plant material, plant extracts or individual active compounds found in the plant.

Depending on the results of the *in vitro* test it may either be decided to carry on with *in vivo* trials or to stop the project if no effect was detected. However, according to an Austrian company working with phytogetic feed additives, the *in vitro* tests are sometimes neglected as positive results may not be replicated in *in vivo* trials, and *vice versa*, *in vivo* tests may show positive results even though *in vitro* experiments would have

shown (discouraging) negative results (Šimerdová, 2010, and pers. com). The seemingly contradictory results of *in vivo* and *in vitro* tests are often due to, sometimes unknown, interactions between active compounds in the feed additive and the gut environment or aspects of *in vivo* drug delivery and mode of action (Šimerdová, pers. com).

Provided laboratory, *in vivo* and field tests show good results of the anticoccidial effects of the feed additive it is decided to complete the EFSA dossier and apply for product approval. If the application is declined, it may be decided to stop the project. However, at this stage it is more likely that a company will decide to adjust the product formulation according to the reasons for rejection, if possible and if economically feasible. On the other hand, if the application is approved, a market survey can be carried out as a basis for deciding to lay idle the product until the market changes (e.g. a ban on coccidiostats) or to proceed with the production and marketing of the product.

Alongside the decisions to carry on the feed additive development, it also has to be decided when to apply for patents. This could occur anywhere between the R&D phase and the final tests of the product, depending on the ability to keep the knowledge of the product development within the company and the presence of similar ongoing product development among competitive companies. Patents may regard the use of the feed additive, the product itself, and/or the production process. In each case there are several aspects that influence when a patent should be applied for, such as costs of applying and the expiration date of a new patent, which calls for an application late in the process. The development of the final product and its marketing may take several years, which implies that a patent approved early in the process may expire before the sale of the product has given a sufficient return on the investment, allowing for competitors to take their share of the profits.

The following sections of this report will focus on the last process in the decision tree, the production phase.

### **3. Production costs analysis of *A. annua* derived feed additive**

In this chapter a quantitative microeconomic model is used to assess the production costs of two plant derived feed additives from a Danish production of *A. annua*. The costs of other phases in the development of new feed additives, as described in chapter 2, will also shortly be commented. The model consists of the phases of production shown in Fig. 2, but is divided into two parts. The first part considers the cultivation of the plant and grinding of the dried leaf material. Studies suggest that dried, ground leaves are more efficient to deliver artemisinin to the bloodstream than the pure form of artemisinin (Elfawal et al., 2012). The dried leaves is an intermediate product in the second part of the cost analysis, where the production chain is expanded with plant extraction and microencapsulation of the extract into a powder ready to be

mixed with a standard poultry feed. Fig. 2 corresponds with the last phase in the decision tree shown in Fig. 1, excluding marketing.



Figure 2. Production phases of a plant derived feed additive made ready to be mixed with a standard feed.

### 3.1 Methodology

The production costs of the *A. annua* feed additives are based on a review of literature and previous studies, online databases, and interviews with private companies engaged in phyto-genic research, plant extraction and drying technologies. To the extent possible, cost data for the analysis is obtained from Danish companies and Danish agricultural databases, while data on yield in the agricultural production and in the extraction processes are based on the results from relevant working packages in the Artemisia research project. The online database 'Farmtal Online' (FTO) is the main source of cost estimates in the agricultural production of *A. annua*. The database, which is managed by the Danish Knowledge Centre for Agriculture (Videncenter for Landbrug), is an aid to Danish farmers during budgeting and planning and contains cost estimates at farm level for agricultural inputs, machinery and labor for different agricultural practices. Data regarding the cost of extraction and drying of the plant extract is not as readily available. A literature search did not identify any adequate studies and therefore cost estimates have been obtained from private companies that establish and run extraction and drying plants.

Yield levels from the plant production as well as the processing of the plant material until and including extraction are obtained from trials and experiments carried out in the Artemisia research project. Results from work package 3 feed into phase 1 to 4 in Fig. 2, while work package 4 feeds into phase 5. Thus, it is assumed that the yields obtained in the agricultural field trials and in the extraction processes can be replicated at a larger scale. Yield figures for *A. annua* is available from other parts of the world, but these are not compatible with the highly mechanized and controlled agricultural production as well as the climate and soil conditions found in Denmark. Most uncertainty is found toward the end of the production chain, as it was not within the scope of the project to carry out plant extraction at a pilot scale and microencapsulation of the extract was not carried out at all. Processing efficiencies are therefore based on estimates from a private, Danish company.

Both artemisinin and the essential oil component of the *A. annua* leaves are important for the therapeutic properties of the studied feed additives. However, since all studies on the use of *A. annua* in broiler health and performance report the artemisinin content and rarely the essential oil contents, the economic assessment of different dosages of feed additives in this report are also based on artemisinin content. Thus,

the combinatorial effect of the artemisinin, artemisinin-related compounds and the essential oil component is not reflected in the resulting cost estimates.

### 3.2. Dry leaves model

This section introduces the production of ground, dried leaves of *A. annua* as a simple feed additive. Dried leaves from the plant have been reported to have a high nutritional potential as a feed supplement due to a high content of protein, minerals and antioxidants, as well as being a source of natural antimicrobial compounds (Brisbe et al., 2009). However, studies on the efficacy of dried *A. annua* leaves as a growth promoter and coccidiostat in the poultry production have shown contradictory results. Brisbe et al. (2008) found that the inclusion of 10 – 20 % dried *A. annua* leaves in the feed increased feed intake and growth and reduced coccidiosis among broilers. Similar positive effects on coccidiosis were found by Allen et al. (1997)<sup>3</sup> and Almeida et al. (2012) with addition of 5 % and 3 % dried leaves. The positive effect on growth could not be confirmed by Engberg et al. (2012), who added between 0,5 % and 2 % dried leaf material to a standard feed and found negative effects on feed intake and growth, as well as no effect on the bacteria causing necrotic enteritis. Engberg et al. (2012) did not investigate the effect on coccidiosis.

In a study of histomoniasis, Thøfner et al. (2012) found an inhibiting effect of dried leaves powder on clonal cultures of histomonas protozoa, with an increasing effect of higher powder dosages. However, the *in vitro* results could not be replicated in an *in vivo* experiment. Cherian et al. (2013) found that dried *A. annua* leaves, added at 2 and 4 % of feed, had an antioxidant potential and improved gut pH towards more protective against pathogenic bacteria. Further, unlike Engberg et al. (2012), they did not find a negative effect on feed intake and body weight when adding 2 % or 4 % of dried leaves to a standard feed. In malaria research, the use of dried *A. annua* plant material as a whole-plant-therapy against malaria parasites in rodents have been shown to be more effective than the administration of pure artemisinin (Elfawal et al., 2012). Besides artemisinin, the derivative compounds like dihydroartemisinin and artesunate also show toxicity towards parasites (Olliaro et al., 2001). The positive effect of the whole-plant-therapy is suggested to be caused by a many-fold increase in the uptake of artemisinin and the derivative compounds in the blood stream, as well as the synergistic effects of several compounds at once, indicating the potential of dried plant material as a natural combination therapy against parasites (Elfawal et al., 2012; Rasoanaivo et al., 2011).

There is a large need for further studies on the efficacy of dried *A. annua* material as an antiparasitic agent and as a growth enhancer among broilers, the latter perhaps aided through masking of the strong flavor of the dried plant. Assuming future research will focus more on these matters, it is interesting to take a preliminary look at the production costs of a feed additive consisting of ground, dried plant material of *A. annua* that can be mixed with a standard feed. Besides the final grinding and sieving of the dried leaves, the

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<sup>3</sup> Only infections with *Eimeria tenella*, the most common species of coccidia, were found to be reduced by the dried leaves. Allen et al. (1997) did not find an effect on *E. acervulina*, another common species of coccidia.

production phases are also found in the production of a plant extract feed additive. Fig. 3 presents the different activities and practices for each of the production phases.

