



## Perspectives of Using Conifer Needle Extract as Phytobiotic as an Alternative to Antibiotics in Biological and Industrial Farms

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### Abstract

This study demonstrated a trend towards dose-dependent, desirable changes in the chicken microbiome resulting from the addition of pine needle extract to chicken feed. Chicken meat is a widely consumed protein source that is cost-effective, yet intensive poultry production relies heavily on antibiotics, fostering antibiotic-resistant bacteria, which may also influence the occurrence of antibiotic-resistant bacteria in humans. Additionally, this study describes the development of a phytobiotic feed or phytopremix (PP) based on standard feed. Conifer needle extract (CNE) from *Pinus sylvestris* L. and *Picea abies* (L.) H.Karst. was shown to possess proven high biological efficacy and an absence of any adverse reactions with regular use. The developed PPs were used for hens, chickens, sheep, and organic feeds produced on organic farms using CNE in various concentrations. CNE obtaining methods are well-known and described in literature, the most optimal method can be considered with extraction process with subsequent solvent separation. This simple method allows obtaining the most complete CNE containing more than a hundred hydrophobic and hydrophilic biologically active compounds in natural proportions. The mixture of complementary feed and conifer needle extract can be stored for 3 months at a temperature of 25 °C, in a dark place. The optimal dose for consumption may be 30 mg CNE/kg of animal weight.

**Keywords:** Feed additives; Alternative to antibiotics; Phytobiotics; Coniferous needle extract; Effectiveness

**Abbreviations:** CNE: Conifer needle extract; FP: Phytobiotic complimentary feed or phytopremixes; FDA: Food and Drug Administration; AGPs: Antibiotic growth promoters; PFA: Phyto-genetic feed additives; EO: Essential oil; FCR: Feed conversion ratio; EDS: Egg drop syndrome; USA: The United States of America; CPNE: Conifer Pine Needle Extract; EFSA: European Food Safety Authority; ESFC: E-Submission Food Chain; AMRL: Antimicrobial resistance; WHO: World Health Organization; MRL: Maximum residue limits; GIT: Gastrointestinal tract; MUC2: Mucin 2; TFF3: Trefoil Factor 3

### Introduction

The biggest problem in the agricultural sector is the uncontrolled use of antibiotics. A possible solution is to develop and propose a clear strategy for replacing antibiotics with products of natural origin, which appear to have dualistic bacteriostatic properties – inhibit the development of pathogens, and stimulate the development (proliferation) of beneficial microflora. This is especially important for poultry and livestock. Chicken meat occupies one of the central places in the production of poultry. On average, 25 billion animals

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are raised for the production of chicken meat, which amount is more than 122 million tons. In the period from 2012 to 2024, the growth of global poultry meat production amounted more than 20 thousand tons [1].

Traditionally, antibiotics have been used in subtherapeutic doses to stimulate growth and as a protective antibacterial factor, preventing epidemic diseases [2, 3]. At the same time, bird's gastrointestinal tract with *cecum* structure features can serve as a natural incubator for the selection and development of pathogenic or super pathogenic microbial flora and transmission it to other animals and humans. This selective microbial intervention and viral invasions have become a global problem [4]. Consequently, in some regions, regulatory documents prohibiting the use of antibacterial agents in poultry and for other farm animals keeping or raising processes are in the development stage. Moreover, biological farms have completely abandoned the use of any antibacterial agents and growth stimulants of the animal organism [5 – 7]. Thus, in September 2018, the FDA prepared the framework document “Supporting the use of antimicrobials in veterinary settings: goals for 2019 – 2023.” On October 6, 2018, the European Parliament extended restrictions, prohibiting the prophylactic use of antibiotics in livestock by 2022. The ban also applies to imports. China announced a timetable for implementing a plan to stop adding antibiotics to animal feed by 2020 [8].

Therefore, it is necessary to develop new promising technologies as an alternative to antibiotic growth promoters (AGPs) [9 – 11] especially for health care and agriculture. The ideal alternative to antibiotics should match the effectiveness of AGPs, ensuring productivity and the availability of nutrients. The ability to modulate immunity and the intestinal microbiome may be particularly important. These technologies should demonstrably stimulate the resistance of the animal's body to infections and effectively use standard feed to produce 1 (one) kg of poultry meat, which should be cost-effective.

## Discussion

### Phytobiotic Feed Additives

Phytobiotic or phyto-genetic feed additives (PFA) [11] are natural biological active substances obtained from plant materials and included into animal feed to increase their productivity [12]. These can be various annual or perennial plants or their parts, classified as herbs, flowers, non-woody, non-resistant plants from which leaves or flowers are used, or spices, non-leafy parts of plants, seeds, fruits, bark, root, etc. [12, 13]. They are used in solid, dried and ground form or as extracts, crude or concentrated. Depending on the process used to obtain the active ingredients, PFA can also be classified as essential oils (EOs), volatile lipophilic substances obtained by cold extraction, steam or alcohol

distillation, and oleoresins, extracts obtained from non-aqueous solvents [12, 13]. The main bioactive compounds of PFA are mainly polyphenols, and their composition and concentration vary depending on the plant, plant parts, geographic origin, harvest season, environmental factors, storage conditions, and methods of processing [12, 14]. PFA are used as natural growth promoters in pig and poultry production [12, 15]. A variety of herbs and spices, e.g. thyme, oregano, rosemary, marjoram, yarrow, garlic, ginger, green tea, black cumin, coriander and cinnamon, have been used in poultry production for their potential use as an alternative to AGP. Broilers fed a diet supplemented with a mixture of 14 herbs showed increased body weight gain and improved feed efficiency [16]. Positive results were obtained with the use of oregano in feed production [17], black cumin seeds [18], fermented *ginkgo biloba* leaves [19], as well as dried and crushed *Scrophularia striata* and *Ferulago angulata* [20]. Various free fatty acids derived from plant extracts improve poultry growth. Studies conducted with the inclusion of sugar cane extract [21], anise extract [22] chestnut wood extract [23], *Forsythia suspensa* extract [24] and *Portulaca oleracea* extract [25] showed a significant increase in body weight and a decrease in the feed conversion ratio (FCR) [26]. However, some other PFAs such as grape pomace, cranberry fruit extract, *Macleaya cordata* extract, garlic powder, grape seed and yucca extracts, tested as growth promoters, did not show any effect on performance parameters [27 – 33].

In addition to herbs and spices, various essential oils, thymol, carvacrol, cinnamaldehyde, essential oils of clove, coriander, star anise, ginger, garlic, rosemary, turmeric, basil, caraway, lemon, and sage, have been used individually or in mixtures to improve animal health and performance. Unfortunately, there have been mixed results of using EO in poultry diets. Using a mixture of thymol and cinnamaldehyde in feed has improved live weight gain in broilers [34, 35].

Similar results were obtained when adding oregano EO to the diet [36, 37], coriander EO [38], mixtures of cloves and cinnamaldehyde [39], thymol and star anise essential oils [40], and herbal essential oil blends [41, 42]. Adding EO improves feed efficiency, and reduced egg drop syndrome (EDS) [40, 43 – 45]. Results from other studies have not shown any positive effects of EO inclusion on performance [46 – 50]. Results can be explained by differences in the quality and composition, type and origin of EO, environmental conditions and the time of the studies [51].

Misfortunately, only one commercial phytonutrient mixture (containing carvacrol, cinnamaldehyde, and capsicum oleoresin) has been approved in the EU so far, as the first plant-based feed additive for improving broiler performance [52, 53]. Studies conducted with this commercial mixture consistently improved in growth and feed efficiency [52, 54, 55].

