Pathways to phase-out contentious inputs from organic agriculture in Europe

Deliverable 4.8, 4.9, 4.10, 4.12, 4.13

Version 1.0

A sampler of 5 Mini paper deliverables

D4.8 Mini papers for stakeholders - enhance animal's immune system

D4.9 Mini papers for stakeholders - dairy cows

D4.10 Mini papers for stakeholders - beef cattle

D4.12 Mini papers for stakeholders - pig production

D4.13 Mini papers for stakeholders - poultry, meat and milk production alternative molecules and bedding

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1. Summary

Eleven mini-papers for stakeholder uptake were prepared based on the literature review and trials conducted within Organic-PLUS:

- 2 mini-papers related to enhance animal's immune system
- 3 mini-papers related to dairy cows
- 2 mini-papers related to beef cattle
- 2 mini-papers related to pig production
- 2 mini-papers related to poultry, meat and milk production, alternative molecules and bedding

In addition, 2 more mini-papers have been produced linked to the mini-papers before:

- 1 short-report in French about the results of the survey in France
- 1 dissemination article in Spanish about the results of the survey in Spain

2. Introduction

To summarise the relevant information regarding natural sources of alternatives to synthetic vitamins, antiparasitics and antimicrobials, several mini-papers were prepared for each topic to help in the dissemination of the results among stakeholders and farmers.

All eleven mini-papers are included in the present document. Each mini-paper stands alone to help in disseminating them easily.

The two extra mini-papers are already freely accessible at:

1. Résultats préliminaires de l'enquête Organic-PLUS sur les élevages biologiques français (juin 2019): https://organicplusnet.files.wordpress.com/2019/12/9-o-survey-on-organic-livestock-in-france-long-version-frensh-rc3a9sultats-prc3a9liminaires-de-lenquc3aate-organic-plus.pdf

2. Magazine 'rumiNEWS' (ruminates.com) target Spanish and Latin America vets, stakeholders or consumers. Article published online 10th February 2022 https://rumiantes.com/el-sector-ganadero-ecologico-espanol-percepciones-y-problematica/ or https://organicplusnet.files.wordpress.com/2023/07/o-el-sector-ganadero-ecologico-espanol.pdf

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ORGANIC RESEARCH CENTRE

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Enhancing the immune system of animals - introducing immunology and species variations

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INTRODUCTION

The field of immunology is expanding exponentially. A myriad of laboratory studies are unravelling the intricacies of communication, function, interaction and collaboration between the cells we recognise as having an immune function.

All organisms are subject to attack by other organisms and the immune system has evolved to provide defence and protection. It is a complex, and not truly understood, series of biological processes that are dynamic, interrelated and regulated by multiple modulators. All species, including bacteria and plants have immune systems, but each species has certain commonalities and differences.

An understanding of immunology is vital to interpret the likely effects of the addition of substances, that have an effect upon the immune system, to an animal's diet. The immune system is highly connected and regulated and alterations in one area will always have ramifications in another.

The immune system

The immune system is organised into a series of multi layered defences. It is broadly divided into the **innate** (nonspecific, immediate, recognises intruders) and **adaptive** systems (take longer to develop but are specific for the pathogen and have memory).

The first layer of defence is a **physical barrier** - skin, tears, urine, stomach acid and the mucous membranes lining the respiratory, gut and urogenital systems. These membranes contain substances such as host defence peptides and lysozymes which can neutralise microorganisms.

There are two families of **host defence peptides** in vertebrates; defensins (αβ, θ) and cathelicidins. They are released at the mucosal surface and essentially form a chemical barrier limiting infection. They have anti microbial and immune modulating activities and provide a link between the innate and adaptive immune systems.

If a pathogen gets past the physical barriers then the next layer of defence approaches. Non antigen specific phagocytes (cells that ingest pathogens)- neutrophils, macrophages - and dendritic cells, natural killer cells and eosinophils, basophils and mast cells migrate to the site of entry. The phagocytic cells recognise patterns that are highly conserved in many organisms, termed **pathogen associated molecular patterns** (PAMPs), but are not found in mammalian cells. All cells carry molecules on their surfaces which are used for interaction and communication. Part of the phagocyte array includes **pattern recognition receptors** (PRRs) on their surfaces which recognise the pathogen PAMPs. Binding between the PAMP and PRR initiates the killing mechanisms in neutrophils and macrophages.

Neutrophils are the first white cells to respond to signals from stressed cells or resident macrophages, they phagocytose pathogens and release anti-microbial factors. Neutrophils may also form extracellular traps, complexes of proteins that ensnare extracellular microorganisms, limit their spread and expose them to antimicrobial compounds. They have an important role in activating and programming other cells in the immune system through the release of cytokines and also engage with non-immune system cells.

Macrophages migrate to and circulate within almost every tissue. They specialise in the detection and phagocytosis of bacteria and other pathogens, they can initiate inflammation or dampen it, facilitate anti parasitic responses and present antigen to T cells in the adaptive response.