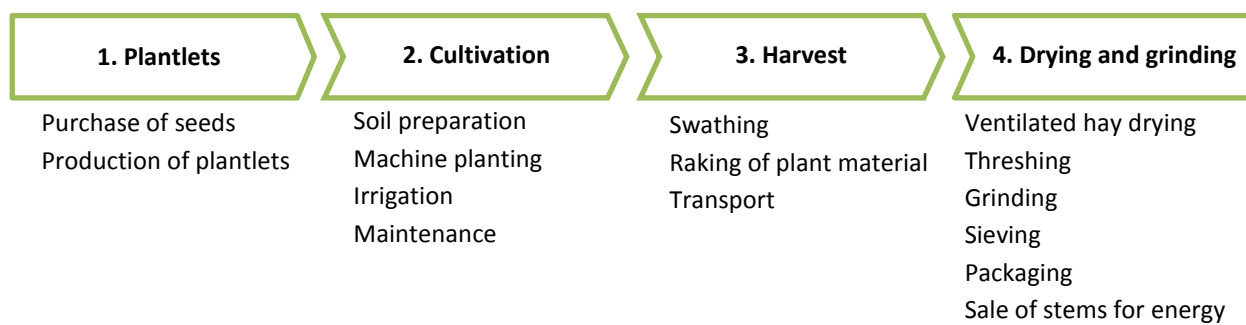


Figure 3. Overview of the production chain for dried plant material, including cost components in each phase.

The following subsections will shortly describe the cost components in each phase. Costs of investments in machinery and materials are included in figures from the FTO database. Only in the case of drying facilities is the investment cost included in the assessment. A summary of the costs and sources of price information is presented in Table 1 following the descriptions of all phases. It is assumed that the cultivation of *A. annua* takes place on an average Danish farm and the existing machinery is adequate for the production, e.g. there are no requirements for additional investments, besides drying facilities.

### **Production of plantlets**

Seeds are bought from Mediplant; a private, Swiss research institute specialized in medicinal plants. The *A. annua* cultivar Artemis (F2 seeds, Mediplant, Switzerland) showed the best combination of artemisinin content and yield from field trials at the Research Station Aarslev, Denmark, and is therefore selected as the most suitable plant material for a Danish cultivation. Estimates of yield and artemisinin content are based on trials and tests involving this specific cultivar. Given the very small size of the seeds (approx. 15.000 seeds/g), the production of plantlets are outsourced to a private plantlet production company at a unit price of DKK 0,5 including delivery (H. Hansen, pers. com.). The highest consistent yields were obtained at a plant density of five plants per m<sup>2</sup> (Grevsen et al., 2012). This corresponds to 50.000 plants/ha, which means that five grams of seeds are required per ha assuming a germination rate of no less than 70 %.

### **Cultivation**

Before planting, the soil is plowed and harrowed to properly prepare the seedbed and increase survival rate of plantlets. Fertilizers are applied corresponding to 100 kg N per ha. To a certain point, increased nitrogen application enhances both plant biomass as well as artemisinin concentration in the leaves (Davies et al., 2009). The plantlets are machine planted in ultimo May or primo June, which requires around 37 man-hours per ha assuming four persons are involved and capable of planting 5.400 plants per hour including time for loading. This approximates the number of man-hours used for machine planting of vegetables grown in open field (O. Scharff, pers.com). Machine costs are based on the use of the farmer's own machinery at marginal costs or rental of planter. It is assumed that no extra planting is required, which

is also the experience from the field trials. Depending on the weather conditions around the time of field establishment, irrigation may be required. In this analysis, it is assumed that irrigation is needed for a total of 10 days during the first three weeks, 10 mm per day. In another field trial site on the Southern part of Funen, Denmark, rabbits were found to be a problem. The use of nonwoven nets, as those often used in cabbage production, is one possible solution; however, here it is assumed not to be necessary. Besides the costs of machine planting, all other costs are derived from the FTO database for machine and labor costs 2013.

### ***Harvest, drying***

The harvest is carried out in October, subject to weather conditions. At this time, the plants at the field trials in Aarslev had reached a height of about 150 cm, with a total biomass production of around 51 tons/ha. This is above yield standards from Africa and Asia but not uncommon for high yield varieties, also when grown in temperate climates (Kumar et al., 2004). There are at least two general harvest solutions depending on choice or availability of facilities to dry the plant material. One is to swath and bale the plant material which may subsequently be dried using different bale drier techniques, either with warm air inlet spikes that dry big bales from the inside and out or by placing two round bales on top a warm air outlet (F. Gergersen, pers. Com). Both systems are in use in Denmark and other European countries to dry hay, e.g. in the production of feed for dairy cows in organic milk production.

The other harvest solution is to swath the plants at 40 cm and leave the plant material on top of the stems, enabling an all-round drying in the field, though subject to weather conditions. This method was used in the field trials and resulted in a reduction of water content from 74 % to 54 % after a two week period. The plants may then be raked and transported to a hay drying facility, e.g. a barn or silo that is equipped with an air flow system that allows for inlet of warm air at the base and an effective outlet of humid air at the top (Oekologi.dk). Drying at relatively low temperatures ensures a better preservation of essential oils and active compounds (Khangouli et al., 2008). The costs of the different combinations of harvest and drying are in the range of DKK 3.400 to 11.000 per ha, with barn drying being the low cost option (oekologi.dk). Barn drying is chosen for this analysis, as it is also a more flexible solution that only requires a modest investment.

The investment costs for the drying facilities are based on several case studies of hay drying facilities in Denmark (Oekologi.dk). With a capacity of drying material from 300 ha, the investment includes refurbishing of an existing barn and installment of warm air ventilators at a total cost of DKK 750.000. Assuming a 30 % scrap value, a 3 % interest rate and a linear depreciation over a ten year period, the average annual cost of the investment is DKK 67.125. It is assumed that the drying facilities are used at full capacity.



### **Threshing and packing**

As the highest content of artemisinin is found in the leaves, the dried plants are threshed and sieved, effectively separating leaves from the stems and yielding about 4 tons of dried leaves per ha, again using numbers from the field trials. The leaf yield may be substantially higher if multiple harvests are carried out instead of just one (Kumar et al. 2004), however, given the operation costs in Denmark, this option is not pursued here. The costs of threshing, sieving and subsequent grinding of the leaves are based on machine cleaning of grain (FTO). It is assumed that the residual stems can be sold as biofuel at the market price of straw used for straw boilers, which is currently 0,5 DKK/kg. After grinding the dried leaves are packed in large bags of 115 kg. The costs of the threshing, sieving, grinding and packing processes may be reduced depending on level of automation. Installment of new machinery to automate this 4-stage process will call for an investment at a certain cost, which has not been estimated in this assessment.

The effects of grinding and of the storage environment on the preservation of efficacy and content of essential oils have not been investigated, but are important aspects to consider. Studies of storage effects on various dried medicinal plants show varying results, from reduced antibacterial properties after 6 months to no effect even after several years of storage (Fennell et al., 2004). The cost of storage is not included in this assessment.

### **Cost summary**

Table 1 summarizes the costs of the production of dried, ground *A. annua* leaves. In order to provide an overview of the costs in each phase of production, also at the farm, the unit costs and machine and labor costs are combined in each phase. The average gross margin of a spring barley production has been added to the total costs in order to show the farmer's required sales value per ha. A Danish *A. annua* cultivation will most likely compete with spring barley.

