



Organic-PLUS

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Authors:

Federico Righi, Rosario Pitino, Giulio Grandi, Afro Quarantelli, Andrea Summer, Judith Conroy, Sara Burbi, Carmen L. Manuelian, Massimo De Marchi

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1. Summary

Organic production and the labelling of organic products in the European Union are regulated by the Regulation (EU) 2018/848 of May 30th 2018, and although the principles of organic farming seem easy to follow, the regulation includes several exceptions, as in some instances a compromise has to be made to ensure animal health and welfare. To help fully realise the principles of organic livestock farming, effective alternatives to the use of synthetic vitamins, anti-infective and immune-stimulators, and bedding materials have to be examined and developed. A literature review of potential plant alternatives to synthetic vitamins, anti-initiotics and antiparasitics has being conducted to support further activities within the Organic-PLUS project, notably the selection of components to be tested in *in vitro* studies. Based on the literature review, 3 Factsheets for stakeholder uptake have been prepared on potential molecules from plant products to be employed as alternative sources of (1) vitamins, (2) antiparasitics, and anti-infective or immuno-stimulants for use in organic farming.



2. Introduction

Organic production and the labelling of organic products in the European Union are regulated by the Regulation (EU) 2018/848 of May 30th 2018. Although the principles for organic farming seem easy to follow, the regulation is comprised of several exceptions since in some instances a compromise has to be taken to ensure animal health and welfare. To help in fully achieve the principles of organic livestock farming, effective alternatives for the use of synthetic vitamins, anti-infective and immune-stimulators, and bedding materials have to be examined and developed.

A literature review on potential plant alternatives to the use of synthetic vitamins, antibiotics and antiparasitics has being conducted to support further activities within the Organic-PLUS project, notably the selection of components to be tested in in vitro studies.

The literature review is available in D4.2. To summarize the relevant information regarding natural sources as alternatives to synthetic vitamins, antiparasitics and antimicrobials, a factsheet has been prepared for each topic (Factsheet 1 for vitamins; Factsheet 2 for antiparasitics, and Factsheet 3 for antimicrobials) to help in the dissemination of the results among stakeholders and farmers.



NATURAL VITAMINS IN ORGANIC LIVESTOCK



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No [774340 - Organic-PLUS]

F. Righia, C.L. Manuelianb, R. Pitinoa, A. Quarantellia, A. Summera, M. De Marchibauniversity of Parma; bDAFNAE, University of Padova

SUMMARY

Vitamins organic are compounds essential for animal health and performance. Conventional farming uses synthetic vitamins to fulfil livestock requirements because they are cheaper and usually more stable than natural those from sources. The European regulation for organic livestock production indicates that vitamins animal feed in correspond to those naturally occurring in feedstuff, with some exceptions to assure animal health. They also allow the use of algae and food industry byproducts, which are relevant sources of vitamins and antioxidant compounds. Economically relevant by-products in the Mediterranean area are those from the olive oil, citrus, wine, and carob food industries. Based on scientific evidence from several research trials, the use of products these is а reasonable alternative to the use of synthetic vitamins to assure adequate vitamin intake of livestock and to ameliorate animals' oxidative status. However, there is very little information on the vitamin content characterisation of these products and very few studies have evaluated their impact animals' performance and products such as milk, meat and eggs. This document briefly summarises the results available for this topic.

INTRODUCTION

Some vitamins must be included in animal diets and are considered dietary essentials, but the microbiota of some animal species are able to synthesise some of them at a sufficient level for them to be absorbed directly from the animals' gut mucosa surface. Thus, nutritional requirements differ between species. For instance, rumen microbiota in healthy adult cattle are able to synthesise adequate amounts of vitamin C and many B vitamins, fulfilling cows' requirements for those vitamins.

What does the European legislation say?

The origin of those vitamins that can be used in organic livestock production is regulated by Regulation (EU) 2018/848 of May 30th 2018. It establishes that supplementation diet vitamins should correspond to those naturally occurring feedstuff. in However, it does allow for monogastrics to be given synthetic vitamins identical to natural vitamins; and for ruminants, the use of synthetic vitamins A, D, and E identical to natural vitamins with prior authorisation of the Member States. based on the assessment of the impossibility for organically raised ruminants to obtain the necessary quantities of the said vitamins through their regular feed. Also listed in Annex VIII are the food additives that could be

used in organic livestock farming, such as extracts from plants and products of animal origin. In Annex V, non-organic feed materials that could be used under certain conditions, which include food industry by-products from non-organic production, are listed. Important by-products in the Mediterranean region are those from the olive oil, citrus, wine, and carob food industries.