The mechanism of action of PFA was not described and was highly dependent on the composition of the active ingredients in the product used. In general, the positive effects of PFA were attributed to their antimicrobial and antioxidant properties. Was proven that the inclusion of PFAs in the diet alters and stabilizes the intestinal microflora and reduces microbial toxic metabolites in the intestine due to their direct antimicrobial properties against various pathogenic bacteria, which leads to the relief of intestinal problems and immune stress, thereby improving the vital functions of the macro organism [31, 34, 56 – 58]. Another important positive effect of PFA inclusion in the poultry diet is the reduction of oxidative stress and increased antioxidant activity in various tissues, contributing to improved health [19, 24, 28, 47, 56, 59, 60]. PFA appears to have immunomodulatory effects, increasing immune cell proliferation, cytokine expression, and antibody titers [61 – 64]. Adding PFAs to the diet increases the production and activity of intestinal and pancreatic enzymes and had a choleric effect [46, 50, 65, 66]. PFA also improved intestinal histology, increased villus height and intestinal absorptive surface area [67, 68]. Increased secretion and absorption of digestive enzymes lead to improved digestibility of nutrients and maintenance of normal intestinal barrier function, resulting in increased productivity [24, 32, 35, 45, 48, 69, 70].

Considering objective facts obtained from many different sources, the need for mandatory and systematic inclusion of PFA in the diet of farm animals is not only an alternative to antibacterial agents. They are naturally and affects the entire homeostasis of the macro organism and microbiota in a very gentle, balanced mode. At the genetic level, they change and stabilize the metabolic processes of lipids, proteins and carbohydrates, the biochemistry of “small molecules”, unique biopolymers, restore humoral immunity, suppress the growth of tumor cells, enhance phagocytic activity, promote normal cell proliferation and affect apoptosis processes.

In 2012, the EU adopted a strategy for the development of the bioeconomy, the main direction of which is “the use of renewable biological resources and waste in the production of products with high added value to create innovative food products, animal feed, bioproducts, biomedicine, bioveterinary science, etc.” [71 – 73]. One such renewable bioresource is forestry waste green biomass of pine and spruce. About 400 thousand tons of green biomass in Latvia remains in the forests, as a waste, annually [74]. Industrial use of this valuable natural resource was irrelevant due to the belief in the unlimited possibilities of synthetic chemistry (without considering consequences), subjective distrust of the possibilities of effective use of natural biologically active complexes and the relatively high cost of production. It should be noted, that commercial wood makes up only 60 % of the tree mass, from which 40% is green mass, which is only rarely used, mainly for producing of essential oils [74 – 78].

## Characterization of Conifer Needle Extract (CNE)

Baltic pine *Pinus sylvestris* L. and Norway or European spruce *Picea abies* (L.) H.Karst. are relict species of coniferous trees, showing high resistance to unfavourable biotic and abiotic environmental factors. Their leaves in the form of coniferous needles have been traditionally used in folk medicine; have high medicinal value due to the presence of flavonoids, terpene trilactones and phenolic compounds, etc. The tree crown, especially the perennial leaf part, is the most important specific part of the tree. Thanks to the needle and the processes occurring there, the entire life cycle and ensure the growth of biomass. During the phylogenesis of conifers, which is more than three hundred million years, a unique set of biologically active compounds in natural ratios was formed in the needles secondarily, naturally, which are used for life in epochal climatic conditions and protection from various pests. The composition of the biomass of a plant / tree can be divided into two parts: structural and living elements. The term “living elements” can mainly be extended to the crown and bark of a tree, and their processing is forest biochemistry [74, 75, 79 – 82].

The founders of forest biochemistry were the Leningrad Forestry Academy scientists Solodky, F. T., and Agranat, A. L. In 1956, they first developed and implemented an industrial technology for processing pine and spruce wood greenery and identified the main uses for the resulting biologically active products. This method involved extracting biologically active substances from ground conifer needles with a hydrocarbon solvent, separating conifer wax from the extract, distilling off the extracting solvent, and treating the residue with an aqueous solution of sodium hydroxide [82 – 85].

This method allows for the complete isolation of possible hydrophobic and hydrophilic wood greenery components in natural proportions. Components obtained in natural proportions, are more than 100 biologically active compounds [75]. This complex includes a lipid (hydrophobic) with the unique composition of fat-soluble biomolecules of vitamins and provitamins: carotenoids (vitamin A), tocopherols (vitamin E), vitamin K group, squalene, polyprenols, phytosterols. Organic acids may include more polar acids than resin and fatty acids: dibasic oxo- and oxyacids. The aldehyde fraction is dihydroabietinal, pimarinal, isopimarinal, labdanoids, tricyclic diterpene alcohols, phytosterols. The oxide fraction contains manoyl- and 13-epimanoyloxides, etc. [74, 75, 83, 85 – 88].

These plant-origin biologically active compounds have proven antioxidant, antifungal, antibacterial, insecticidal and herbicidal properties [74, 75, 89 – 103]. Essential oils are complex chemical mixtures of volatile and non-volatile components. These components are widely used to treat various diseases in neurology, infectology, dermatology, etc.

[75]. Possessing antioxidant [75, 89 – 92], antimicrobial [89, 93 – 97], antifungal [98 – 100], larvicidal / herbicidal [75, 101], anti-inflammatory effects [75, 102, 103].

Wood greenery contains a significant amount of hydrophilic water-soluble substances belonging to various chemical compound classes. There are six main chemical substance groups: vitamins, nitrogen-containing substances, acids, carbohydrates, phenolic and ash-containing substances [83].

Wax can be used to produce natural / ecological coatings [104], and recycled pine needles can be used to produce thermal insulation materials [105, 104]. Therefore, the processing of pine needle mass is a waste-free production that meets all BIO criteria. The resulting products are a unique biologically active complex that can be widely used in agriculture, the food industry, medicine, veterinary science, etc.

Naturally, within the framework of the EU / USA state and with the support of private funding, it is necessary to pay attention to this untapped natural resource, and recheck and refine the technology at the modern level. Moreover, complete the research that has been started, following the established procedure; issue all permits for the widespread use of pine needle extract. For instance, our group had developed Conifer Pine Needle Extract (CPNE) dossier and already submitted it in the European Food Safety Authority (EFSA). In this dossier, we described evidences that prove and recommend using CPNE in nutrition as a Biologically Active Additive. CPNE obtained from *Pinus sylvestris* L. / *Picea abies* (L.) H.karst. needles by extraction method either with hydrocarbon solvent Nefras, or a 1 : 1 mixture of hexanes and ethyl acetate. CPNE dossier assigned code in E-Submission Food Chain (ESFC) platform is EFSA-Q-2025-00156 and, at the moment, dossier is under review.

Conifer needle extract was obtained by extracting process of *Pinus sylvestris* L. and *Picea abies* (L.) H.karst. needles with an organic solvent. According to organoleptic parameters, CNE is a thick, homogeneous ointment-like mass at room temperature and at temperatures above 25 °C, liquid with a characteristic coniferous odor and olive- or dark green color [81 – 84]. The obtained natural product is a dark green mass with the distinct smell and taste of pine and is called a chlorophyll-carotene paste. The main chemical components of CNE are sodium chlorophyllin and other chlorophyll derivatives (4 – 16 g/L), carotene and other carotenoids (200 – 1200 mg/L). Vitamins, such as vitamin E (300 – 500 mg/L), vitamin K group (12 – 20 mg/L), sodium salts of fatty, resin dibasic, oxo-, and oxyacids (44 – 60 %), minerals (5 – 7 %), waxes (5 – 8 %), phytosterols, polyphenols, polyprenols and squalene of dry basis [83, 84]. It is determined that the sample extract also contains the following elements (% of dry body weight): Na 2.764; K 0.056; Ca 0.006; Mg 0.008; Mn 0.001;

Zn 0.001; Fe 0.013; Cu 0.0007; Ni 0.0005; Co 0.00007; Cd 0.00005, Pb 0.0002 [75, 83, 84].

### Preparing Process of Coniferous Branches

The technology of processing woody greens to produce coniferous needle extract, coniferous wax and raw essential oil includes the following technological operations: harvesting woody greens, preparation for extraction: grinding and freeze-drying.