Table 1 identifies the production of plantlets as the single, most expensive element in the production chain, constituting approx. 60 % of the total costs. The unit price of 0,50 DKK/plantlet is estimated by a Danish plantlet producer based on the costs of production of other plants with small seeds. However, plantlet companies do not yet have experiences with production of *A. annua* plantlets. Management improvements and increased production efficiencies may come with experience and allow for a lower per unit price, which will improve total costs substantially. A unit price of DKK 0,4 would reduce the total production costs with more than 12 %. Further sensitivity analyses are carried out for the complete production chain in section 3.4.

**Table 1. Production costs of a coccidiostat feed additive based on dried, ground leaves of *Artemisia annua* cultivated and processed in Denmark.**

	Quantity/unit	Costs DKK/ha	Yield/ha	Data source
<b>Plantlets</b>				
Seeds	5 g./ha	1,541		Mediplant.ch
Plantlet production	50.000/ha	25.000		JH Planter
<b>Cultivation</b>				
Plowing & harrowing	1	1.025		Farmtal Online
Fertilizer	100 kg N/ha	985		Farmtal Online
Machine planting	37h/ha + machine	7.389		Scharff (2013)
Irrigation 3 weeks	10 d, 10 mm/d	2.180		Farmtal Online
Interrow hoeing	3	1.050		Farmtal Online
<b>Harvest &amp; drying</b>				
Swathing at 40 cm	1	475	44,4 tons	Farmtal Online
Raking after field drying	1	140	25,1 tons	Farmtal Online
Home transport	1	200		Farmtal Online
Insertion in boxes	2 hours/ha	1.100		Farmtal Online
Ventilated hay drying	25,1 tons/ha	1.380	11,0 tons	Oekologi.dk
<b>Threshing &amp; packing</b>				
Threshing & sieving	11 tons/ha	281	4,0 tons leaves	Farmtal Online
Grinding	4 tons/ha	133		Farmtal Online
Packaging & handling	4 tons/ha	512		Estimate
<b>Investments</b>				
Drying facility, annual cost	per ha	224		Oekologi.dk
<b>Total costs and yield</b>				
Total costs incl. investments		43.614		
Sale of stems for energy	7,0 tons/ha	3.509		Farmtal Online
<b>Cost price</b>	<b>4,0 tons/ha</b>	<b>40.106</b>		
<b>Gross margin &amp; sales value</b>				
Avg. of spring barley	1 ha	1.500		Farmtal Online
Required sales value	1 ha	41.606		

### **Costs per broiler**

Leaves of *A. annua* cv. *Artemis* cultivated in the Artemisia Project were analyzed with high performance liquid chromatography (HPLC) and found to have an average concentration of artemisinin of 1,925 % of the leaf dry weight. This is a high percentage compared to earlier studies of the Artemis cultivar (Simonnet et al., 2008; Larson et al., 2013). With an average of 4.026 kg of dry leaves from the field trials, the total artemisinin content is approx. 77 kg/ha, which is also in the high end but comparable to other studies (e.g. Kumar et al., 2004). Table 2 shows the costs per treated broiler based on artemisinin content and dosages of dried leaves used in studies of disease control in broilers by Almeida et al. (2012), Cherian et al. (2013), and Brisbe et al. (2008). Almeida et al. (2012) found positive effects on coccidiosis with a daily intake of leaves corresponding to no less than 4 mg/kg live body weight of artemisinin, which is approx. 5 g of dried leaves per kg feed with the artemisinin content obtained in the Artemisia Project. Though the broilers studied by Almeida et al. (2012) had a much longer growth period (74 days) than in a conventional Danish broiler production and therefore a higher accumulated intake of artemisinin, the concentration of

artemisinin in the broiler at any point of time would be similar or higher with the lowest dosage in Table 2<sup>4</sup>. In the other extreme, Brisbe et al. (2008) likewise found anticoccidial effects, but also growth enhancement, when adding 10 % of *A. annua* leaves to the broiler feed, corresponding to 40 g/kg feed taking into account the differences in artemisinin content found in their study and in the Artemisia Project.

**Table 2. Production costs, including investment in drying facilities, of the dried leaves feed additive per treated broiler at different dosages. It is assumed that each broiler chicken consumes 3.200 g of standard full feed until day 31.**

Dosage leaves	Artemisinin per broiler	# treatments/ha	Costs per treatment
5 g / kg feed	0,31 g	251.638	0,165 DKK
20 g / kg feed	1,23 g	62.909	0,661 DKK
40 g / kg feed	2,46 g	31.455	1,323 DKK

The figures in Table 2 are based on the production of a typical conventional broiler in Denmark, with a growth period of 36 days, a slaughter weight of 2200 g., and a total consumption of 3.630 g of feed given a feed conversion ratio of 1,65 (VFL, 2012). It is assumed that the dried leaves are added to a standard full feed from day 1 until 5 days prior to slaughter and that the accumulated consumption of standard full feed at day 31 is 3.200 g. This means that the dried leaves are assumed to be administered prophylactically, contrary to several studies on the anticoccidial efficacy of dried *A. annua* leaves that are set up to investigate curing of an existing infection. Allen et al. (1997) finds a prophylactic effect of small amounts of dried leaves and very small amounts of pure artemisinin (2 ppm of feed), and suggest that a preventive use requires smaller amounts of feed additive than a treatment against coccidiosis. Under the assumption of a complete and continues use of feed additive, the costs per treated broiler vary from DKK 0,165 to 1,323 for dosages from 5 to 40 g/kg feed. In chapter 4, the costs of the dried leaves feed additive are compared to the costs of the conventional coccidiostats that are currently used in broiler feeds.

### 3.3 Plant extract powder model

Several studies have found anticoccidial effects of extracts of *A. annua* and other *Artemisia* spp., e.g. Arab et al. (2006) and Thøfner et al. (2013). Furthermore, *in vivo* studies carried out in the Artemisia Project found inhibitory effects of *A. annua* extracts on *Clostridium perfringens*, the bacteria causing necrotic enteritis (Fretté et al., 2011, Engberg et al., 2012). A similar effect of the dried leaves could not be found by Engberg et al. (2012). The combinatorial effects on both coccidiosis and necrotic enteritis have become more important since several antibiotics against *C. perfringens* have been banned in the poultry production in the EU, e.g. flavomycin.

Extracts are difficult to store over longer periods of time and the administration of the extract to broilers, e.g. via drinking water, is a more cumbersome procedure than adding a product to a standard feed mixture.

<sup>4</sup> Artemisinin has a short elimination half-life and once present in the blood stream and intestines the compound is broken down completely within a couple of days (Elfawal et al., 2012).

Therefore, this section presents the production of a powder made by spray drying the extract of *A. annua*, thereby encapsulating the essential oils and the artemisinin in microcapsules. The plant extraction produces a substance with a high content of the active compounds, which can then be made readily available as a powder for blending with a standard broiler feed. Microencapsulation through spray drying is widely used in both the food and pharmaceutical industries to transform a liquid substance into a solid powder. The microencapsulation offers protection, masking of strong flavors, and control of release of the substance of interest, as well as a number of other advantages (Ré, 1998; Szejtli and Szente, 2005).

The production of an *A. annua* feed additive in the form of a powder has several advantages over the ground, dried leaves. During the processing of the plant extract, which among other procedures involves distillation of the raw extract, the concentration of artemisinin can be controlled, thereby making it possible to market a standardized product. This is more difficult in the production of a dried leaves product, since the concentration of artemisinin in the leaves is influenced by the growing conditions that may differ from year to year. Through the choice of the material that forms the wall of the microcapsules, the so called carrier material, several characteristics of the feed additive can be improved compared to a feed additive consisting of the dried leaves or the plant extract itself, such as storage stability, control of delivery in the gut environment of the poultry, and palatability.