Why use food industry by-products?

The possibility of using food industry byopens a door to the products philosophy underlying environmental sustainability and organic farming. Industry waste can be reduced, low value product are given a higher value - both from an economical and nutritional point of view -, carbon footprint is reduced by using local waste products, as well as the feed to food competition in livestock production[2]. However, food industry by-products have so far been investigated more for their potential in energy production than for their use as animal feed or diet additives[2].

BY-PRODUCTS AS VITAMIN ALTERNATIVES

Algae contain α -tocopherol (Vitamin E fraction), β-carotene (Vitamin precursor), niacin and thiamine (Vitamin B-complex), and Vitamin C; and byproducts have Vitamin C, α-tocopherol and carotenoids. Moreover, they are polyphenols which rich in have antioxidant properties and can simulate and enhance the antioxidant activity of Vitamin A, E and C, apart from exerting a sparing effect on them. Table 1 summarises the animal species from which the effect of food industry byproducts has been studied.

Table 1. Studies evaluating food industry byproducts for use in animal feed.

By-product	species	n. of studies
Olive oil	cows, water buffalos, sheep, poultry, pigs, rabbits, fish	22 (50% of them in ruminants)
Citrus	beef, sheep	5
Wine	poultry	4
Carob	cow, lamb, pigs	6

Olive oil industry by-products

Olive groves (Olea europaea L.) are an important crop in the Mediterranean area which contains the world's 3 greatest olive producers. In 2017, 21 megatonnes of olives were produced globally (Figure 1)^[3]. Of this total, 31% were from

Spain, 13% from Greece and 12% from Italy. Almost all olives harvested are destined to produce oil.

The olive oil industry produces several by-products such as olive oil mill wastewater, olive pomace, olive cake, olive leaves and olive stones. Their chemical composition depends on the olive variety and the oil extraction method. In general, they have a low protein content but high energy, which could negatively affect animals' feed intake. Diet supplementation using some of these by-products has been evaluated in cows, water buffalos, sheep, poultry, pigs, rabbits and fish. The effect of supplementation with these by-products at a low level (<10%) resulted in an improvement in the fatty acid profile in milk and meat^[4]. This means a lower proportion of saturated than unsaturated fatty acids, usually without affecting animal productive performance. However, in rabbits it has also been reported to reduce feed intake as well as growth rate, carcass weight and dressing out percentage.

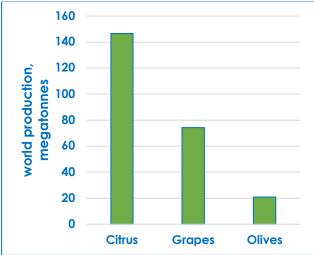


Figure 1. Citrus, grape and olive worldwide production in 2017. Data from FAOSTAT[3].

Citrus industry by-products



Citrus (Citrus spp.) are one of the most abundant fruit crops, with a

worldwide production of 147 megatonnes in 2017 (Figure 1), with China (27%), Brazil (14%) and India (8%) the greatest producers^[3]. About 18% of global citrus production is from Mediterranean countries^[3].

The citrus industry produces a by-product named citrus pulp which comprises peel, pulp, pith and seeds. This by-product is rich in pectin and soluble carbohydrates; it has a high energy and a low protein content. This by-product has only been tested in lambs and beef. In lambs, it improved the meat fatty acid profile, meat

oxidative stability and antioxidant status. In beef, it improved forage intake, digestion and ruminal pH.

Wine industry by-products

Grapes (Vitis spp.) are one of the most valued fruits in the world based on hectares cultivated and their economic value. Italy, France and Spain represent 24% of worldwide grape production (74 megatonnes in 2017; Figure 1), and are among the 5 greatest producers worldwide^[3].

The wine industry produces a byproduct named grape pomace

which comprises

skins, stems and seeds. The study of animal diet supplemented with grape pomace has focused on chickens showing its potential to reduce lipid oxidation of the meat during refrigerated storage. This is an important trait for the poultry industry because poultry meat is richer in polyunsaturated fatty acid compared with other species.