Harvesting of woody greens is one of the most important and time-consuming operations involved in the production of CNE. Harvesting of woody greens is carried out manually, and branches are harvested with a maximum twig diameter of no more than 8 mm. Branches covered with needles or leaves with a diameter of up to 50 mm are fed to the chopper knives using a conveyor. The chopper knives cut them into pieces of a certain length and separate the side branches. The crushed mass is introduced into the loading cyclone by air flow. In the air flow created by the fan, heavier wood particles are separated from the wood greens. The chopped wood greenery, 2 – 20 mm long, separated from the woody part of the branches, is fed from the cyclone to a two-section knife crusher by a screw device, and the conditioned wood greenery (2 – 7 mm long, 90 – 95 % of the raw material) is loaded into the extractor through the cyclone.

The process of lyophilization or freeze-drying was used to study the possibility of preserving the needles properties for as long as possible.

### Extraction Process

Crushed conifer greenery is extracted with a hydrocarbon extractant in an extractor with a remote heat exchanger. As an extractant the Nefras-C1, with b. p. 70 – 110 °C is used, and the extraction time is 3.5 – 4.0 h. When the extraction process is complete, the extract is transferred to a conifer wax settling vessel. The settling time is 16 hours at a temperature of 10 – 15 °C. The resulting conifer wax crystals are separated from the extract by filtration. The wax sediment, separated from the extract of biologically active substances, is used to manufacture the “conifer wax products”, and the solvent is distilled from the extractive solution. The solvent is distilled at a temperature of 70 – 80 °C. As the amount of extractant solvent decreases, the pressure in the vessel decreases. At the end of the distillation, direct steam is introduced into the vessel. At the end of the distillation process, a sample to determine the residual content of solvent in the extract is taken. The permitted residual Nefras-C1 content should be not more than 0.01 %.

After solvent distillation process, the extract is sent to a neutralizing vessel for the production of wood green extract. Neutralization of the free acids in the extract is carried out with a calculated amount of aqueous sodium hydroxide

solution. The product pH is in the range 7.5 – 8.0, and the moisture content is 38 – 42 %. The obtaining product, which meets quality specifications, is decanted into containers and transferred for the production of feed additives [83 – 85].

### Development of Complementary Feed Types for Poultry and Livestock

Comprehensive approach is proposed to obtain the best results and to reduce antibiotic consumption among poultry and livestock. Firstly, the development of various phytopremixes. Secondly, addition of sodium chlorophyllin to drinking water. Thirdly, the use of conifer essential oils in the air circulation and air disinfection system.

In collaboration with leading Latvian cereal products manufacturer JSC “Dobeles dzirnavnieks” were developed several phytopremixes formulations for poultry and livestock, containing various organic supplementary feeds, coniferous needle extract, and specified their preparation technology. In total, eight feed phytopremix formulations were developed.

Complementary phytopremix was prepared by mixing the thick coniferous needle extract with the supplementary feed. The samples prepared by the mixing method contained 10 % conifer needle extract. Premixes containing conifer needle extract in portions was added to the calculated amount of supplementary feed in portions. After adding each portion of supplementary feed, the mass was stirred until a homogeneous mixture was obtained [107].

Organic supplementary compositions and some photographs of developed phytopremixes external appearance are presented in Fig. 1 – Fig. 4.

#### 1. Organic supplementary feed for calves aged 0 – 2 months.

Organic supplementary feed contains organic wheat, barley, soybeans, beans, oats, soybean meal, calcium carbonate, *Saccharomyces cerevisiae*, defluorinated monocalcium phosphate, sodium chloride, magnesium oxide, vitamins A, D<sub>3</sub>, E, trace elements - zinc, manganese, copper, iodine, cobalt and selenium (Fig. 1).

#### 2. DOFEED 2.5 % supplementary feed for poultry.

Intended for organic farms. Bio supplementary feed contains defluorinated monocalcium phosphate, calcium carbonate, sodium chloride, vitamins A, D<sub>3</sub>, E, B vitamins, and vitamin K<sub>3</sub>, trace elements – zinc, manganese, copper, iodine, cobalt, selenium, iron, digestive enzymes – endo-1,3,4-B-gluconase, endo-B-xylinase and 6-phytase (Fig. 2).

#### 3. Complete feed for laying hens for organic farms

Complete feed contains organic wheat, soybeans, oats, calcium carbonate, organic barley, beans, soybean meal, *Saccharomyces cerevisiae*, vitamins A, D<sub>3</sub>, E, B vitamins,

and vitamin K<sub>3</sub>, trace elements – zinc, manganese, copper, iodine, cobalt, selenium, iron, digestion promoters – endo-1,3,4-B-gluconase, endo-B-xylinase and 6-phytase (Fig. 3).

#### 4. Complete feed for 0 – 8 week old chickens for organic farms.

The complete feed contains organic wheat, soybean meal, oats, *Saccharomyces cerevisiae*, calcium carbonate, vitamins A, D<sub>3</sub>, E, B vitamins, and vitamin K<sub>3</sub>, trace elements: iron, zinc, manganese, copper, cobalt, selenium, digestion promoters - endo-1,3,4-B-gluconase, endo-Bxylinase and 6-phytase (Fig. 4).

#### 5. Complete feed for young birds for organic farms.

The complete feed contains organic wheat, oats, soybeans, soybean meal, *Saccharomyces cerevisiae*, calcium carbonate, vitamins A, D<sub>3</sub>, E, B vitamins, and vitamin K<sub>3</sub>, trace elements: zinc, manganese, copper, iodine, cobalt, selenium, iron, digestion promoters - endo-1,3,4-B-gluconase, endo-B-xylinase and 6-phytase.

#### 6. Complete feed for 0 – 28 days old broilers for organic farms

The complete feed contains organic wheat, soybean meal, oats, *Saccharomyces cerevisiae*, calcium carbonate, vitamins A, D<sub>3</sub>, E, B vitamins, and vitamin K<sub>3</sub>, trace elements: zinc, manganese, copper, iodine, cobalt, selenium, iron, digestion promoters - endo-1,3,4-B-gluconase, endo-B-xylinase and 6-phytase.

#### 7. Complete feed for 28 – 63 days old broilers of Grower organic farms

The complete feed contains organic wheat, soy flour, barley, soybeans, oats, *Saccharomyces cerevisiae*, calcium carbonate, vitamins A, D<sub>3</sub>, E, B vitamins, and vitamin K<sub>3</sub>, trace elements: zinc, manganese, copper, iron, iodine, cobalt, selenium, digestion promoters - endo-1,3,4-B-gluconase, endo-B-xylinase and 6-phytase.

#### 8. Complete feed for broiler for Finisher organic farms

The complete feed contains organic wheat, soybean meal, soybeans, oats, *Saccharomyces cerevisiae*, calcium carbonate, vitamins A, D<sub>3</sub>, E, B vitamins, and vitamin K<sub>3</sub>, trace elements: zinc, manganese, copper, iodine, cobalt, selenium, iron, digestion promoters - endo-1,3,4-B-gluconase, endo-B-xylinase and 6-phytase.

According to comprehensive approach mode, phytobiotics should be used as well as additives to animal drinking water. Sodium chlorophyllin is recommended to be added into animal drinking water. Sodium chlorophyllin is highly soluble in water, and it is able to normalize hemoglobin content in the blood, restoring strength reserves and improving well-



**Figure 1:** Organic supplementary feed for calves aged 0 – 2 months, which contains 10 % of pine needle extract.



**Figure 4:** Complete feed for 0 – 8 week old chickens for organic farms, which contains 10 % of pine needle extract.



**Figure 2:** DOFEED 2,5 % supplementary feed for poultry, which contains 10 % of pine needle extract.



**Figure 3:** Complete feed for laying hens for organic farms, which contains 10 % of pine needle extract.

being. In Latvia there is a registered food supplement “Hofi”, containing 0.05 % of spruce (*Picea abies*) needle sodium chlorophyllin and drinking water [108].