Natural killer cells are found mainly in the peripheral circulation, they attach to cells that have been invaded by a pathogen (they also recognise and kill tumour cells). On binding to the invaded cell, they release a burst of chemicals; perforin punches holes in the cell membrane, and granzymes then enter inducing apoptosis (or cell suicide). Natural killer cells are also involved in initiating the 'acute phase response' and then the adaptive immune response.

Mast cells are distributed through the body, particularly associating with surfaces that interface with the external environment. Mast cells recognise PAMPs and anaphylatoxins and on activation release granules containing inflammatory mediators producing allergic reactions, defending against parasites and recruiting other cells of the innate response. In contrast to resident mast cells **basophils** circulate but have many of the same actions and are particularly implicated in responses to ticks and filarial worms.

The granules in **eosinophils** contain cytotoxic proteins which play a beneficial role in defence against parasitic nematodes. Eosinophils are pro inflammatory but also modulate adaptive immune responses producing immunoregulatory cytokines and presenting antigen to T cells.

Dendritic cells the "sentinels of the immune system" are specialists in capturing, processing and presenting antigen to the cells of the adaptive immune system

Cytokines are a large family of 'communication proteins' which form the core of communication in the immune system. They attract cells of the immune system to where they are needed and depending on their structure/role they can initiate further immune response, encourage inflammation or be antiinflammatory and induce responses in non-immune system cells. Cytokines therefore provide a direct link between the innate immune system and the adaptive immune system.

Interferons (IFN) are members of the cytokine family. They are signalling proteins, classified according to their molecular structure and further differentiated by their amino acid sequences. Type 1 IFNs are produced by many cells in the body and are mostly involved with viruses. There is only one Type 11 IFN; IFNγwhich is only produced by T cells or NK cells and has a role in regulating overall immune function.

The activation of the innate immune system induces the '**acute phase response'** characterised by inflammation, an increase in temperature (fever) and circulating white blood cells, release of hormones, synthesis of acute phase proteins in the liver and behavioural changes. These changes include lethargy, lowered food and water intake, changes in social behaviours and a heightened reaction to pain. An increased temperature in the body helps to kill bacteria and microorganisms, helps prevent the formation of bacterial biofilms and accelerates the proliferation of many immune cell types.

Acute phase proteins may be produced in response to inflammation arising from infection, injury or stress. They mediate immune function and are involved in tissue repair.

If the innate system is functioning well most pathogens will have been detected and neutralised before the adaptive response is fully activated.

The activity of the innate immune system provides time for the more targeted antigen specific response from the **adaptive** arm to develop. The adaptive response focuses on specific antigens and with its capacity for generating memory cells ensures that once an antigen has been responded to, when it appears again, the response to it will be rapid.

Adaptive immunity is mediated by lymphocytes, the **T cells** (so named as they finish their development in the thymus) and **B cells** (first discovered in the Bursa fabricus of birds), in an elegant sequence of events. T cells orchestrate '**cell mediated immunity**' and B cells '**humoral' immunity**'.

Cluster of differentiation molecules (**CD**) are expressed on the surface of many cells. The molecules have different structures and each type has a different function. They have been assigned numbers as their structure and function has been elucidated e.g.CD 56 is a NK cell marker, CD 95 is involved in apoptosis, CD3 is associated with T cells and CD21 required for B cell activation.

Cell mediated immunity is coordinated and actioned by T cells which are classified according to their role.

T helper (Th) cells identified by CD4 produce cytokines that encourage other T and B cells grow and divide. **Cytotoxic T cells** (Tc) bear the CD8 marker and specialise in destroying virus infected cells

T cells don't respond to antigen directly, it must be presented to them by an **antigen presenting cell** (APC) this may be any cell but macrophages, dendritic cells and B cells are particularly adapted for this role. Once an APC has internalised antigen it then binds it to a **major histocompatibility complex** (MHC) molecule which is transported to the cell membrane and displayed. There are three types of MHC molecule, MHC1 and MHC 11, which present antigen to T cells and MHC111 which does not. The T cell binds to MHC 1 or MHC 11 via its receptors and is activated; the more T cells bind MHC the greater the response.

All T cells bear **T cell receptors** (TCR) on their cell surfaces which are complex chains of polypeptides classified as αβ or γδ chains, depending upon their structure. Within each chain there is a 'variable' region thus there are a large number of different forms of αβ or γδ chains available to bind to the different antigens presented by the MHC molecule.

As the activated T cells divide, the majority become effector cells active in the immune response but some, after antigen clearance, become **memory T cells** (T_{MEM})which can persist for years, either in the tissue where they were first activated or in the circulation.

A further important, recently discovered T cell, designated the '**virtual memory T cell'**(T_{VM}) has the characteristics of memory cells but has never seen antigen, but in response to both antigen specific and innate stimuli it can function as an effector cell. It is speculated that these cells are important for neonates which as yet have few pathogen specific T cells. The T_{VM} can generate a response, producing cytokines and cytotoxicity whilst an effector T cell population develops.