Carob industry by-products

Carob tree (Ceratonia siliqua L.) is a typical crop in the Mediterranean area. Carobs worldwide production in 2017 was 0.14 megatonnes, with the most productive

countries being Portugal (31%), Italy (21%) and Morocco (16%)[3].



The processing of the pods produces carob pulp as a by-product. The carob pulp presents a high sugar content and low protein and fat contents. Moreover,

its fatty acid profile includes essential fatty acids for animal nutrition such as linoleic and α -linolenic acids. Diet supplementation with carob pulp has been investigated in cows, lambs and pigs showing an improved fatty acid profile of the meat; that is, a greater proportion of unsaturated than saturated fatty acid, and a reduction of n-6/n-3 ratio.

Conclusions

Algae, olive oil, citrus, wine and carob industry by-products contain Vitamin E and A's precursors, Vitamin C, some vitamins from the B-complex and Polyphenols polyphenols. antioxidant properties and can simulate and enhance the antioxidant activity of Vitamin A, E and C. Although there is very little information on the vitamin characterisation of these products and their impact on animal performance and products. There is evidence that algae, olive oil, citrus, wine and carob industry by-products are a feasible alternative to the use of synthetic vitamins in assuring the adequate vitamin intake of livestock and to improve animals' oxidative status.

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TAKE HOME MESSAGES

- Food industry by-products seem a reasonable alternative to the use of synthetic vitamins to assure the adequate vitamin intake in livestock.
- By-products are rich in energy but have a low protein content.
- They enhance the antioxidant activity of Vitamins A, E and C.
- There is very little information available on the vitamin characterisation of by-products and their impact on animal performance and products.

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For further information, please contact those responsible for the Livestock work of Organic-PLUS, massimo.demarchi@unipd.it

PROJECT WEBSITE

www.organic-plus.net





NATURAL ANTIPARASITICS IN ORGANIC LIVESTOCK



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F. Righia, G. Grandib, C.L. Manueliana, R. Pitinoa, M. De Marchia

^aUniversity of Parma; ^bSwedish University of Agricultural Sciences; ^cDAFNAE, University of Padova;

SUMMARY

The control of parasites in livestock is still reliant on the application of synthetic emergence compounds. The parasites (helminths and arthropods) resistant to virtually all the available compounds together with the fact that organic farming should be free from these substances. make finding alternative treatments an uraent matter. The literature is rich in studies of the use of plant and plant-derived substances as antiparasitics. Their use is often documented in traditional medicine (ethnomedicine). Despite the increasing amount of published studies, standardised procedures are lacking, as well as an agreement in the scientific community that could lead to a recognised status of antiparasitic products for at least some of the plant studied. Here we present examples of plants and plant-derived substances (essential oils and extracts) and their activity against a broad group of parasites, mainly helminths and arthropods.

INTRODUCTION

Parasitic infection in livestock impairs growth performances, fertility and productivity (milk, wool, meat) with a wide range of impacts on either calves/heifers, dairy cattle, sheep and swine. These parasites can be generally classified as endoparasites (protozoa and metazoa) and ectoparasites (arthropods). There is an urgent need to explore alternatives to conventional treatments since anthelmintic and antiparasitic drug resistance is emerging worldwide and the routine use of antiparasitic drugs in organic farming is unclear.

Plants naturally produce over 60,000 chemical compounds to deter herbivores, to destroy microbial pathogens and to communicate with other organisms. Based empirical and scientific evidence, the exploitation the of diversity bioactivity plant of secondary metabolites may be a viable alternative to synthetic antiparasitic products. The consumption of medicinal plants either in the form of plant parts or as extracts has been related to anti-nutritional and immune-modulatory effects. effects on hosts can occur after plant or plant products administration and, as a result, the possibility of using plant products to control parasitism livestock is still under investigation. Over the last 7 years there has been a substantial increase in the number of publications on this topic, showing that this is a very active research field.

What does the European legislation say?

Organic livestock production regulated by Regulation (EU) 2018/848 of May 30th 2018, which indicates that chemically synthesised allopathic veterinary medicinal products prohibited for preventive treatment. However, the regulation does not specify what is allowed or forbidden regarding antiparasitic products, thus making even more urgent the need to define alternatives to conventional antiparasitic drugs.

Table 1. Phytochemical compounds with antiparasitic activity.