In addition, the use of pine and / or spruce essential oils in the air circulation and air disinfection system are also recommended. According to literature data, antioxidant and antimicrobial activities of Himalayan cedar needles (*Cedrus deodara*) essential oil were determined, and evaluated. The essential oil revealed strong antimicrobial activity against typical food-borne microorganisms, with minimum inhibitory concentration and minimum bactericidal concentration values of 0.2 to 1.56 and 0.39 to 6.25 µg/mL, respectively [90]. In addition, Norway or European spruce (*Picea abies*) essential oils also showed antimicrobial activity against *Escherichia coli* strains growth [109].

### Avian Digestive System

To understand the advantage of the phytobiotics application, special attention should be paid to the digestive tract of birds. The efficient conversion of feed into its main components for optimal nutrient absorption is a vital for both broilers and broiler breeder production and welfare. Gut health is an intricate and complex area, combining nutrition, microbiology, immunology and physiology, plays a key role. When gut health is compromised, digestion and nutrient absorption are affected, which, in turn, can have a detrimental effect on feed conversion, leading to economic loss and a greater disease susceptibility. In addition, recent changes in legislation on the use of antimicrobials, differing feed requirements and birds that are more efficient, highlight the need for a better understanding of gut function and health. The purpose of this section is to highlight the importance of gut health and to describe key factors that are important for developing and maintaining optimal gut function.

A bird's gastrointestinal (GI) tract morphology, digestive strategy, and metabolic capability have been intimately intertwined during evolution to match the nutrient content and physical attributes of food available in its natural habitat. When compared across species, the GI tract is the most anatomically diverse organ system. However, distantly related species consuming similar food items often display morphological convergence because of similar nutritional and ecological selection pressures. The GI tract has sufficient morphological plasticity to accommodate changes in nutritional needs during the life cycle and to adapt to the diet's changing physical and nutritional characteristics. The digestive tract is essential in converting food into nutrients that the body needs, for maintenance, growth, and production. Once food is eaten, it must be broken down into its basic components. This is done through both mechanical and chemical means.

If we speak in detail, avian digestive tract is a continuous tube that opens at either end (beak and vent) to the outside world and consists of a mouth, esophagus, crop, proventriculus, ventriculus or gizzard, intestine, caeca, rectum and cloaca (Fig. 5 and Fig. 6). The most interesting thing in the structure of the bird's intestine is the species-specific development of the ileocecal angle. Two long blind appendages are closed on one side - appendices, which in their length can make up to 1/3 of the length of the entire small intestine. They, like an incubator, contain a specific microbiome responsible for most of the functions of the macroorganism - the main ones are immunity and stress resistance. As food progresses through these organs, a specific sequence of digestive events occurs, including grinding, acidifying, hydrolysing, emulsifying, and transporting of the products.

Avian digestive systems have a couple of key management points. The first, the gut is responsible for the digestion and absorption of nutrients. The second, if gut function is impaired, digestion and absorption of feed will be reduced, and bird performance and welfare will be compromised [110 – 111].

As previously emphasized *ceca* (in singular *cecum*) (Fig. 6) are two blind pouches located where the small and large intestines join. Some of the water remaining in the faecal material is reabsorbed here. Another vital function of the ceca is the fermentation of any remaining coarse materials. In the ceca several fatty acids and eight B vitamins: thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folic acid and vitamin B<sub>12</sub> are produced. However, ceca is located so close to the end of the digestive tract, very little of produced nutrients are absorbed and available to the chicken [110].

The ceca empty their contents twice or thrice times a day, producing pasty droppings that often smell worse than regular ones. The number of times cecal droppings are released, and

their color and texture tell you that the chicken's digestive tract is functionally normal.

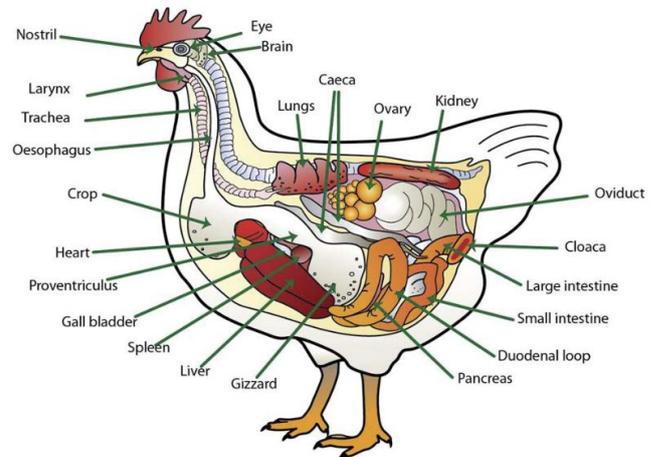


Figure 5: Model showing the internal organs of the female chicken.

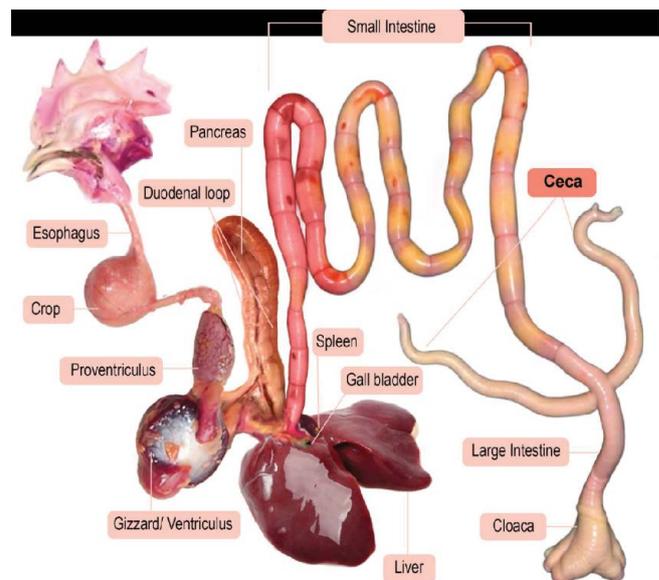


Figure 6: The Gastrointestinal tract of a chicken.

### Intestinal Microflora

A vital stage in the normal development of a bird's organism is the colonization / settlement of beneficial microorganisms in the intestine, and the formation of a microbiome. Beneficial bacteria, referred to as microflora, normally populate both the small and large intestines. This population of microflora is important since it aids in digestion. Intestinal disease normally occurs when the balance of normal microflora is upset or the quantity of foreign organisms significantly exceeds the normal microflora amount. As a result, enteritis or inflammation of the intestine may develop, which producing symptoms include diarrhea, increased thirst, dehydration, loss of appetite, weakness, and weight loss or slow growth.

When chicks hatch, their digestive tracts are virtually sterile. Consequently, if a mother hen raises chicks these “beneficial” bacteria appear, and they would obtain beneficial microflora by consuming some of their mother’s faecal material. Unfortunately, this is not possible in artificial incubation and brooding. Probiotics are usually used to raise this beneficial microflora. Probiotics comprise the normal beneficial microflora that inhabit the chicken’s digestive tract. By spraying it in the shipping boxes or supplying it in the first feed, the chicks receive the “good” bacteria they need to fight off infection by pathogenic bacteria, such as *Salmonella*.

The gut consists of a diverse range of bacteria, fungi, protozoa, and viruses. The development of the gut microbiota begins upon hatching; bacteria are picked up from the environment, feed, and people. Each of these areas can affect gut microbiota development.

The first bacteria to enter the gastrointestinal tract are the pioneering bacteria, which rapidly multiply and colonise the gut environment. The composition of the pioneering bacterial community undergoes a succession of changes as the gut develops and oxygen levels fall. It can take up to 3 – 4 weeks for the microbiota to form the climax (or adult) microbiota, but during this period, stability is seen in the gut if chicks are provided with optimal brooding conditions along with good feed and water quality.