On binding MHC1 a T cell is activated to become a cytotoxic T cell (Tc). A series of signals lead to the destruction of the infected cell, the Tc releasing granules that perforate the cell wall and interact with the cell constituents leading to apoptosis. When a T cell is activated by binding MHC11 it becomes a T helper cell. As always in immunology, complexity reigns and Th are further divided into several classes with different functions depending on the panel of cytokines they secrete and the immune response/activity.

Th1 are stimulated by virus and intracellular bacteria antigens, they secrete IFNγ which activates macrophages, NK cells and encourages proliferation of Tc cells, they promote inflammation and dampen the Th2 response.

Th2 secrete cytokines that encourage B cell proliferation and differentiation and thus the production of antibody. They stimulate mast cells, eosinophils and basophils and hence are involved in parasite elimination. They are anti-inflammatory and dampen the Th1 response.

Th17 promote inflammation via inflammatory cytokines. They recruit neutrophils to kill extracellular bacteria and fungi.

T reg cells characterised by CD4 and CD25 secrete regulatory cytokines are anti-inflammatory and promote immune tolerance.

T follicular helper cells (T_{FH}) are found in follicles in the spleen and tonsils and stimulate B cell production of high affinity antibodies.

Humoral immunity describes the activity of B cells which generate specific antibodies targeting pathogens and toxins.
B cells can recognise antigen directly, their TLRs recognising PAMPs but more usually they are activated by Th2 cells and cytokines. An activated B cell differentiates into a plasma cell which then secretes various classes of **immunoglobulins** (antibodies), the exact classes being determined according to cytokine signals.

Immunoglobulins, although all glycoproteins, have differing structure and chemistry. Each has two small (light) chains and two larger (heavy) chains. The heavy chain has one variable region, the light chains 3-4 depending upon the antibody class. An antibody is Y shaped, the upper arms are responsible for antigen recognition, binding and neutralisation (preventing a pathogen from progressing), the stalk of the Y interacts with other components of the immune system.

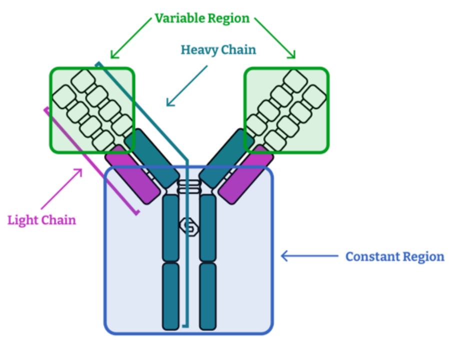

Figure 1. Structure of an antibody

The five classes of immunoglobulins in mammals are IgG, IgM, IgA, IgD and IgE. During the course of a response, a series of antibodies may be produced. **IgM** a large pentameric molecule usually found in the vascular system, appears early in a response and provides protection before the other classes are synthesised. Levels then drop as other antibodies take over. Depending upon the pathogen and thus signalling from T cells and cytokines a 'class switch' occurs and different immunoglobins are produced.
IgG, the most abundant antibody is smaller and can diffuse between vascular and interstitial tissues, and contributes to immunity against bacteria, viruses, parasites and some fungi. There are a number of sub classes of IgG each with a particular function.
IgA has two forms, IgA1 is found largely in the serum and IgA2 in mucosal secretions (secretory IgA) where it is the first line of humoral defence against pathogens entering through the mucosa.
IgE is regarded as the most important humoral defence against nematode parasites and is directly involved in allergic reactions binding to high affinity receptors on mast cells and basophils.
IgD does not circulate but is bound to B cells initiating the immune response and mobilising IgM and may have a role in class switching.
A plasma cell can secrete thousands of antibodies in a very short time.

B cells also differentiate into long lived memory cells which migrate back to the bone marrow until familiar antigen is encountered again.

Antigen specific antibody production requires a period of time, but certain B cells on recognising PAMPs can differentiate into short lived plasma cells that produce low affinity antibodies, providing a quicker line of defence until more antigen specific antibodies are generated.
As our understanding of the immune system grows, we realise that the arms function together to create and balance responses. That the innate, cell mediated and humoral immunity work in concert is critical to the successful functioning of the immune system.