Microencapsulation protects the core material – the artemisinin compounds and essential oils – against oxidation and other degenerating processes, and increases the storage stability. This, on the other hand, increases shelf life of the product and thereby adds more flexibility in the production and distribution lines of the final mixed feed. The carrier material can be decisive for the control of the release of the core material and thereby the optimization of the active substances' mode of action. Engberg et al. (2012) reported a reduction in feed intake of broilers when pure extracts were used, likely due to the strong and bitter taste of the plant extract. Microencapsulation of the extract can reduce or eliminate the bitter tastes, e.g. when using maltodextrin or cyclodextrin, two starch sugars, as the carrier material (Szejtli and Szente, 2005). Furthermore, the possible dust-related health and operational issues of working with finely ground dried leaves are avoided with a microencapsulated extract with fluid flow properties. Finally, in the extent that essential oils, artemisinin and other artemisinin-related compounds can be contained in the microcapsules, the assumed benefits of the whole-plant-treatment are maintained in the extract powder.

The following subsections describe the phases of the production of the *A. annua* feed additive in its final form as a powder, starting with the production of dried leaves as presented in section 3.2 (Fig. 4). Though described as two phases in Fig. 4, the extraction and microencapsulation are carried out in one continuous process using a processing plant designed and described by a Danish process engineering company. The costs of investment in the plant as well as the operational costs are provided by the same company. A common alternative to investing in a processing plant is to outsource the extraction and

microencapsulation processes to a toll manufacturer. Therefore, two alternative production chains are presented based on a) own investment and production and b) use of toll manufacturing.

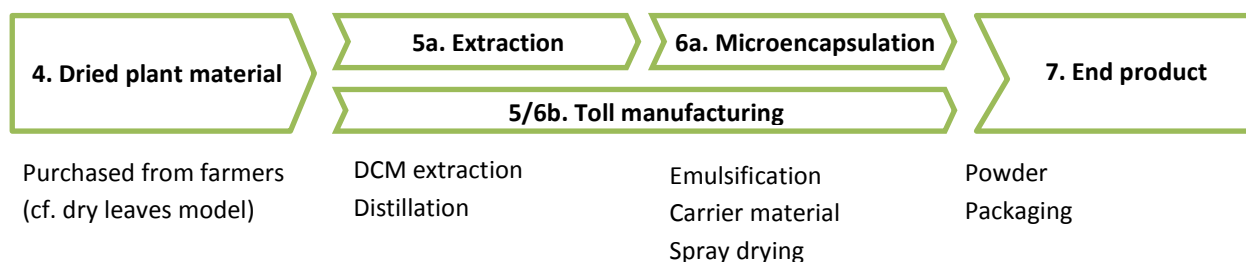


Figure 4. Overview of the production chain for a feed additive based on a spray dried, plant extract of *Artemisia annua*.

### **Dried plant material**

The dried leave material is produced as described in section 3.2, with the exception that the leaves, separated from the stems, are not ground. The costs, as summarized in table 3, include the gross margin to farmers as described earlier.

### **Extraction and microencapsulation**

There are several techniques available to carry out plant extractions, such as solvent extraction that make use of a large variety of solvents, hydro distillation, supercritical fluid extraction, and microwave assisted and ultrasonication assisted extractions (Gupta et al., 2012). Solvent extraction was used in the Artemisia Project. The highest extraction efficiencies in terms of artemisinin content and amount of extract per kg of dry leaves were obtained using dichloromethane (DCM) as solvent. Further, the DCM extract was found to have the highest effect on inhibition of histomoniasis among broilers (Thøfner et al., 2012). This technique is therefore also applied in the production chain described in Fig. 4 and table 3.

Based on data from the Artemisia Project and requirements for the final product, a Danish company described a possible extraction and microencapsulation plant as well as the type and amount of inputs including energy needed for the process. The inputs and their costs, using one ton of dried leaves as reference, are summarized in Table 3. The processing plant consists of i) a continuous, counter-current extraction unit, capable of extracting 345 kg of dried leaves per hour and re-cycling 95 % of the DCM solvent; ii) a distillation tower that refines the extract through partial vaporization and condensation; and iii) a spray drying unit, where an emulsifier (lecithin) and the carrier material are added before the solution is spray dried. Finally, iv) a packaging unit is attached to the plant. The plant is run by two technical operators per shift and a laboratory technician during day hours. An alternative to spray drying is freeze drying, which has the advantage that the essential oil component is not subjected to high temperatures during drying. However, even when high temperatures are used in spray drying, the core material is not directly subject to the heat. Furthermore, the costs of freeze drying was found to be much higher than for spray drying, and this technique was therefore not pursued further.

An estimation of the extraction efficiency of the described processing unit requires trials on pilot scale, which was not within the scope of the Artemisia Project. Instead, data from laboratory DCM extractions is used to estimate the amount of extract per kg of dried leaves and the content of artemisinin in the extract. HPLC showed an average artemisinin content in the dried leaves of *A. annua* cv. Artemis of 1,925 %. However, DCM extraction followed by filtering and refinement with a rotary evaporator yielded roughly 70 g of extract per kg dried leaves, with an artemisinin content of 20,21 %. This corresponds to an effective extraction of artemisinin of 57 kg/ha and 1,42 % of the dried leaves. This gives an extraction efficiency of approx. 74 %, which is within the range normally achieved with standard extraction methods (60 – 80 %) (Cutler et al., 2006). New methods, such as ultrasonic extraction, can improve the efficiency (Briars & Paniwnyk, 2013). A major part of the dried leaves ends up as residual material after the extraction. This can be used either as manure or for energy but requires that environmentally hazardous residues from the extraction process are removed, so the residual material complies with the so called 'Sludge regulations' (E. Olesen, pers. com.). The associated costs of cleaning the residual plant material are assumed to half the value of the material when used for bioenergy.

There is a wide selection of carrier material, incl. Arabica gum, gelatin, starches, maltodextrin, and protein isolates from whey, milk and soy. The choice of material depends on a variety of requirements for the final product, incl. flavor and taste, storage stability, hygroscopic characteristics, size of microcapsules, control of release, and proportion of carrier material versus core material. Maltodextrin is chosen as carrier material for this analysis, as it is commonly used in the encapsulation of food stuffs. However, pilot scale testing of spray drying with different carrier materials is necessary for the identification of the most suitable material or mix of materials.

### ***Investment or toll manufacturer***

The investment in the processing plant is a large cost component. Based on price estimates from a Danish company, the total investment needed to purchase and install the extraction, spray drying and packing plant sums up to DKK 106 million<sup>5</sup>, excluding buildings. With a 30 % scrap value after ten years, a 3 % interest rate and a linear depreciation over a ten year period, the average annual cost of the investment is DKK 4.484 per ton processed leaves assuming the plant runs at full capacity, 7.000 hours a year (K. H. Hansen, pers. com.). An alternative to the large investment in facilities and the costs of hiring specifically skilled labor is to outsource all or part of the processing to a toll manufacturer. Two of Europe's largest plant derivative companies only have equipment to carry out test productions and use toll manufacturers for commercial productions (K. R. Wendler, pers. Com.). The plant derivative company buys the raw material, in this case the dried *A. annua* leaves, retains rights to the product throughout the manufacturing

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<sup>5</sup> The total price given by GEA Niro was 64 mil DKK for a plant with a capacity of 115 kg/hour leaves, and with an estimated cost factor of 1,4 for every factor 2 in capacity, with a maximum capacity x 3 with the same staff requirements.

process, and bears all the inventory and selling risks. The toll manufacturer uses its own facilities and skilled labor force, and purchases all other materials used in the manufacturing process, which follows directives and makes use of know-how from the plant derivative company. The advantage of using a toll manufacturer is not only to avoid the large investment in facilities and skilled labor, but also to benefit from the experience of the toll manufacturer that enables a more cost efficient operation than if the plant derivative company had to run the manufacturing process themselves. A further advantage of toll manufacturing is increased flexibility in the production, as the plant derivative company does not have to consider meeting the maximum capacity of the processing plant. In the summary in table 3 it is assumed that the cost of toll manufacturing is equal to the total operational costs for the extraction and microencapsulation plant described above.