The bacterial community of the intestinal microbiota form a protective barrier, which lines the gut, preventing the growth of less favourable or pathogenic bacteria such as *Salmonella*, *Campylobacter* and *Clostridium perfringens*. This principle is commonly known as competitive exclusion. Theories suggest that the commensal (or beneficial) microbiota dominate attachment sites on the gut cells, reducing the opportunity for attachment and colonisation by pathogens. Another proposed mechanism is that the intestinal microbiota can secrete compounds, including volatile fatty acids, organic acids and natural antimicrobial compounds, known as bacteriocins that either inhibit the growth of, or make the environment unsuitable for, less favourable bacteria. Studies using germ-free animals have also shown that the intestinal microbiota is important in the stimulation and development of the immune system. The commensal microbiota is thought to maintain the gut immune system in a state of “alert” to react quickly to pathogens. The gut microbiota is also considered an important factor in the development and maturation of the immune system. Animals lacking a gut microbiota have been shown to be more susceptible to disease and have poorly developed immune tissues. In addition, protection against disease and stimulation of the immune system, the intestinal microbiota can influence host growth rates by producing extra nutrients through the fermentation of the plant fibers that the birds cannot digest [110, 112].

## Gut Health Additives

Many products are available to support gut health. These products can either be added to water, added to feed at the feed mill or top-dressed on feed at the farm. Gut health additives vary in their action, making which makes choosing the right product difficult. Some gut health products provide or stimulate beneficial bacteria, some promote the development of the gut tissues, some aid digestion and others inhibit pathogens. Consequently, when deciding which product to use, it is critical to investigate what is causing the gut health issue and ensure that any potential product can help to solve the problem.

These products are often called “alternatives to antibiotics”, and they are successfully used in programs targeting a reduction in antibiotic use. However, it is important to recognise that their use is more preventative, and thus, their use should be considered an alternative strategy. As part of a strategic approach, the aim is to administer a product that offers a solution to the needs of the gut at significant points in the chicken’s life.

The gut has three predominant stages, development, transition and maintenance (Fig. 7). During the development stage, the aim is to promote bacterial colonisation and stimulate tissue and immune development. The transition stage refers to periods, where there are fluctuations in the gut environment in response to impact factors, such as a feed change, vaccination and handling. These events can cause a change in the intestinal environment and increase the risk of malabsorption and bacterial overgrowth. The maintenance stage refers to the period when the gut has stopped developing and reached balance. However, there is still the risk of disruption due to management or pathogen challenges, so it is still important to maintain support of the gut tissues at this time [111].

In the last decade, the use of various kinds of phytobiotics as an alternative to antibiotic therapy in poultry has become increasingly common, it help to restore and improve chicken intestinal microflora and promote normal growth and development of poultry. Phytobiotic premixes are produced using various plants and / or plant parts, such as fresh or fermented leaves, seeds, and essential oils obtained from these seeds, etc. [11 – 33].

Antibiotic residues remain in poultry meat and pose significant public health risks, contributing to the growing global challenge of antimicrobial resistance (AMR). It was proven that antibiotic residues stimulate allergic reactions, toxicity, disruption of human gut microbiota, and the promotion of antibiotic-resistant bacteria. Long-term exposure to antibiotic residues, even at low levels, exacerbates these risks, affecting individual health and broader public health systems.

Nowadays, the annual mortality from antibiotic-resistant infection is 700 thousand people, but by 2050, it may reach 10 million lives. According to WHO data, this is the biggest threat to human health, food security and further global development, and a greater threat to the global economy. Regulatory frameworks, establishing of maximum residue limits (MRLs) and stringent monitoring, are crucial for ensuring food safety. An alternative could be minimising antibiotic residues with the subsequent use of phytobiotics in poultry [113].

Due to the possible solution to this serious threat to human health, the high demand for poultry meat, and the large number of poultry farms worldwide, the demand for phytobiotics has increased significantly. Unfortunately, not all feed additive production can meet market needs. The output of phytobiotics from plant biomass could be the rational option, because the amount of plant biomass is large. In this case, the coniferous greenery biomass, which is the waste of logging, can serve as a potential raw material for coniferous needle extract. If we evaluate total cost prize of preparing logging green biomass with continuous wood greenery, processing to obtain CNE, and with subsequent addition it to the feed in poultry farming, it will become obvious, that coniferous needle extract has a high production potential for creation of cost-effective biologically active feed additive [95, 107].

CNE also known as chlorophyll-carotene paste, and it is well-investigated product that has been used in Russia since 1956 and has various applications as a remedy in different areas, for example, in gastroenterology to reduce dyspepsia symptoms in patients, and modulate human GIT microbiome, destroying pathogen bacteria and fungi like *Candida* [114, 115]. In addition, chlorophyll-carotene paste has been used as a wound healing treatment, with deodorising effects, which can stimulate wound edge epithelisation. Over and above, CNE could be used to modulate beneficial microbiota, as well as an anti-inflammation remedy. According to published information conifer needle extract, increased blood hemoglobin count and reduced inflammation markers [82, 115].

Furthermore, a published investigation of essential oils extracted from *Picea abies*, which was tested by isothermal calorimetry showed antimicrobial activity. Provided results unambiguously confirm the antibacterial activity of *Picea abies* extracts on the growth of *Escherichia coli*. The extracts inhibited not only the growth but also interfered with the metabolic activity of the microorganism [109].

According to published *in vitro* investigation results, the antimicrobial activity of CNE was assessed against 15 bacterial strains that were previously isolated from clinical material of animal origin (*Pseudomonas aeruginosa*; *Enterococcus faecium*; *Enterococcus faecalis*; *Proteus mirabilis*; *Escherichia coli*; *Klebsiella pneumoniae*; *Staphylococcus aureus*; *S. haemolyticus*; *Enterobacter cloacae*; *Citrobacter*

*freundii*; *Bacillus cereus*; *Salmonella enterica*; *Acinetobacter baumannii*; *Aeromonas hydrophila*; *Pasteurella multocida*). Positive outcomes were observed with increasing doses, such as a reduction in the relative abundance of *Campylobacter* [95, 107]. It was found that the investigated poultry willingly ate probiotic food. Subsequently, the cecal contents of investigated chickens were collected in a special sterile box for microbiome analysis. This study demonstrated trends toward dose-dependent desirable changes in the chicken microbiome, resulting from supplementing chicken feed with pine needle extract. Some positive outcomes were observed with increasing doses of CNE, such as a reduction in the relative abundance of *Campylobacter*. The antimicrobial activity of pine needle extract was also demonstrated against *Enterobacter cloacae*, *Bacillus cereus*, *Salmonella enterica*, and *Acinetobacter baumannii* strains. Consequently, this valuable type of plant biomass can be converted into high value-added nutritional supplements [95, 107]. However, the mechanism of pine needle extract was not described properly, and the antimicrobial activity should be investigated further. We assume that CNE may act in a similar way as resveratrol [116] (Fig. 8). Potentially pine needle extract similarly as a resveratrol could improve intestinal microbial barrier by inhibiting the growth of pathogens and modulating the composition of intestinal dominant flora. Promotes goblet cells to secrete MUC2 and increases TFF3 in mucous layer [116].

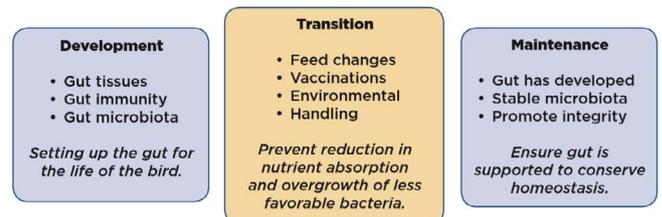


Figure 7: Understanding the needs of the gut at different points in the bird's life and the main goals of gut health support at these times.