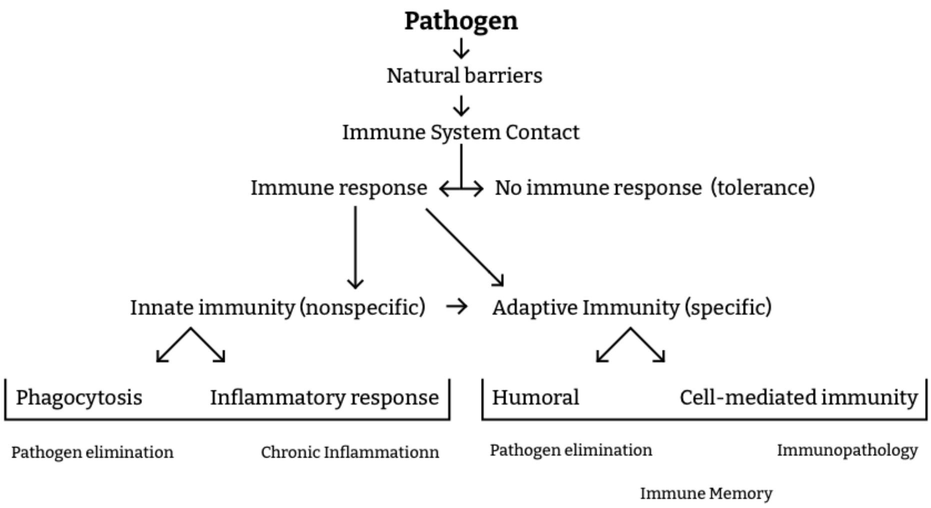

Figure 2. A summary of the main actions of the immune system

The immune response has an energetic cost. In general, innate immunity carries a low metabolic cost to activate, but a high metabolic cost to use. Whereas adaptive immunity has a high metabolic cost to activate, but a low cost to use. Thus, different species place a different emphasis on different parts of the immune system. Shorter lived species balance their immune systems more towards the innate response e.g. chickens, species with a longer life span e.g. ruminants have a greater tendency to invest in adaptive immunity.

Differences in the immune system between species

Our understanding and knowledge of the differences in immune system reflects the amount of research available. There are probably greater differences in structure and function than are currently recognised. Birds and mammals evolved from common reptilian ancestor over 200 million years ago, they have some common immunological systems and some key differences. Immune systems and responses have evolved as each species has changed and adapted to different environments.

Immense amounts of money have been invested in mouse models but there are some critical differences between mice, humans, livestock and poultry. Indeed, recent studies have even suggested that immune responses between laboratory bred mice and wild mice differ. Our understanding of the differences between species is far from complete and care should be taken when extrapolating results. Nor should we forget that the same pathogen invading different hosts may trigger different responses and outcomes. In addition to differences in immune responses/systems between species, there are also differences between breeds, strains, gender and age.

An obvious difference between species is in the transfer of immunity to the foetus and thus neonates. In humans, the majority of transfer is in utero. The porcine epitheliochorial placenta has no direct connection, the foetal tissue presses against the wall of the uterus and there is no transfer, the ruminant synepitheliochorial placenta has areas of erosion in the maternal tissue and some degree of communication but as in pigs the majority of the transfer of immunity occurs postnatally and primarily in the colostrum. In poultry maternal antibodies present in the yolk provide protection for the chick.

Host defence peptide families differ in pigs and cattle, neither have aor θ but pigs have 34 genes for βdefensins and cattle over 100. β1 defensin is not detectable until 4-5 weeks of age in young piglets. Bovine neutrophils contain unique β-defensins found in large granules in the cells. In sheep, two unique β-defensins have been characterised SBD1, found in pulmonary tissue and SBD2 found in the gut.
Pigs have ten genes coding for cathelicidins. Avian cathelicidins were first identified in 2005 and four genes have been described in chickens.

Pattern recognition receptors (PRRs) differ between species. In the pig a particular receptor – the toll like receptor (TLR, has been shown to differ between the domestic breeds and also between wild boar and domestic pigs. In poultry, the expression of TLR4 varies between breeds and TLR15 varies within different lines of chickens.
Interferons (IFN) are members of the cytokine family involved in signalling. There is a range of IFNs and their associated genes in different species. Table 1 illustrates the differences between cattle and pigs. Interestingly mice have 9 IFN-1ζ genes humans have none, humans have no IFN-1ωgenes, mice have one and neither humans nor mice have IFN-1δ genes.

Table 1 The number of genes coding for type 1 IFN varies. Cattle have 51, pigs 39.

IFN – 1 structure	Cattle	Pigs
IFN α	19	18
IFNβ	8	1
IFNδ	0	11
IFNε	0	1
IFNκ	1	1
IFNω	19	7
IFN τ	0	0
IFN ζ	0	0
IFN Total	**51**	**39**

The functions and levels of **acute phase proteins** differ between all species. For example, in pigs the acute phase protein pig-MAP increases when the animals are stressed by transport or dietary change.

Porcine **neutrophils** differ from other species in that they are smaller and less granular and have a higher activation threshold. It has been shown that there are differences in both morphology and function between different breeds of pigs e.g. the neutrophils from large whites are more efficient at phagocytosing *E. coli* than those from the Meishan.

The **heterophil** replaces neutrophils in chickens. The granules in the heterophil differ in that they contain no catalases or myeloperoxidases, but do contain similar lysosomal and non-lysosomal enzymes as well as unique gallinacins. They are extremely efficient at phagocytosing bacteria. Heterophils have a key role in signalling and thus activation of further immune responses. Research has shown that heterophils in different lines of chickens have altered activity and those lines in which activity is lower have a greater susceptibility to infections such as *Enterococcus gallinarum, Campylobacter jejuni* and *Eimeria tenella*. Similar differences between commercial and wild turkeys have also been observed.
The pathogenesis of lesions as a result of heterophil activity differs from neutrophils, the latter causing liquidity and abscesses, the former walled off granulomas.
As birds do not have resident pulmonary macrophages, the heterophil is the first line of defence in the avian respiratory tract.