- <i> </i> -
Phytochemical compound
Saponins
Benzyl isothiocyanate
Cysteine proteinases
Isoflavones
Artemisin
Phenolic compounds
Tannins
Alkaloids

Reliability of results on the antiparasitic properties of plant products

The great variety of models and methods available to test the anthelmintic properties of plants and the lack of measures to minimise experimental variability lead to a general inconsistency between studies on the anthelmintic activity of plant products. When testing plant products, it should also be considered that the in vitro results sometimes are not confirmed in vivo.

ANTIPARASITIC EFFECTS OF PLANTS

Recent reviews highlight that products derived from plant extracts will probably become a viable alternative for the

control of parasites of veterinary interest in the near future. Their mode of action depends on their content of different phytochemicals (Table 1).

Plants with anthelmintic compounds

Plants with anthelmintic efficiency in livestock and humans are:: extracts from lichens and ferns (e.g. Dryopteris filix-mas) against tapeworm infections; trees and shrubs (e.g. Salix spp.) against parasites; oil from herbaceous plants like wormseed or goosefoot (Chenopodium ambroisoides) against **Ascaris** infections; Caraway (Carum carvi), thyme (Thymus spp.), mint (Mentha spp.) against Trichostrongylus larvae in vitro and in vivo (sheep); plants from Artemisia genus (active compound: santonin) and Tanacetum vulgare (active component: thujon) against several **nematode** (Ascaris, Enterobius, Trichostrongylus, Ostertagia) and tapeworms; Daucus carota, Brassica spp., Allium spp. and all kinds of berries against parasites; the Cucurbitaceae family, particularly pumpkin cucumber seeds (active compound: cucurbitine) against tapeworm infections; **Nicotiana rustica**, particularly leaves, (active compound: nicotine) against nematode in ruminant until mid-1950s: pasture plants (active compound: proanthocyanidins) such as Hedysarum coronarium (sulla) and Lotus pedunculatus (lotus major) against nematodes both in vivo and in vitro; and tanniferous plants, the activity of which against internal parasites could be explained by the increased availability of diaestible protein to the animal or by a direct anthelmintic effect on resident worm populations or on free-living stages in animals.

Other plants showing antiparasitic activity are[2]: Zingiber officinale (containing zingiberene) against H. contortus; Melia azedarach (rich in mliacaprin, scopoletin, meliartenin) has also larvicidal and ovicidal activity on H. contortus; Nigella sativa L. (rich in thimoquinone) against tapeworms and several nematodes: **Flemingia** (containing aenistein) procumbens against intestinal parasites of poultry trematode species; Ocimum and sanctum (containing eugenol, caryophyllene and urosilic acid) against several larvae of **mosquito** species; Azadirachta indica (containing azadirachtine) against F. gigantica and helminths of livestock contortus) and ticks (B. microplus); Calotropis (containina procera calotropin and calactin) against nematodes **Oesophagostomum** columbianum Bunostomum and trigonocephalum in sheep Ostertagia, Nematodirus, Dictyocaulus, Taenia, Ascaris and Fasciola; Artemisia annua (rich in artemisinin and quercetin) against F. hepatica and gastrointestinal **nematodes** in small ruminants **Plasmodium** spp. and other important protozoan parasites.

A review from Serbia^[3] mentioned the efficacy of white mugwort (Artemisia absinthium) mugwort and black (Artemisia vulgaris) traditional as anthelmintics treatments. Moreover, decoction of the rhizome of genuione brachens. Male Fern (Dryopteris filixmas), represents one of the strongest natural drugs to control tapeworms and flukes through the active compounds filicin and filmarone, which are toxic for the worms, and oleorescin that paralyses parasite musculature.

Plants for ectoparasitic control

At the moment, farmers rely mainly on chemical acaricides and repellents for the control of ectoparasites (e.g. ticks, mites, lice). However, as in other cases, ectoparasites resistant ticks are found with growing frequency in relation also to an increased application of acaricides. In places where small scale farms are the largest proportion of livestock enterprises – as in developing countries across the world – ethnoveterinary plant use for ectoparasites control is very important. Evidences of some activity against ectoparasites has been reported for the following plants:

Topical application of natal laburnum (Calpurnia aurea) juice of leaves and bark for **tick** control (to kill in compromise mobility) Ethiopia: Otostegia integrifolia for mosquito repellence: Jetropha curcas in Latin America, Africa and Asia against ticks decreasina their egg production and acaricidal activity, in particular against Callosobruchus maculatus and R. annulatus: Nicotiana tabacum in South-Western Nigeria for the prevention of lice and treatment of larvae and nymphs of R. appendiculatus on the ears of calves.