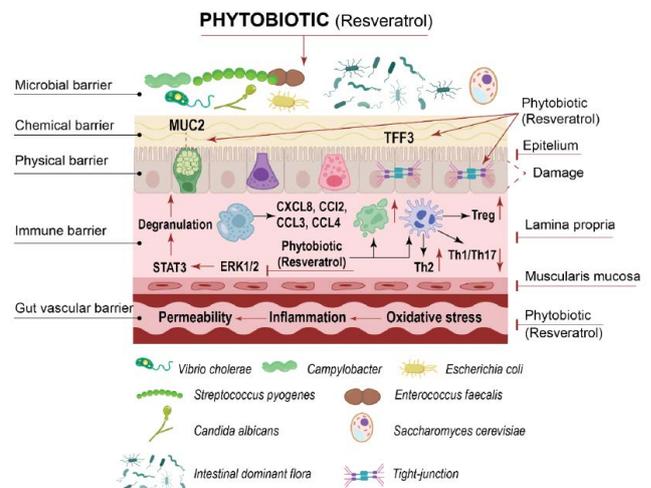


Figure 8: Effects of resveratrol on the intestinal barrier.

## Conclusions and Applications

1. Conifer needle extract is a naturally occurring phytobiotic product, with various effects, including antioxidant, antimicrobial, antifungal, larvicidal / herbicidal, and anti-inflammatory effects, traditionally used in folk medicine.
2. Conifer needle extract show potential antimicrobial action. It demonstrates trends toward dose-dependent desirable changes in the animal microbiome. According to presented aggregated information, coniferous needle extract feasibly can become a cost-effective, biologically active phytobiotic premix ingredient for poultry and livestock. It gently affects the gut, stimulates beneficial bacteria, some of them promote the development of the gut tissues, and inhibit pathogens. Consequently, this valuable plant biomass can be converted into high-value-added nutritional supplements.

However, the mechanism of the antimicrobial activity of conifer needle extracts remains largely unknown and should be investigated in further studies.

## Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interests.

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## Author Contributions

R. J.: writing – 1 original draft preparation, editing, supervision; B. V.: writing – original draft preparation; R. V.: writing – original draft preparation; M. Y. K.: writing – original draft preparation, editing supervision; I. D.: formal analysis; Č. T.: writing – review and editing, visualisation; M. D.: writing – original draft preparation; R. A.: writing – original draft preparation. All authors have read and agreed to the published version of the manuscript

## References

1. Chicken meat production worldwide from 2012 to 2025 (in 1,000 metric tons). Agriculture statistics.
2. Habiba U, Khan A, Mmbaga EJ, et al. Use of antibiotics in poultry and poultry farmers—a cross-sectional survey in Pakistan. *Frontiers in Public Health* 11 (2023): 1–10.
3. Mehdi Y, Letorneau-Montminy MP, Gaucher ML, et al. Use of antibiotics in broiler production: Global impacts and alternatives. *Animal Nutrition* 4 (2018): 170–178.
4. Grady NG, Petrof EO, Claud EC. Microbial therapeutic interventions. *Semin Fetal Neonatal Med* 21 (2016): 418–423.
5. Singh S, Kriti M, Anamika KS, et al. A One Health approach addressing poultry-associated antimicrobial resistance: Human, animal, and environmental perspectives. *The Microbe* 7 (2025): 100309.
6. Stallone RA, et al. The Effects on Human Health of Subtherapeutic Use of Antimicrobials in Animal Feed. *National Academy of Sciences* (1980): 376.
7. Aidara-Kane A, Minato Y. WHO Guidelines on use of medically important antimicrobials in food-producing animals. *World Health Organization* (2017): 88.
8. Chen S, Zhang J, Wu Y. National Action Plan in Antimicrobial Resistance Using Framework Analysis for China. *China CDC Weekly* 5 (2023): 492–498.
9. Wegener HC, Aarestrup FM, Jensen LB, et al. Use of Antimicrobial Growth Promoters in Food Animals and Enterococcus faecium resistance to therapeutic antimicrobial drugs in Europe. *Emerging Infectious Diseases* 5 (1999): 329–335.
10. Butaye P, Devriese LA, Haesebrouck F. Antimicrobial Growth Promoters Used in Animal Feed: Effects of Less-well-known Antibiotics on Gram-Positive Bacteria. *Clinical Microbiology Reviews* 16 (2003): 175–188.
11. Steiner T, Syed Basharat AS. Phytogenic Feed Additives in Animal Nutrition. *Medicinal and Aromatic Plants of the World* (2015): 403–423.
12. Windish W, Schedle K, Plitzer C, et al. Use of phytogenic products as feed additives for swine and poultry. *Journal of Animal Science* 86 (2008): 140–148.
13. Van der Klis JD, Vinyeta E. The potential of phytogenic feed additives in pigs and poultry. *Proc. European Society of Veterinary & Comparative Nutrition* (2014): 18.
14. Applegate AJ, Applegate MD. A Study of Thoughtful Literacy and the Motivation to Read. *The Reading Teacher* 64 (2010): 226–234.
15. Franz C, Baser KH, Windisch W. Essential oils and aromatic plants in animal feeding – A European perspective. *Flavour & Fragrance Journal* 25 (2010): 327–340.
16. Guo J, Bryan B, Polymenis M. Nutrient-specific effects in the coordination of cell growth with cell division in continuous cultures of *Saccharomyces cerevisiae*. *Archives of Microbiology* 182 (2004): 326–330.