Some **NK** cells have receptors named 'Killer immunoglobulin-like receptors' (KIR) which are essential for immune-surveillance. Cattle have a number of genes coding for KIR whereas pigs have only one. NK cells expressing a p46 molecule on their surfaces are particularly efficient at lysing virus. Pigs have a very high proportion of NKp46 cells compared to other species.

The **MHC** molecule is much less variable in chickens in comparison to mammals, suggesting that poultry rely on different/further antigen recognition strategies.

The **cytokines** in chickens differ from those in mammals, research suggests that certain cytokine families evolved separately as different sequences are found in poultry.

T cell repertoires in ruminants, pigs and chickens express high numbers of γδ **TCRs** (humans have high numbers of TCR αβ). T cells are classically characterised by the expression of CD8, but in the pig, significant numbers of T cells also express CD4, which appear to be porcine memory cells. The
δchain of the γδ TCR has a large potential for recombination producing a very diverse repertoire of TCRs. Studies have shown, that in pigs, this wide repertoire diminishes with age suggesting that the TCR γδ population is selected over time in the individual according to environmental exposures.

B cells develop in the Bursa fabricus in the chicken, whereas in mammals, they develop in the bone marrow.
The **immunoglobulin** IgG is only present in mammals; it is replaced by **IgY** in birds (and reptiles).
The diversity of the immunoglobulin heavy chain is more restricted in both pigs and cattle. The genes coding for the diversity belong to different families, IGHV3 in pigs, IGHV in cattle.

The 'inverted' **lymph nodes** of pigs differ from ruminant lymph nodes (and other mammals except rhinos, elephants and dolphins) in that lymphocytes exit them directly into the blood rather than into the lymphatic system. Chickens do not have lymph nodes but rather lymphoid tissue.

CONCLUSIONS

The immune system is a complex and dynamic organisation of cells and mediators. It is finely balanced and tuned, and changes in one area of the system can impact responses in other areas. The innate and adaptive immune responses differ between species so care must be taken when extrapolating results from studies, as due to the differences they may not directly apply.

References

References are available from the author.

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Enhancing animals' immune systems

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INTRODUCTION

The development of an infection depends upon the outcome of the interaction between the invading pathogen and the immune system of the defending animal. Rigorous selection in the dairy, pig and poultry industries has resulted in animals with an accelerated metabolism that utilises feed efficiently for growth and production. However, they have fewer resources for the support and maintenance (seen by some as a 'waste' of energy) that enables an animal to function well and cope with changing conditions and challenges. As the environment changes, so does energy partitioning, but often 'high performance' animals no longer have the ability to cope, and are less resilient.

Furthermore, in many poultry and pig systems that are housed in intensive 'biosecure' environments, immune responses have been negatively impacted by management and selection. Animals with poor responsiveness are more susceptible to disease, while animals with heightened responses (selected for response to vaccination) overreact to other challenges.

Rather than a sole focus on high risk, short term, high output relying on 'a pill for every ill', the objective should be a healthy animal with a long productive life. Managing animals with health and welfare as a goal alleviates many of the stressors that negatively impact immune performance, while supporting and **enhancing animals' immune function**. A depressed immune system increases the risk of disease, but an overactive immune system can lead to auto immune diseases, chronic inflammatory diseases, sepsis and severe allergic reactions.

Nutrition

Proper nutrition underpins health and performance. A broad diet of ingredients that the animal has evolved to eat, helps maintain a healthy gut and a healthy gut biota. Mounting an immune response is energetically expensive and requires particular nutrients for success. Under nutrition reduces the capacity of the immune system to respond to an insult, thus poorly fed animals have a lower resistance and a greater likelihood of disease.

"*though the cow cannot classify forage crops by variety name or by tonnage yield per acre she is more expert than any biochemist at assessing their nutritive value*" (Albrecht 1958 in Provenza 2008)

Specific nutrients known to affect immune function include **arginine, tryptophan, methionine, valine, threonine, vitamin A, b-carotene, folic acid, vitamin B6, vitamin B12, vitamin C, vitamin E, riboflavin, iron, sodium, zinc, chromium, copper, manganese** and **selenium**. These may support or stimulate the immune system.

The gut mucosa is lined with mucous which contains glycoproteins rich in threonine. Potential infections increase the demand for mucin and thus threonine. The demand can increase by more than 10% and in laying hens under challenge approaches 30%. Evidence suggests that additional amino acids in the diet can alleviate the impacts of disease on growth.

Zinc, copper, manganese, selenium and iron are all critical components of antioxidant enzymes and are required to ensure good function. An excess or deficiency can impact immune system activity. The killing ability of neutrophils is reduced in copper deficient animals, antibody production is reduced and cytokine production impaired. Copper deficient animals have been shown to be more susceptible to viral, bacterial and parasitic infections. Zinc is a co factor in over 300 enzyme systems as well as influencing inflammatory actions and protein synthesis. As an enzyme co-factor zinc also has a role in humoral and cell mediated immunity, so a deficiency is associated with lessened T cell function, lower antibody responses and may hinder the development of adaptive immunity. Selenium levels not only influence the host immune response, low levels decreasing cellular function and harming cell membranes, they may also trigger changes in the viral genome increasing virulence.