The use of white mugwort and black mugwort (ground fresh leaves, mixed with lard and rubbed over the cattle skin) as a repellent of flies has been reported from Serbia, and the use of stinking hellebore (Helleborus L., Ranunculaceae family) together with hellebore (Veratrum album L., Liliaceae) and tobacco (Nicotiana tabacum L.,

Solanaceae) to eradicate cattle lice infestation and mange.

Antiparasitic plants in fish farming

A further research area that should be considered when studying the use of plants as alternatives to synthetic antiparasitics is the use of plant products in fish farming. Information is available regarding the use of plant compounds for oral administration or immersion, active against the most economically important parasite species in fish farming, namely protozoans, myxozoans and helminths (monogeneans). Several studies have demonstrated the effects of both essential oils and different kinds of extracts in treating fish parasites^[4].

PROJECT WEBSITE

www.organic-plus.net

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For further information, please contact those responsible for the Livestock work of Organic-PLUS, <u>massimo.demarchi@unipd.it</u>

TAKE HOME MESSAGES

- A variety of plant products have traditionally been used for therapeutic purposes to control both endoparasites and ectoparasites.
- Scientific validation of the efficacy of these plant products is often lacking.
- Various evidences demonstrate that a scientific effort needs to be made to characterise and validate the use of various plant products.

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ORGANIC NATURAL ANTIMICROBIALS

IN ORGANIC LIVESTOCK This project has received funding from the European Union's Horizon 2020 research and innovation program



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F. Righia, C.L. Manuelianb, R. Pitinoa, M. De Marchib aUniversity of Parma; bDAFNAE, University of Padova;

SUMMARY

Antimicrobials represent one of the most important discoveries, to both human and animal health. However, spread of multi-resistant microorganisms and the development of alternative livestock farming systems such as organic farming has led to the need to exploit new alternative and natural compounds from plants, minimally replace synthetic processed. to molecules. Among these, the wide group of phytochemicals has been recently investigated, mostly in order to replace the use of antimicrobial-growth promoters in swine and poultry. These compounds are called phytobiotics by some authors. Among them, essential shown oils have very interesting antimicrobial properties against several pathogenic bacteria. Many in vitro studies have been published essential oils and other plant extracts. However, areater information is still needed before allowing the use of these alternatives on a daily basis at farm level. They appear to be very promising tools in contributing to the reduction of antimicrobial-resistance and at the same time, to meet food safety requirements without compromising animal health and welfare. This document briefly summarises the results available for this topic.

INTRODUCTION

Phytochemicals are non-nutritive plantderived compounds which have an important role in plants defence. Phytochemicals are considered, along with other compounds and additives, as possible alternatives to antibiotics with the advantage of being from natural sources. Because they are derived from plants, they are sometimes called phytobiotics. Some of the preferred characteristics of natural antimicrobials are listed in Table 1.

Characteristics natural **Table** 1. that antimicrobials should meet:

Properties for natural antimicrobials			
non-toxic, with no side effects on animals			
short term presence of residues			
be stable in the feed and animal			
gastrointestinal tract			
low environmental impact			
without influences on palatability			
without disturbing physiological intestinal flora			
efficacious against pathogenic bacteria			
enhance the body resistance to diseases			
ameliorate feed efficiency and enhance			
animal growth			
good compatibility			
without contributing the development of			
antimicrobial-resistance			

What does the European legislation say?

livestock production Oraanic regulated by Regulation (EU) 2018/848 of May 30th 2018, which indicates that chemically synthesised veterinary medicinal products are prohibited for preventive treatment. Moreover, when they are prescribed the withdrawal period is twice that for conventional farming; and if animals receive more than 3 courses of treatment within a year (or more than 1 course if the productive lifecycle is <1 year), they should undergo a conversion period to be considered organic again.

PHYTOBIOTICS IN LIVESTOCK

Several in vitro and in vivo studies have been conducted to investigate the antimicrobial properties of plantderived compounds. Great attention has been paid to thyme, oregano, rosemary, marjoram, yarrow, garlic, ginger, green tea, black cumin, coriander and cinnamon, especially in the poultry sector for their potential application as arowth promoter alternatives[2].