17. Florou-Paneri P, Dotas D, Mitsopoulos I, et al. Effect of Feeding Rosemary and Alpha-Tocopheryl Acetate on Hen Performance and Egg Quality. *Journal of Poultry Science* 43 (2006): 143–149.
18. Khalaji S, Mojtaba Z, Mahmood S. Estimation of Standardised Ileal Threonine Equivalency Values of a Multi-Enzyme and Its Effects on Broiler Chick's Performance. *Italian Journal of Animal Science* 10 (2011): 47–50.
19. Cao F, Zhang X, Yu W, et al. Effect of Feeding Fermented Ginkgo biloba Leaves on Growth Performance, Meat Quality, and Lipid Metabolism in Broilers. *Poultry Science* 91 (2012): 1210–1221.
20. Rostami F, Ghasemi H, Taherpour K. Effect of *Scrophularia striata* and *Ferulago angulata* as Alternatives to Virginiamycin on Growth Performance and Immune Response of Broiler Chickens. *Poultry Science* 94 (2015): 2202–2209.
21. El-Abasy M, Motubu M, Shimura K. Immunostimulating and Growth-Promoting Effects of Sugar Cane Extract in Chickens. *Journal of Veterinary Medical Science* 64 (2002): 1061–1063.
22. Durrani F, Sultan A, Ahmed S, et al. Efficacy of Aniseed Extract as Immune Stimulant and Growth Promoter in Broiler Chicks. *Pakistan Journal of Biological Sciences* 10 (2007): 3718–3721.
23. Schiavone A, Guo K, Tassone S, et al. Effects of a Natural Extract of Chestnut Wood on Digestibility and Performance Traits of Broiler Chicks. *Poultry Science* 87 (2008): 521–527.
24. Wang L, Piao X, Kim S, et al. Effects of *Forsythia suspensa* Extract on Growth Performance and Antioxidant Activities in Broiler Chickens. *Poultry Science* 87 (2008): 1287–1294.
25. Zhao X, He X, Yang X, et al. Effect of *Portulaca oleracea* Extracts on Growth Performance and Microbial Populations in Broilers. *Poultry Science* 92 (2013): 1343–1347.
26. Li Y, Ma R, Qi R, et al. Novel Insight into the Feed Conversion Ratio in Laying Hens and Construction of Its Prediction Model. *Poultry Science* 103 (2024): 1–15.
27. Goni I, Brenes A, Centeno C, et al. Effect of Dietary Grape Pomace and Vitamin E on Growth Performance and Nutrient Digestibility in Chickens. *Poultry Science* 86 (2007): 508–516.
28. Brenes A, Viveros A, Goni I, et al. Effect of Grape Pomace Concentrate and Vitamin E on Digestibility of Polyphenols and Antioxidant Activity in Chickens. *Poultry Science* 87 (2008): 307–316.
29. Leusink G, Rempel H, Skura B, et al. Growth performance, meat quality, and gut microflora of broiler chickens fed with cranberry extract. *Poultry Science* 89 (2010): 1514–1523.
30. Juskievicz J, Gruzauskas R, Zdunczyk Z, et al. Effects of dietary addition of *Macleaya cordata* alkaloid extract on growth performance in broilers. *Journal of Animal Physiology and Animal Nutrition* 95 (2011): 171–178.
31. Viveros A, Chamorro S, Pizarro M, et al. Effects of dietary polyphenol-rich grape products on intestinal microflora in broiler chicks. *Poultry Science* 90 (2011): 566–578.
32. Issa K, Omar JM, A M. Effect of garlic powder on performance and lipid profile of broilers. *Open Animal Science* 2 (2012): 62–68.
33. Chamorro S, Viveros A, Centeno C, et al. Effects of dietary grape seed extract on growth performance and amino-acid digestibility in broiler chicks. *Animal* 7 (2013): 555–561.
34. Tiisonen K, Kettunen H, Bento MH, et al. The effect of feeding essential oils on broiler performance and gut microbiota. *British Poultry Science* 51 (2010): 381–392.
35. Amerah AM, Gilbert C, Simmins H, et al. Influence of feed processing on the efficacy of exogenous enzymes in broiler diets. *World's Poultry Science Journal* 67 (2011): 29–46.
36. Malayoglu H, Baysal S, Misirlioglu Z, et al. Effects of oregano essential oil with or without feed enzymes on growth performance of broilers. *British Poultry Science* 51 (2010): 67–80.
37. Hashemipour H, Kermanshahi H, Golian A, et al. Effect of thymol and carvacrol feed supplementation on performance and immune response in broiler chickens. *Poultry Science* 92 (2013): 2059–2069.
38. Ghazanfari S, Mohammadi Z, Moradi AM. Effects of coriander essential oil on performance and intestinal microbiota of broilers. *Brazilian Journal of Poultry Science* 17 (2015): 419–426.
39. Chalghoumi R, Belgacem A, Trabelsi I, et al. Effect of dietary supplementation with probiotic or essential oils on growth performance of broiler chickens. *International Journal of Poultry Science* 12 (2013): 538–544.
40. Kim SJ, Lee KW, Kang CW, et al. Growth performance and blood characteristics in broiler chickens fed diets containing different nutrient density with or without essential oils. *Asian-Australasian Journal of Animal Sciences* 29 (2016): 549–554.
41. Alcicek A, Bozkurt M, Cabuk M. The effect of a mixture of herbal essential oils on broiler performance. *South African Journal of Animal Science* 34 (2004): 217–222.

42. Khattak F, Ronchi A, Castelli P, et al. Effects of natural blend of essential oil on growth performance and carcass quality of broiler chickens. *Poultry Science* 93 (2014): 132–137.
43. Cabuk M, Bozkurt M, Alcicek A, et al. Effect of a dietary essential-oil mixture on performance of laying hens in the summer season. *South African Journal of Animal Science* 34 (2004): 215–221.
44. Isabel B, Santos Y. Effects of dietary organic acids and essential oils on growth performance and carcass characteristics of broiler chickens. *Journal of Applied Poultry Research* 18 (2009): 472–476.
45. Amad AA, Manner K, Wendler KR, et al. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. *Poultry Science* 90 (2011): 2811–2816.
46. Lee JT, Bailey CA, Cartwright AL.  $\beta$ -Mannanase ameliorates viscosity-associated depression of growth in broiler chickens. *Poultry Science* 82 (2003): 1925–1931.
47. Basmacioglu H, Tokusoglu O, Ergul M. The effect of oregano and rosemary essential oils on performance and lipid oxidation of meat in broilers. *South African Journal of Animal Science* 34 (2004): 197–210.
48. Hernandez F, Madrid J, Garcia V, et al. Influence of two plant extracts on broilers' performance, digestibility, and digestive organ size. *Poultry Science* 83 (2004): 169–174.
49. Zhang AW, Lee BD, Lee SK, et al. Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance and meat quality of broiler chicks. *Poultry Science* 84 (2005): 1015–1021.
50. Jang IS, Ko YH, Kang SY, et al. Effect of dietary supplementation of ground grape seed on growth performance and antioxidant status in broiler chickens. *Korean Journal of Poultry Science* 34 (2007): 1–8.
51. Franz C, Baser KH, Windisch W. Essential oils and aromatic plants in animal feeding – a European perspective. *Flavour & Fragrance Journal* 25 (2010): 327–340.
52. Bravo D, Pirgozliev V, Rose SP, et al. A mixture of carvacrol, cinnamaldehyde, and capsicum oleoresin improves energy utilization and growth performance of broiler chickens fed a maize-based diet. *Journal of Animal Science* 92 (2014): 1531–1536.
53. Commission Implementing Regulation (EU) 2015/1490. Authorization of carvacrol, cinnamaldehyde and capsicum oleoresin as a feed additive for chickens for fattening. *Official Journal of the European Union* 231 (2015): 4–5.
54. Karadas F, Pirgozliev V, Rose SP, et al. Dietary essential oils improve the hepatic antioxidative status of broiler chickens. *British Poultry Science* 55 (2014): 329–334.
55. Pirgozliev V, Bravo D, Rose SP. Rearing conditions influence nutrient availability of plant-extract supplemented diets when fed to broiler chickens. *Journal of Animal Physiology and Animal Nutrition* 98 (2014): 667–671.
56. Zhang ZF, Kim IM. Effects of multistrain probiotics on growth performance and nutrient digestibility in broilers. *Poultry Science* 93 (2014): 364–370.
57. Zhao FQ, Zhang ZW, Wang C, et al. The role of heat shock proteins in inflammatory injury induced by cold stress in chicken hearts. *Cell Stress and Chaperones* 18 (2013): 773–783.
58. Lu H, Adedokun S, Ajuwon L, et al. Anti-inflammatory effects of non-antibiotic alternatives in coccidia-challenged broiler chickens. *Journal of Poultry Science* 51 (2014): 14–21.
59. Mueller D, Gerber J, Johnston M, et al. Closing yield gaps through nutrient and water management. *Nature* 490 (2012): 254–257.
60. Liu Y, Song M, Almeida F, et al. Energy concentration and amino-acid digestibility in corn coproducts fed to growing pigs. *Journal of Animal Science* 92 (2014): 4557–4565.
61. Kim Y, Seok J, Kwak W. Evaluation of microbially ensiled spent-mushroom substrates as roughage for ruminants. *Journal of Animal Science & Technology* 52 (2010): 117–124.
62. Lee K, Lee S, Lillehoj H, et al. Effects of direct-fed microbials on growth performance and immune characteristics in broiler chickens. *Poultry Science* 89 (2010): 203–216.
63. Pourhossein Z, Qotbi A, Seidavi A, et al. Effect of dietary sweet-orange peel extract on immune-system responses in broiler chickens. *Journal of Animal Science* 86 (2008): 105–110.
64. Cho S, Choi Y, Park S, et al. Carvacrol prevents diet-induced obesity by modulating gene expression in mice fed a high-fat diet. *Journal of Nutritional Biochemistry* 23 (2012): 192–201.
65. Malayoglu H, Sarica S, Sanlier S, et al. The use of oregano essential oil and enzyme mixture in corn–soybean diets of broiler chicks. *European Poultry Science* 78 (2014): 1–14.
66. Hashemipour H, Kermanshahi H, Golian A, et al. Effect of thymol and carvacrol feed supplementation on performance and immune response in broiler chickens. *Poultry Science* 92 (2013): 2059–2069.