### Cost summary

Table 3 summarizes the costs for the full production model of the plant extract powder, including the cost of dried *A. annua* leaves as reported in Table 2. The costs in are based on the processing of one ton of dried leaves.

**Table 3. Production costs of a plant extract feed additive based on *A. annua*. All costs are based on the processing of 1 ton of dried leaves.**

	Quantity	Costs DKK	Data source
<b>Dried <i>A. annua</i> leaves</b>			
Purchased (Table 2)	1 ton	10.266	
<b>Extraction &amp; Microencapsulation</b>			
DCM solvent 95% reuse	152 kg	167	GEA Niro Online price
Water	713 l	28	Vandcenter Syd
Maltodextrin	609 kg	1.674	Online price
Lecithin	87 kg	383	Online price
Steam (3 & 14 bar)	2.609 kg	183	US Dep. Energy
Power	522 Kwh	485	Dansk Energi
Packaging (25 kg)	28 bags	111	Estimate
Technical operators	5,8 hours	1.649	Lund et al.
Laboratory technician	1,5 hours	562	Lund et al.
<b>A: Investment</b>			
Processing plant, annual costs	Per ton	4.484	GEA Niro
Total costs incl. investments		19.994	
Value of residual material	920 kg/ton	-230	
<b>Cost price</b>	<b>696 kg powder</b>	<b>19.764</b>	
<b>B: Toll manufacturer</b>			
Outsourced processing	Per ton	5.244	Costs as above
<b>Cost price</b>	<b>696 kg powder</b>	<b>15.506</b>	

Table 3 provides an overview of the cost component and an indication of the total costs for a plant extract powder manufactured as outlined above. It should be noted that the costs of extraction and microencapsulation as well as the investments cost are based on specific production plant units and

processes as proposed by a Danish process engineering company. There are other manufacturing processes available, both for the extraction and the microencapsulation. Pilot scale trials are necessary to identify the best processes and materials as well as to create greater certainty of the costs.

### **Costs per broiler**

Table 4 shows the production cost per treatment at different dosages. The dosages are based on concentrations of extracts and artemisinin in studies by Arab et al. (2006) and Thøfner et al. (2013). Arab et al. found anticoccidial effects of an extract of another artemisinin-containing plant *Artemisia sieberi* Bess., when administered to broiler chickens at daily artemisinin concentrations of 3 mg/kg body weight. This corresponds to approx. 1,1 g/kg feed of the extract powder, taking into account differences in artemisinin content. Thøfner et al. (2013) added 200 and 300 mg *A. annua* extract per kg feed and found a dosage dependent, anticoccidial effect. The 300 mg/kg feed corresponds to approx. 3,0 g extract powder per kg feed. Thøfner et al. (2013) also investigated the effect of adding 100 mg/kg feed of pure artemisinin, corresponding to 4,9 g extract powder per kg feed. The dosages in Table 4 correspond to artemisinin concentrations of 23, 61 and 100 ppm. For comparison, Allen et al. (1997) found a positive, prophylactic effect on several coccidia infections<sup>6</sup> with a concentration of pure artemisinin in the feed as little as 2 ppm, i.e. 0,002 g/kg feed.

**Table 4. Production costs of a plant extract feed additive per broiler at different dosages. The dosages are based on previous studies of efficacy of *A. annua* plant extracts. It is assumed that each broiler chicken consumes 3.200 g of standard full feed until day 31.**

Dosage powder	Artemisinin /broiler	# treatments/ton dried leaves	Costs per treatment	
			Own investment	Toll manufacturer
1,1 g/kg feed	0,07 g	193.258	0,102 DKK	0,080 DKK
3,0 g/kg feed	0,20 g	72.868	0,271 DKK	0,213 DKK
4,9 g/kg feed	0,32 g	44.449	0,445 DKK	0,349 DKK

The total artemisinin per broiler, number of treatment per ton of processed leaves at different dosages, and the costs per treatment are based on a feed consumption of 3.200 g per broiler until day 31 with the extract powder added throughout the period. The total artemisinin contents per broiler are lower than for the dosages of dried leaves, see Table 2. This does not necessarily indicate that the plant extract has a higher efficacy against coccidiosis, but at least partly is a reflection of the choice of artemisinin and dried leaves content in the reviewed studies. So far, no study has compared the anticoccidial efficacy of different types of administration of *A. annua*, e.g. extract versus dried plants. The costs per treated broiler vary from DKK 0,102 to 0,445 for feed additive dosage of 1,1 – 4,9 g/kg feed, when investments are made in own process facilities. The costs are reduced to DKK 0,08 to 0,349 when a toll manufacturer is used.

<sup>6</sup> E. acervulina, E. tenella and a double infection of both.



### 3.4 Sensitivity analysis

The costs per treatment summarized in Table 2 and 4 are based on a set of assumptions regarding cultivation, processing, yields, artemisinin content etc. derived from the results of the Artemisia Project. In order to assess the impact on costs from changes in different production parameters, a rough sensitivity analysis is carried out for the most important parameters. The amount of dried leaves from the field may vary yield levels and leaf percentage of total plant. The yield of *A. annua* varies substantially under different growing conditions and practices, even for the Artemis cultivar. The fresh weight yield of plant material per ha and dry weight of leaves reported in section 3.2 are based on average values from 15 trial plots with the Artemis cultivar during two consecutive years. The reported values of 50 and 5 tons/ha, respectively, are based on yields ranging from 36 to 75 tons/ha of fresh weight material and 2,7 to 6 tons/ha of dried leaves. Yield figures for the same cultivar grown elsewhere in Europe are within the same range, e.g. Simonnet et al. (2008). The contents of artemisinin in the dried leaves (1,92 %) and in the DCM extract (20 %) as found in the Artemisia Project were high compared to earlier studies, and it may not be possible to sustain these high concentrations at large production scales. For comparison, Delabays et al. (2001), Simonnet et al. (2008) and Graham et al. (2010) report artemisinin content of the Artemis cultivar at 1,33; 1,43 and 2,0 % leaf dry weight.

Tables 5 and 6 show the percentage change in costs per treatment given changes in agricultural fresh weight yield/ha and artemisinin content in the dried leaves. The extract powder is assumed to be processed by a toll manufacturer. The total artemisinin content per broiler is held constant for each feed additive, which means that the amount of leaves or extract powder change as the artemisinin content changes. The studied change in the two production components takes into account that the artemisinin content obtained in the Artemisia Project is relatively high compared to other studies, i.e. a commercial production is more likely to obtain lower concentrations than higher, while the fresh weight yield per ha is equally likely to be higher or lower. The reduction in yield and artemisinin content may also illustrate losses in the production process, e.g. from the threshing and drying of leaves to reduced extraction efficiencies.