Plants and plant products with high concentrations phytochemical of compounds can be used in solid, dried and around form or as extracts (crude or concentrated), and also as essential oils and oleoresins, depending on the process used to derive the active ingredients. Among essential oils, two major classes have been identified: terpenes (e.g. carvacrol and thymol) phenylpropenes and (e.g. cinnamaldehyde and eugenol).

In vitro trials on phytobiotics

Minimum inhibitory concentration, pharmacokinetic and quorum sensing studies have been employed to evaluate phytobiotics (e.g., tannins, Quebraco extract) against bacterial

pathogens in swine, Salmonella and other bacteria responsible for diarrhoea in animals, and bacteria responsible for mastitis in dairy cattle, including Staphilococcus spp., Streptococcus spp. and E. coli. Essential oils from thymol, eugenol and carvacrol have also been tested against E. coli and Salmonella spp. (Figure 1).



Figure 1. An example of MIC determination, from Thosar et al.^[3].

In vivo trials of phytobiotics in

Swine. Several experiments have been conducted to evaluate the use of essential oils



in swine, including oregano, cinnamon, mexican pepper alone or blended, capsaicin, carvacrol, and thymol to counteract diarrhoea and other diseases.

Poultry. Herbs, spices, other plant extracts and essential oils are used as

feed additives in the poultry industry, where they are perceived to be growth promoters. They have been used to control pathogenic bacteria,



including **Clostridium perfringens** and **E. coli**, and to stabilise the ecosystem of gastrointestinal micro-biota^[4].

Cattle. Very few researchers have investigated the use of herbs in the treatment and prevention of diseases in



cows. Studies have focused on specific bacterial species like Staphylococcus aureus that causes mastitis in dairy

cattle^[5]. An example is *Morinda citrifolia* juice, that when fed to cattle, has been demonstrated to be effective in reducing the total bacterial count in milk.

Rabbits. Multiherbal products have been shown to increase growth performance in rabbit, while other plant products (e.g. **cumin seed**) increased

immune response against Pasteurella multocida and Staphylococcus aureus; and spirulina and thyme reduced the count of

Clostridium coccoides and Clostridium leptum in cecal content.

Aquaculture. Phytochemicals have been shown to act in aquaculture in



immunomodulation and as antioxidants, as well as being antiparasitic, anaesthetic and reducing tress. Supplementations with essential oils have shown potential in promoting the health of the gastrointestinal tract in fish. For example, thymol and carvacrol

appeared to positively affect the gut microbiome.

ANTIMICROBIAL CAPACITY OF FOOD INDUSTRY BY-PRODUCTS

Several studies have confirmed the antimicrobial activity of some bioactive compounds contained in plant by-products. Phenolics and flavonoids of pomegranate fruit skins have shown antimicrobial properties against L. monocytogenes,

S. aureus, E. coli, Yersinia enterocolitica,
Pseudomonas
fluorescens. Extract of
grape pomace at 10%

the growth of *Enterobacteriaceae*, *S. aureus*, *Salmonella*, yeasts and moulds in beef patties during 48 hours of storage

has been shown to inhibit

at 4 °C. Grape seed and Mate Yerba extracts promoted the growth of aastrointestinal beneficial bacteria in poultry. The antimicrobial properties of olive pomace and olive juice powder have also been confirmed.

Conclusions

alternatives Most studies on antimicrobials in livestock have been focused on bioactive compounds from plants, such as essential oils. Several positive effects on animal health and productivity of these secondary metabolites shown have been different species of food-producing animals and could contribute to reduce the use of antimicrobials at farm level. important Nevertheless. it is limitations in overcome some

experimental studies such as diet and essential oil composition, which can influence experiments outcomes. There is still a shortage of knowledge about essential oil metabolism, and scientific evidence showing the potential link between feeding essential oil and animal health is not solid yet. The mechanisms by which essential oils affect gut microflora and the gutassociated immune system are not yet fully understood. Research on this topic should contribute to the strategy to fight antimicrobial-resistance, but also to find new solutions for a more effective organic farming.

TAKE HOME MESSAGES

- An enormous variety of plant products with antimicrobial properties is available.
- Specie-specific studies have been conducted to assess their effects on the main diseases of farm animals.
- In vitro studies have been conducted on a wide range of bacteria.
- More information is needed on the impact on animal performance and their products.

For further information, please contact those responsible for the Livestock work of Organic-PLUS, massimo.demarchi@unipd.it

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