67. Ghazanfari S, Driessen-Mol A, Strijkers G, et al. The evolution of collagen-fiber orientation in engineered cardiovascular tissues visualized by diffusion-tensor imaging. *PLoS ONE* 10 (2015): 1–15.
68. Mugugesan G, Ledoux D, Naehrer K, et al. Prevalence and effects of mycotoxins on poultry health and performance. *Poultry Science* 94 (2015): 1298–1315.
69. Jamroz D, Orda J, Kamel C, et al. The influence of phytogenic extracts on performance and gut microbial status in broiler chickens. *Journal of Animal Feed Science* 12 (2003): 583–596.
70. Placha I, Takacová J, Ryzner M, et al. Effect of thyme essential oil and selenium on intestinal integrity and antioxidant status of broilers. *British Poultry Science* 55 (2014): 105–114.
71. European Commission. *Innovating for Sustainable Growth: A Bioeconomy for Europe*. COM(2012)60 (2012).
72. Trigo E, Chavarria H, Pray C, et al. The bioeconomy and food systems transformation. *Sustainability* 15 (2023): 1–12.
73. McGauran T, Dunne N, Smyth B, et al. Feasibility of the use of poultry waste as polymer additives and implications for energy, cost and carbon. *Journal of Cleaner Production* 291 (2012): 1–30.
74. Klavins L, Almonaityte K, Šalaševičienė A, et al. Strategy of coniferous needle biorefinery into value-added products. *Molecules* 28 (2023): 1–25.
75. Bhardwaj K, Silva A, Atanassova M, et al. Conifers phytochemicals: A valuable forest with therapeutic potential. *Molecules* 26 (2021): 3005.
76. Koutsaviti A, Toutoungy S, Saliba R, et al. Antioxidant potential of pine needles: Essential oils and extracts of *Pinus* species. *Foods* 10 (2021): 142.
77. Mofikoya O, Mäkinen M, Janis J, et al. Chemical fingerprinting of conifer needle essential oils by ultrahigh-resolution mass spectrometry. *ACS Omega* 5 (2020): 10543–10552.
78. Hoi N, Duc H, Thao D, et al. Selectivity of *Pinus sylvestris* extract on estrogen-responsive breast-cancer cells. *Pharmacognosy Magazine* 11 (2015): 290–295.
79. Akaberi M, Boghrati Z, Amiri M, et al. A review of conifers in Iran: Chemistry and biological importance. *Current Pharmaceutical Design* 26 (2020): 1584–1613.
80. Díaz-Sala C, Cabezas J, de Simon B, et al. The uniqueness of conifers. *Woodhead Publishing* (2013): 67–96.
81. Zulak K, Bohlmann J. Terpenoid biosynthesis and specialized vascular cells of conifer defense. *Integrative Plant Biology* 52 (2010): 86–97.
82. Bespalov V, Alexandrov V. Coniferous chlorophyll-carotene paste: A review of medical applications. N.N. Petrov State Scientific Research Institute of Oncology (2006).
83. Jagodin VI. *Fundamentals of chemistry and technology of processing wood greens*. Leningrad University Press (1981).
84. Roschin VI, Vasiliev SN, Pavlutsкая IS, et al. The method of processing coniferous species woody greens. Patent RU2015150 C1 (1994).
85. Solodky FT. *Production of coniferous chlorophyll-carotene paste*. Moscow (1956).
86. Roschin VI, Vasiliev SN, Pavlutsкая IS, et al. The method of processing coniferous species woody greens. Patent RU2015150 C1 (1994).
87. Trigo E, Chavarria H, Pray C, et al. The bioeconomy and food systems transformation. *Sustainability* 15 (2023): 1–12.
88. McGauran T, Dunne N, Smyth B, et al. Feasibility of poultry waste as polymer additives. *Journal of Cleaner Production* 291 (2012): 1–30.
89. Levin ED, Repyah SM. *Processing of woody greens*. Moscow (1984).
90. Levin ED, Repyah SM. Conifers chlorophyll-carotene paste technical specifications, GOST 21802-84. Moscow (1984).
91. Gindulin IK, Shegolev AA. *Methods of studying woody greens*. Ekaterinburg (2023).
92. Dziejdzinski M, Kobus-Cisowska J, Szymanowska-Powałowska D, et al. Polyphenol composition and antimicrobial properties of *Pinus sylvestris* shoots extracts. *Emirates Journal of Food and Agriculture* 32 (2020): 229–237.
93. Zeng WC, Zhang Z, Gao H, et al. Chemical composition and antimicrobial activities of pine needle essential oil. *Journal of Food Science* 77 (2012): 824–829.
94. Kwak CS, Moon SC, Lee MS. Antioxidant and antitumor effects of pine needles (*Pinus densiflora*). *Nutritional Cancer* 56 (2006): 162–171.
95. Lee MT, Lin WC, Yu B, et al. Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals – A review. *Asian-Australasian Journal of Animal Sciences* 30 (2016): 299–308.
96. Haman N, Morozova K, Tonon G, et al. Antimicrobial effect of *Picea abies* extracts on *E. coli* growth. *Molecules* 24 (2019): 4053.

97. Kim NY, Jang MK, Jeon MJ, et al. Verification of antimicrobial activities of pine needle extracts against resistant *Staphylococcus aureus*. *Journal of Life Sciences* 20 (2010): 589–596.
98. Rubens J, Kibilds J, Jansons M, et al. Application of Baltic pine needle extract as a gut-microbiota-modulating feed supplement for chickens. *Plants* 12 (2023): 1–13.
99. Zeng WC, Jia LR, Zhang Y, et al. Antibrowning and antimicrobial activities of water-soluble pine needle extracts. *Journal of Food Science* 76 (2011): 318–323.
100. Kacaniová M. Antibacterial and antiradical activity of essential oils from different origins. *Journal of Environmental Science and Health Part B* 49 (2014): 505–512.
101. Soultanov V, Kulyashova L, Nikitina T, et al. Antimycotic activity of conifer needle complex against *Candida* species. *Natural Product Communications* 13 (2018): 75–78.
102. Faldt J. Volatile constituents in conifers and conifer-related wood-decaying fungi. PhD Thesis, Royal Institute of Technology, Stockholm (2000).
103. Ismail A, Hamrouni L, Gargouri S, et al. Chemical composition and biological activities of essential oils of *Pinus patula*. *Natural Product Communications* 6 (2011): 1531–1536.
104. Jagodin VI. Fundamentals of chemistry and technology of processing wood greens. Leningrad University Press (1981).
105. Jagodin VI, Roschin VI, Vasiliev SN, et al. The method of processing coniferous species woody greens. Patent RU2015150 C1 (1994).
106. Koutsaviti K, Giatropoulos A, Pitarokili D, et al. Greek *Pinus* essential oils: Larvicidal activity and repellency against *Aedes albopictus*. *Parasitology Research* 114 (2015): 583–592.
107. Suntar I, Tumen I, Ustun O, et al. Wound healing and anti-inflammatory activities of essential oils from *Pinus* species. *Journal of Ethnopharmacology* 139 (2012): 533–540.
108. de Cassia da Silveira e Sa R, Andrade LN, de Olivero RD, et al. Anti-inflammatory activity of phenylpropanoids found in essential oils. *Molecules* 19 (2014): 1459–1480.
109. Belums S, Platnieks O, Sevcenko J, et al. Sustainable wax coatings made from pine needle extraction waste for nanopaper hydrophobization. *Membranes* 12 (2022): 1–12.
110. Muizniece I, Lauka D, Blumberga D. Thermal conductivity of patterned pine and spruce needles. *Energy Procedia* 72 (2015): 256–262.
111. Kaur S, Konwar R, Negi PJ, et al. Utilization of biodegradable insulation materials for developing solar water heater for hill climates. *Energy for Sustainable Development* 67 (2022): 21–28.
112. Development of bioefficient animal feed for organic farms. Project Report (2021). Riga.
113. Latvian Food Supplements Registry. Health drink “Ho-fi”. Latvian Registry (2021).



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