**Table 6. Increase in per treatment costs of dried leaves containing 1,2 g artemisinin at different fresh weight (FW) yields and artemisinin contents in dry weight %.**

Change in treatment cost of leaves at 1,2 g art.			
Artemisinin content, %	FW yield / ha		
	-25 %	51 tons	+25 %
-50 %	169 %	100 %	58 %
-25 %	80 %	33 %	6 %
1,92	35 %	<b>0,661 DKK</b>	-21 %
+10 %	22 %	-9 %	-28 %

**Table 5. Increase in per treatment costs of powder with 61 ppm artemisinin at different fresh weight yields and artemisinin contents in dry weight %.**

Change in treatment cost of powder 61 ppm			
Artemisinin content, %	FW yield / ha		
	-25 %	51 tons	+25 %
-50 %	146 %	100 %	72 %
-25 %	64 %	33 %	15 %
1,92	23 %	<b>0,213 DKK</b>	-14 %
+10 %	12 %	-9 %	-22 %

The price of each treatment changes similarly to changes in the leaves' artemisinin content; when the content is halved, the cost per treatment is doubled as the total amount of artemisinin is fixed. Contrary, a

change in agricultural yield impact the cost of the dried leaf feed additive more as the cost of cultivation makes up a larger part of the production costs. The two extreme changes represented in each of Table 5 and 6 (upper left and lower right) correspond to costs ranging from DKK 0,167 – 0,524 for the extract powder and DKK 0,476 – 1,782 for the dried leaves.

The unit costs of agricultural practices based on data from FTO include labor costs and it is therefore difficult to analyze the individual effect of wage increases. There may also be general cost increases due to rising costs of inputs such as energy, as well as cost reductions due to e.g. improved efficiency. Table 7 provides an overview of the change in costs per treatment in the event of a 20 % cost increase or reduction in each of the different production phases.

**Table 7. Changes in treatment costs given changes in costs of +/- 20 % in specific production phase.**

<b>+/- 20 % change in production costs</b>		
	<b>Dried leaves</b>	<b>Powder</b>
Plantlet production	12,76 %	8,50 %
Cultivation & harvesting	6,46 %	4,31 %
Drying, threshing, packing	1,64 %	1,00 %
Extraction & encapsulation		6,76 %

With the same argument as above, the dried leaves feed additive is influenced most by the change in costs in the agricultural production. The production of plantlets is a major cost component in both models. The Danish plantlet manufacturer estimated a price of DKK 0,5 per plantlet, but it is not unlikely that given more experience and a large scale production the price could be reduced to DKK 0,4, resulting in a cost reduction per treatment of more than 12 % and 8 %, respectively.

A reduction in costs may also be realized through higher extraction efficiency. The laboratory DCM extractions performed in the Artemisia Project extracted approx. 74 % of the artemisinin present in the dried leaves. Other techniques are more efficient. Cutler et al. (2006) report supercritical fluid extraction using CO<sub>2</sub> to reach 82 % in efficiency, but at much greater costs than the more widely used solid-liquid extractions, such as the DCM extraction. More recent techniques include microwave and ultrasound assisted extraction which further increase artemisinin content in plant extracts (Briars & Paniwnyk, 2013). Considering the cost of the extract powder feed additive, reaching an extraction efficiency of 90 % would reduce the costs per treatment with 18 %, assuming identical extraction costs.

The change in costs per treatment reported in Tables 5 – 7, as well as the dosages used to calculate the per treatment costs in Tables 2 and 4, are based on the content of artemisinin. As described earlier, there is also an antibacterial effect of artemisinin-related compounds and the essential oils, e.g. camphor. These

are not necessarily negatively affected by a reduction in artemisinin content. This is not taken into account in the cost sensitivity analyses.

### 3.5 Other development costs

As described in the introductory chapter to this report, the emphasis in the economic evaluation is on the cost of producing the *A. annua* feed additives. Section 2 shortly introduced other costs associated with the development and marketing of a feed additive, most notably the cost of R&D and obtaining an EFSA approval based on extensive documentation of the final product's efficacy, characterization of the active substance, etc. Lauritzen (2013) provides a detailed account of the required documentation. It is difficult *a priori* to estimate the costs of R&D and documentation for product approval as these costs will vary substantially between products and type of companies involved. One of the few studies that investigate the R&D costs of new veterinary products estimates the costs of a campylobacter vaccine for broilers to be approx. € 2 mil. (Lund et al. forthcoming). Their estimate is based on R&D experiences from a Danish research project involving universities. This is a common setup that nonetheless differs from vaccine development within a private veterinary pharmaceutical company, where R&D of new products is an ongoing process. Therefore, it may be difficult to isolate the R&D costs for a single product. Acknowledging this, Lund et al. estimate the general cost level for R&D for a new vaccine to be approx. € 1 mil., but the authors note that it may be considered as low and that true costs are nearly impossible to estimate.

In order for a coccidiostat feed additive to be approved by the European Feed Safety Authority (EFSA), an extensive documentation of the product's characteristics and manufacturing is needed, e.g. composition and purity of the additive, properties of the active substance, stability, interaction with other substances, health and safety issues, etc. The full list of required documentation can be found in EFSA's guidance for dossiers for coccidiostat feed additives (EFSA, 2011). The accumulated cost in this phase can be as high as the R&D costs, depending on the success of tests and the application for product approval, among other things (cf. the decision tree in section 2.1). Lauritzen (2013) estimates the costs of a complete testing and documentation phase for the *A. annua* feed additive to reach as much as 1 mil. €, but also notes that the realized costs are often less, in the area of € 0,5 mil., due to a number of reasons. These reasons include differing salary levels and use of labor where the cost of labor is lowest, and the re-use of documentation for earlier, related products. Costs may also be cut by not carrying out *in vitro* tests and instead replacing them with literature reviews, moving directly to *in vivo* tests (Lauritzen, 2013). This is practiced among several phytogenic companies in Europe (V. Šimerdová, pers. Com). An Austrian phytogenic company reports costs of testing and documentation to be between € 0,5 – 1 mil., depending on the level of information and data already at hand (K. R. Wenders, pers. Com.). This is in line with Lauritzen (2013). Lund et al. (forthcoming) estimate a lower cost of approx. € 300.000 for testing and documentation in the case of a campylobacter vaccine and assuming the vaccine is approved in the first round.

Both the cost of the R&D phase and the cost of testing and documentation are sunk costs, incurred only once during the development, production and marketing of a product. Costs that are incurred continuously include the cost of distribution. Feed additives that are standard in the conventional broiler production, such as coccidiostats, are predominantly purchased by feed manufacturers and mixed with the feed before sale to retailers or farmers. This means that the distribution of the feed additives is generally limited to a few buyers, who purchase directly from the producer. In their study of a vaccine for broilers, Lund et al. (forthcoming) use an estimate for the distribution costs of DKK 0,075 (€ 0,01) per vaccine. While it has not been possible to find distribution costs of feed additives of any kind from empirical studies, the cost is expected to be relatively low compared to a vaccine due to different distribution networks.

#### 4. Market potential

Assuming that the *A. annua* feed additive has no competition from other coccidiostats, the European production of poultry represents a very large market with more than 6 billion poultry produced per year<sup>7</sup>. Table 8 shows the poultry production in Denmark, Northern Europe, and the EU plus Norway and Switzerland, and the amount and production value of *A. annua* feed additive if provided to all poultry. The figures for the feed additive either as an extract powder or as dried leaves differ substantially. This is due to the difference in artemisinin content in the two types of treatment (see Tables 2 and 4), which is based on a review of studies of anticoccidial effects of *A. annua*. The two dosages used in Table 8 represent medium artemisinin dosages investigated in the Artemisia Project and previous studies.