Chromium is an essential mineral for metabolism, but it also has an effect on immunity. Stressed calves supplemented with chromium had enhanced antibody responses, perhaps due to chromium countering stress induced immunosuppression.

Iron is an important mineral but an excess in pigs has been shown to stimulate microbial growth and increase the susceptibility of piglets to enteric diseases. Vitamin deficiencies have long been linked to immune disorders. Ruminants, pigs and poultry synthesise their own Vitamin C but studies suggest that supplementation with Vitamin C is useful at times of stress and particularly in poultry during heat stress. Vitamin C is an antioxidant, influences immune responses, has a role in maintaining homeostasis and in pigs has been shown to alter lymphocyte proliferation.

Vitamin A affects the function of nearly all immune cells. It is also critical for the maintenance of the mucosa in the gastro intestinal and respiratory tracts. Vitamin E exists in 8 forms, each with differing degrees of biological activity. It supports physiological and immune system functions. In ruminants, as an antioxidant, it is critical in reducing the severity and duration of immune challenge.

Dietary lipids, particularly omega-6 and omega-3 fatty acids, can increase oxidative stress if not properly balanced by antioxidants. Polyunsaturated fatty acids also affect the composition of immune cells, their immune function and the inflammatory response. Omega 3 fatty acids (found in fish oils, linseed) may have positive effects on respiratory infections and gastro intestinal challenges in livestock through their anti-inflammatory actions.

It is noteworthy that litters born to sows fed high levels of omega 6 fatty acids (soybean, corn or sunflower oils) had lower birth weights and increased mortality. Antioxidants are important for reducing oxidative stress in immune cells and for reducing immune-cell apoptosis. Free radicals or reactive oxygen species (ROS) form as a natural consequence of metabolic activity. They are also produced in immune defence as part of the strategy for destroying microorganisms. Phagocytosis and respiratory burst generate large amounts of ROS. In addition, the cell membrane of immune cells contains large amounts of polyunsaturated fatty acids, if ROS interact with the membrane they make it less fluid and the cell less responsive.

Antioxidant systems exist to clear up excess ROS e.g. vitamins A and E, carotenoids and ubiquinone, Vitamin C, uric acid and taurine and the antioxidant enzymes glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) and the thiol redox system. However, under stress the rate of ROS increases and if left unchecked damage will occur to cell membranes, proteins and nucleic acids.

Antioxidants such as Vitamin E, Vitamin C and Vitamin A (and βcarotene a precursor to Vitamin A with immune-stimulatory properties) may all be added to feed to support immune function in livestock. Studies in ruminants indicate that carotenoids improve mammary, reproductive and overall health and that dairy cows supplemented with βcarotene at drying off had fewer udder infections. It is critically important to recognise the role played by gut microbiota as they process nutrients and then release metabolites into the gut and to the mucosa. They have a direct impact on many physiological functions and on immunity.

"The most glaring issue facing using nutrition/ diet to modulate host immunity is that virtually all previous work has ignored the effect of the gut microbiome".
(Kogut 2017)

All species have a gut microbiota, a dynamic and complex environment, it is often termed the 'second genome' of the host. The host diet provides the nutrition for gut communities and shapes their composition. In return they contribute essential nutrients but also have (hitherto largely unrecognised) effects on the immune system, metabolism, energy balance, hormonal activity, body weight and possibly gene expression. The immune system must recognise the difference between friendly gut microorganisms and pathogens. Under the influence of the resident microbiota the mucosal immune system is tolerant of dietary antigens and the residents.

The mucous secreted by the epithelial lining of the gut contains defensins and secretory IgA which neutralises invasive pathogens. Macrophages phagocytose and kill any pathogens that penetrate the gut lining and dendritic cells and regulatory T cells control immune cells and maintain tolerance. Age, environment, diet, digestibility, feeding method, feed additives, pathogen pressures, stress and antibiotics all act on the microbiome community and the alterations have been implicated in several conditions including changing the ratio of beneficial and detrimental species leading to gut disorders and increasing the quantity of bacterial product in circulation leading to low grade inflammation. Chronic inflammation is frequently triggered by surpluses as the metabolites and nutrients are sensed by the same pathogen sensing systems involved in classical inflammation.

The modulation of gut health and immunity goes beyond simply giving probiotics. A study in pigs showed that under non intensive conditions gestation and the first day of life were hugely influential in establishing stable communities, bacterial colonies being acquired from the mother and surroundings. Continued exposure for the first week of life led to improvements in immune health, which was abrogated by antibiotics.

The diversity of species in the gut underpins a stable ecosystem enabling stock to resist infections. Animals treated with antibiotics are more susceptible to infections with intestinal pathogens than un-treated animals, as antibiotics suppress the resident gut flora, creating the opportunity for arriving pathogens to thrive. The provision of a broad wholesome diet will do much to support the gut colonies, immunity and health. However, the constant search for low cost feed stuffs and cheap diets often leads to poorer immune function and to feed induced inflammation and the search for additives.