**Table 8. Production of poultry in different regions, and amount and production value of the *A. annua* feed additives. The amount and value of feed additives are based on 20 g/kg feed of dried leaves, extract powder at 61 ppm, and toll manufacturing.**

Region	Poultry 2011 <sup>B</sup> Millions	Amount, tons		Production value, mil. DKK	
		Powder	Leaves	Powder	Leaves
Denmark	106,1	1.013	6.790	22,6	70,2
Northern Europe <sup>A</sup>	1.369,5	13.074	87.648	291,5	905,7
EU27+NO & CH	6.761,0	64.545	432.702	1.439,0	4.471,5

<sup>A</sup> DK, EE, FI, IE, LV, LT, NO, SE, UK.

<sup>B</sup> 2011 data from EuroStat, except AT, BE, IE, EE, NO, CH with data from 2008 (EFSA).

The domestic market potential in Denmark is an annual production of 106 mil. broilers. Supplying the entire production corresponds to 1.013 tons of extract powder (61 ppm of feed) with a production value of DKK 22,6 mil., or 6.790 tons of ground, dried leaves (20 g/kg feed) with a production value of DKK 70 mil. The agricultural area required to produce the *A. annua* plant material total 362 ha and 1.687 ha, respectively. In case of the extract powder, 362 ha of land to cover the entire Danish production of broilers must be

<sup>7</sup> Data is based on EuroStat and DEFRA. For some countries it is unclear whether it is total poultry production or only broilers. However, the figures give a good indication of the size of production in different regions.

considered a small crop, and even less than the capacity of the extraction and microencapsulation plant described in section 3.3, which can process 2.415 tons of leaves per year at full capacity, corresponding to 600 ha. Expanding to the EU27-level plus Norway and Switzerland, the demand for the *A. annua* feed additive increases to 64.545 tons (DKK 1.439 mil.) of powder or 432.702 tons (DKK 4,471 billion) of dried leaves. The agricultural area required to produce the feed additives will be 4.700 ha and 21.800 ha, respectively.

#### **4.1. Competition with existing products**

The figures above do not represent the true market potential, as the assumption of 'no competition' is far from realistic; poultry producers can choose from a number of coccidiostats. Two of the most widely used products are salinomycin and narasin, the latter often in combination with nicarbazin. There are no studies or reports on the total market value of coccidiostats in Denmark or in other European countries. The UK Veterinary Medicines Directorate (2009) reported the annual sale of coccidiostats in the UK to be between 196 and 277 tons of active ingredients (ionophore and non-ionophores) in the period 2006-2011. The sales value, however, is not reported. The UK sales include from 9 (2006) to 12 (2011) different coccidiostats that are used for animals, though particularly in the poultry production as the report states. Moreover, the sale of coccidiostats is highly correlated with the June census of poultry stocks in UK for the same period, which varied between 152 and 173 million poultry.

Price data for salinomycin and narasin/nicarbazin were obtained from Danish feed producers in order to compare the costs of the *A. annua* feed additive with the conventional coccidiostats and to make an estimate of the total market value of coccidiostats. Both salinomycin and the combination of narasin and nicarbazin are used in feeds suitable for broilers from day 1 until 5 days prior to slaughter. The cost price, i.e. excluding sales markup, reported by the Danish feed producers were 0,31 DKK/g for salinomycin sodium and 0,65 DKK/g for narasin/nicarbazin. It was emphasized that these figures were current cost prices in October 2013, but that they can fluctuate substantially during the course of a year. The two feed additives are mixed with standard feeds at concentrations of 70 mg/kg and 50/50 mg/kg, respectively. In 2006, 86 % of all poultry feed used in the EU was added a coccidiostat (EC, 2008). Assuming this percentage is still valid and that the two feed additives share the poultry market, the total value of the Danish market for coccidiostats is approx. DKK 12,7 mil. (based on the cost prices above). Northern Europe and EU27 plus Norway and Switzerland would total approx. DKK 164 mil. and DKK 809 mil., respectively.

Table 9 shows the cost for each feed additive per produced broiler, including the two *A. annua* products and pure artemisinin. The price for pure artemisinin is the expected 2014-price of \$ 400 per kg, which is lower than current prices. The decrease in price is based on an expectation of an increased production of semi-synthetic artemisinin. To facilitate a comparison of feed additives, the costs of each are based on the same assumptions regarding feeding as described in section 3.2, i.e. 3.200 g of standard full feed until day 31 (see also the text to Table 9). It is common practice in Denmark to supplement the standard feed with

around 18 % of wheat. In this case, the standard feed is a supplementary feed with a higher percentage of coccidiostats, so the amount per kg feed remains the same.

**Table 9. The cost of different coccidiostat feed additives. The *A. annua* powder and dried leaves are based on 61 ppm artemisinin and 20 g/kg feed. Pure artemisinin is based on 61 ppm added to the feed at the expected 2014-price. A broiler is assumed to consume 3.200 g of feed with feed additive until day 31.**

	Salinomycin <sup>a</sup>	Narasin/ Nicarbazin <sup>b</sup>	<i>A. annua</i> powder	<i>A. annua</i> leaves	Pure artemisinin
Cost/broiler, DKK	0,070	0,208	0,213	0,661	0,437

<sup>a</sup> Based on Sacox 120g (70 mg salinomycin sodium per kg mixed feed)

<sup>b</sup> Based on Maxiban 160g (80 mg narasin and 80 mg Nicarbazin per kg mixed feed)

The use of salinomycin has the lowest cost at DKK 0,07 per broiler, while the dried leaves are the most expensive, almost ten times the price of salinomycin. The extract powder has similar costs as the narasin/nicarbazin combination and can thus compete on costs with one of the widely used conventional coccidiostat feed additives. Whether or not it can compete on anticoccidial efficacy, effects on growth performance, etc. is not clear. It has not been possible to find studies that empirically compare the anticoccidial efficacy of conventional and phytogetic coccidiostats. However, just as the *A. annua* products, the synthetic and antibiotic coccidiostats do not fully eliminate the coccidia parasite, but inhibit parasite reproduction and improve poultry health. The pure artemisinin, even at the expected reduced price, does not seem economically interesting to use as a coccidiostat feed additive.

The cost comparison made in Table 9 should be considered with some caution. The costs of the two conventional coccidiostats are based on cost prices from feed producers, and not the actual costs realized by the feed additive producer, which will be lower. On the other hand, the cost estimate of the *A. annua* feed additives is conservative in some components, e.g. the production of plantlets, which is a major cost component. With time and more experience other parts of the production chain may also be more efficient. The cost of R&D, testing and documentation, and other sunk costs (e.g. patents approval) are not included in the cost of the *A. annua* feed additives. While these costs are important when assessing the net present value of a new product given a certain timeline and an expected return on investment, it can be more interesting to compare the fixed and variable running costs of a new product with the current cost of a mature competing product.

## 4.2 EU ban and the organic poultry production

The EU is discussing a ban on antibiotics and the ionophore coccidiostats (EC, 2008). Such a ban will not be implemented as long as there are no equally effective alternatives at hand. If the *A. annua* feed additives can be developed and documented to be such an alternative, the comparison in Table 9 will have less importance and the description of the potential market value much more so. However, even with a ban on synthetic and antibiotic coccidiostats, the phytogetic alternatives will not be without competition. New vaccines for coccidiosis are continuously being developed, most recently so called DNA vaccines that are

believed to be more effective than conventional protein based or live vaccines (Shirley & Lillehøj, 2012). Peek & Landman (2011) describe 16 different vaccines and argue that vaccines, rather than phyto-genic therapeutic agents like *A. annua* extracts, will replace the conventional coccidiostats. The reasons vaccination is far from as widely used as prophylactic use of coccidiostats in broiler production are relatively high costs of vaccinations and lack of the additional benefits that many coccidiostats offer, such as improved performance and anti-clostridial effects. Vaccines are more often used for egg layers and broiler breeder than for broilers. However, in cases with very aggressive coccidia infections a standard administration of coccidiostats may not be effective, and vaccination is sometimes the only solution (World Poultry, 2013).