Stress

Stress "elicits coordinated physiologic responses in the body in an attempt to re-establish homeostasis" (Carroll and Forsberg 2007). Animals are impacted physically (e.g. overcrowding, handling, transportation, hunger, thirst fatigue, disease), physiologically (e g nutrients deficiencies, glandular disorders, endocrine disruptors) and psychologically (e.g. fear, loud noises, restraint) all of which increase levels of stress with a negative effect immunologically.

Psychosocial stress is an important risk factor, it arises through a limited opportunity for natural behaviours, exploration and socialisation issues. Enriched conditions have been shown to improve immune responsiveness (changes in antibody levels and white blood cell populations) and to lower disease susceptibility in pigs.

Thermal stress occurs when animals are exposed to hotter or colder conditions than is comfortable for them. When outside their thermal neutral zone, their immune responses are negatively impacted and they become more susceptible to disease. In pigs temperature stress increases the production of pro inflammatory cytokines and influences the levels of eosinophils, neutrophils and monocytes. Heat stressed poultry exhibit a decrease in immature B cells in bursal follicles and depressed antigen specific responses. It has been shown that in pregnant dairy cattle heat stress impacts the migration and differentiation of cells destined to become immune progenitor cells in the calf. In addition, under temperature stress, the colostral transfer of IgG is impaired as absorption is lower in the calf.

Figure 1. Stage4 heat stressed cattle (Photo adapted from ars.usda.gov)

Weaning is very stressful particularly if the diet change is accompanied by transportation and social mixing. The immune status of young stock is low as they transfer from passive immunity (maternal milk intake) to their own individual protective immunity. Studies have shown an overall depression of the innate immune system at this time and changes in the levels of immune markers including neutrophils, lymphocytes and cytokines.

The immunity of a 14 - 21day old piglet is determined by the levels of antibodies absorbed from the sow, and how fast they are broken down. Half of them are broken down within 8-16 days and all will be gone between 30 - 60 days, but passive immunity varies between individuals, as does the class of antibodies. Piglets should therefore be weaned when they can mount their own immune responses so they are better able to cope with the effects of stress. Studies have shown that weaning before 21 days has long term negative effects on the mucosal development including affecting the production of cytokines. The severity of impact on the mucosa depends upon how it much it has developed prior to weaning. Weaning before 5 weeks was shown to decrease the ability of lymphocytes to undergo clonal expansion initiating the adaptive response. Unfortunately, the point at which the piglet immune system is ready to cope with weaning and when many production systems wean, does not often coincide.

Interestingly piglets fed formula rather than from the sow had smaller lymphoid follicles and germinal centres within the gut lymphoid tissue suggesting less immune stimulation had occurred. A study of the effects of weaning on Holstein calves described a short increase in levels of circulating neutrophils but a suppression of phagocytosis and killing ability. All the calves in the study had raised plasma concentrations of cortisol and haptoglobin (an acute phase protein) indicative of stress.

Dehydration is stressful and can impact immunity directly as mucosal secretions decrease and dry out, making the mucosal linings of the gut, reproductive and respiratory less able to neutralise pathogens. Chronic stress can cause disease and also shift the immune system into suppression. In poultry, stressful events are often reflected in higher levels of morbidity and mortality in the flock.

Immunomodulation

A poorly functioning or suppressed immune system increases the risk of disease. Immunomodulators, by influencing the hosts' immune system, induce, enhance (immunostimulants) or suppress (immunosuppressants) immune responses.

The literature is rich of suggested immunomodulants including minerals, vitamins, plants and plant constituents and other biological compounds such as amino or nucleic acids. All of which are purported to support the natural mechanisms in the host, enhancing the immune response or suppressing over reactive responses.

Immunostimulants enhance the innate immune function and improve host resistance by modulating cytokine responses, specific antigen based responses and reducing inflammatory damage. Immunostimulants can also initiate activation of innate defence by acting on receptors and triggering intracellular genes resulting in the production of antimicrobial molecules.

Immunosuppressants inhibit immune system activity, quietening the immune response. As discussed above stress down regulates both the innate and adaptive arms of the immune system, acting as an immunosuppressant and increasing susceptibility to pathogens. However, immunosuppressants have a valuable role to play in down regulating immune responses, particularly in auto immune diseases and excess inflammation. Turmeric (*Curcuma longa*) has a strong anti-inflammatory action and can alter the expression of transcription factors, cytokine production and antigen presentation down regulating immune response. Liquorice (*Glycyrrhiza glabra*) has anti-inflammatory effects, limits T cell proliferation and has been used in traditional medicine to calm over active responses. Parsley (*Petroselinum crispum*) has a history of use as an immune modulator, supressing chronic inflammation.