Vaccination for coccidiosis is also used in organic broiler production and in small scale productions, such as back yard poultry rearing. The synthetic and antibiotic coccidiostats are not allowed in organic poultry production, which may therefore represent a readily available market for plant derived coccidiostats provided that their price and effectiveness is competitive with vaccination. As for other products, the share of chicken meat and eggs that are certified organic is increasing. The number of organic poultry in the EU increased 6,2 % annually from 2002 to 2007. In 2007 there were 19 mil. heads of organic poultry, of which one third were egg layers, while the remaining were mainly broilers. This is a very small share of the total production of broilers, roughly 0,25 %. The share is even lower in Denmark, where the Danish Agriculture & Food Council estimates that 0,15 % of the broiler production were organic in 2009, corresponding to 150.000 heads. In comparison, organic eggs had a 16,4 % share of the production in Denmark in 2012 according to the official database, StatBank Denmark. Assuming the organic production of poultry has continued to increase with 6,2 % annually, the total number would have reached 25,8 mil. poultry heads in 2012, including 17,2 mil. broilers. This is still a relatively small production.

Organic broilers live almost twice as long as conventional broilers, and also consume more feed, around 6 kg in a 60 day production line (VFL, 2011). If the organic broilers were given the *A. annua* extract powder at concentrations of 61 ppm artemisinin (300 ppm extract), the total amount needed to cover the European production would be 308 tons of powder, with a production value of almost DKK 7 mil., and requiring 110 ha. If the organic producers prefer to use the dried *A. annua* leaves, the total amount of dried leaves needed to supplement the feed with 20 g/kg would be 2.064 tons. This amount would cost DKK 21,3 mil. to produce and require 513 ha. The cost per organic broiler would be DKK 0,399 for the extract powder and DKK 1,240 for the dried *A. annua* leaves.

In practice, the distribution of the *A. annua* feed additives to the organic producers would be more difficult, and thus more costly, than for the conventional market. There are few producers of organic broiler feeds in Europe, partly because proteins from GMO soy is not allowed in organic poultry feed. Many organic broiler producers are therefore producing their own feed. In general, the organic market is characterized as being fragmented and consisting of many small, heterogeneous producers dispersed throughout Europe. This

complicates the marketing of feed additives targeted at organic producers, and do not benefit from the advantage of a simple distribution to a few central feed producers.

Despite being a small market at the moment, the organic broiler production is expected to increase further in the future as consumers and retailers increasingly demand organic products and improved animal welfare. This is an argument for moving into the market, despite the large costs of having a new feed additive approved by EFSA. It would require a relatively small production of *A. annua* to offer the entire organic poultry production an alternative to vaccines and other therapeutic agents against coccidiosis and other poultry diseases. The organic poultry production is also interesting as it provides an initial market, albeit a small niche market but growing, which can facilitate further development of the *A. annua* feed additives while waiting for the EU to phase out the antibiotic and synthetic coccidiostats.

## 5. Concluding remarks

The results of the Artemisia Project show promising effects of the dried leaves and plant extract of *Artemisia annua* against coccidiosis, histomoniasis and necrotic enteritis. Furthermore, the project has shown that it is possible to cultivate the *A. annua* plant in Denmark with high crop yields and high concentrations of the active compound artemisinin in the leaves. Based on these positive results, a production model for two feed additives, the ground, dried leaves and an extract powder of *A. annua*, have been described and assessed with a quantitative microeconomic model.

The results show that the extract powder can compete on costs per treated broiler with the conventional coccidiostats, while the dried leaves cannot. However, the costs are highly dosage dependent and the optimal dosages have yet to be identified. The economic assessment has mainly been carried out for the medium dosages of 3 g/kg feed of powder (61 ppm artemisinin) and 20 g/kg feed of dried leaves (385 ppm artemisinin). The associated production costs of DKK 0,213 and DKK 0,661 per treated broiler, respectively, are competing with costs of DKK 0,070 – 0,208 per broiler for two of the most widely used conventional coccidiostats.

Even with more than 6 billion broilers produced per year in the EU, the market potential for *A. annua* feed additives is limited by the existing coccidiostats premixed in standard broiler feed, and further challenged by ongoing developments of vaccines for coccidiosis. However, an EU ban on antibiotic and synthetic coccidiostats would change the market. Without an EU ban, phyto-genic feed additives can be developed and marketed for the organic poultry production, which albeit having a small market share is increasing as consumers demand higher standards of food safety and improved animal welfare. An emerging industry for *A. annua* feed additives could facilitate first mover advantages once the demand expands.



In conclusion, the assessment of a Danish production of *A. annua* based feed additives for broilers shows a promising economic potential even before an EU ban on conventional coccidiostats is implemented. The results call for further investigations into the best formulation and production of the additives.

#### ***Broader economic impact of phytogetic coccidiostats***

An economically competitive *A. annua* feed additive may also bring broader economic gains to national and the EU poultry industries. A poultry production free of antibiotic and synthetic coccidiostats can facilitate improved access to segments of export markets, where consumers demand products with high food safety standards. An EU ban on antibiotic and synthetic coccidiostats will create a new market for phytogetic coccidiostats, but it may also create barriers for imports of poultry from countries without a ban on these agents. This can result in a higher demand for broilers produced internally in the EU, and national broiler productions that are first movers in the use of alternative coccidiostats and feed additives may see a head start on fulfilling the additional demand.

The production of the *A. annua* feed additive involves a longer production chain than the veterinary pharmaceutical products that are entirely produced in a one-locality production chain at a medicinal manufacturing company. The production chain for the *A. annua* feed additives involves an agricultural production targeted specifically at the processing of the additives. However, the stems of the *A. annua* plant may be used for other purposes than burning for energy as proposed in section 3.2. For example, plant fibers are increasingly used for different purposes, as is the case of the hemp plant, also in Denmark. Thus, a production of *A. annua* feed additives may also imply general welfare gains in form of job creation in the agricultural sector. Assuming an EU ban on antibiotic and synthetic coccidiostats is implemented, the production of *A. annua* feed additives may have a broader economic impacts than a just as a niche production.

#### ***Perspectives for further research, scaling up and collaboration with the industry***

The technology required to perform the plant extraction at large scale as well as the subsequent microencapsulation of the essential oils and artemisinin is available in Denmark. The described combined process of DCM extraction and microencapsulation with maltodextrin has not been tested, but Danish companies have the capacity to develop optimal production facilities once the best processing methods have been identified. Before reaching that point, it is necessary to carry out a number of additional research tasks, including i) improving the documentation of the efficacy of the Danish produced *A. annua* against coccidiosis, histomoniasis, and necrotic enteritis, ii) finding the optimal dosage of artemisinin and essential oils in the plant extract for anticoccidial and antibacterial effects, iii) identifying the best extraction technique, and iv) identifying the most suitable carrier material and optimal microencapsulation technique. These four main research tasks require a substantial amount of work, including pilot scale trials of plant extraction and spray drying, as well as new *in vivo* tests with extract powders. The research must be done in a collaborative effort between research institutions and private companies with suitable

expertise and know-how. This could include collaboration with a one or more phytogetic companies that have expressed interest in the Artemisia Project.

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