Figure 2. Parsley, garlic and capsicum; traditional immunomodulators (from Grieve a Modern herbal)

The successful activity of herbs has largely been assumed to be caused by the activity of bioactive compounds on the pathogen, but more evidence of the role of plant compounds in modulating the immune response is accumulating. For example, phytochemicals have been reported to help regulate inflammation, influence white blood cell numbers and cytokine production. Phytochemicals such as capsaicin and eugenol bind mammalian transient receptor potential channels expressed on cell surfaces and thus modulate inflammation, antibody production and lymphocyte activity by stimulating or inhibiting cytokines. Capsaicin can also act indirectly by binding receptors on neurons and thus regulating the release of neuropeptides that then promote either inflammatory or anti-inflammatory actions. Garlic (*Allium sativum*) has been shown to increase the numbers of CD4 T helper cells when fed as a supplement to pigs and to dairy cattle. Chickens fed turmeric (*Curcuma longa*) and *Capsicum annum* showed increased lymphocyte proliferation when vaccinated and then challenged with *Eimeria tenella*. Capsaicin is also an effective antioxidant as one molecule of capsaicin scavenges two peroxyl radicals. Flavonoids have been shown to affect the function of enzymes involved in generating the inflammatory response, they have antioxidant properties and can inhibit T cell proliferation. Noni (*Morinda citrifolia*) juice fed to neonatal Holsteins potentiated the immune system by increasing expression of CD25 on CD4+, CD8+ and γδT cells. Noni also increased the secretion of IL-1a, TNFβ and IFNγin bovine colostrum and enhanced innate cell mediated immunity.

Some alkaloids may disrupt bacterial enzyme systems, saponins may interact with the sterols in bacterial membranes and tannins interact with proteins. High doses of tannins are cytotoxic and antinutritional but lower doses have antioxidant, antimicrobial and anti-inflammatory effects. Supplements of tannic acid at doses of between 500-2750mg/kg reduced oocyst shedding in chickens infected with *Eimeria maxima*. Inclusion of forages containing condensed tannins has been shown to reduce faecal egg counts in sheep and impact hatch rates and larval development thus reducing pasture contamination.

The presence and activity of plant secondary chemicals is affected by plant growth stage, environment, harvesting conditions and processing, the sum total of which affect the action of the plant on the host. An animal's health, welfare, diet, environment and disease challenges will further affect the efficacy of a given herb.

Studies in pigs have shown that the inclusion of **thyme, oregano, cinnamon** and **tea** have had positive effects on the gut microbiome and thus performance. When **garlic** was included as part of the diet pigs gained weight. In ruminants, plant compounds can affect rumen fermentation, altering the products and proportions of fermentation, thus care should be taken when supplementing stock that there are no negative impacts on the rumen microbiome function. Immunostimulants should never be given to animals until their immune systems have matured, giving them to young stock may adversely affect development.

The literature surrounding potential 'plant medicines' is overwhelming and frequently the veracity of it is underwhelming. Many experiments with herbs/phytonutrients have been carried out in cultures, isolating cells or designated active components of the plant, often using high dose rates to achieve an effect. Results gained in the laboratory petri dish may not translate to the animal in field conditions. These results should be regarded as indicators of actions but not accepted as functional. The activity of the whole plant is often greater than the sum of its parts and is always influenced by the physiology of the host.

Allowing animals to choose

Plants contain a broad array of phytochemicals which provide protection, and help them recover from injury. High intakes of these secondary chemicals by animals can reduce intake, affect metabolism and in certain cases prove fatal. At lower levels, these same compounds can adversely affect pathogens and have positive effects upon the metabolism and immune system, helping promote animal health. Animals seek to remain in balance, internally and externally. It has been suggested that animals have an inherent 'nutritional wisdom' to choose the correct plants, but observations of animals suggest that this is not infallible and is likely augmented by learning.

Flavour and the post ingestive feedback (was that good or bad?) is influenced by the animal's health and physiological status at time of ingestion. If a feed contains chemicals that an animal requires at the time, it will favour it. As circumstances change, the food may not be approached again until it is required once more to reset the balance. Sheep offered high tannin diets have been shown to select substances that attenuate the effects of tannins and when forced to forage plants with high levels of tannins will selectively graze areas where ameliorating substances are available. Sheep will, however, choose to increase tannin consumption (with no amelioration) when parasitised, so as to combat the infection.

By providing a mixed, varied diet, livestock will ingest a **range of secondary compounds as a consequence of the days foraging**. This low level intake will provide nutritional and metabolic benefits and it is likely that the phytochemicals will both support and modulate the immune system. Animals will self-medicate in such diverse situations by choosing to increase their intakes of particular plants as and when they are needed. It is now accepted that livestock are indeed aware of their health status, and may in fact address sub clinical issues before the symptoms are visible to the farmer. The choice of species to ingest may range from prophylactic to therapeutic, actively supporting the immune system and metabolism to regain health.

Figure 3. A stress free dairy cow choosing her diet from mixed pastures

CONCLUSIONS

Livestock provided with broad diets and the ability to choose their intake from day to day as their physiology directs will have the ability to enhance their immune

systems, dealing with pathogens when they are encountered. **Diverse diets** naturally contain compounds that have immunomodulatory effects. Animals can be supplemented with immune enhancing (or quietening) compounds but care should be taken with appropriate dosages and animal age.
Animals managed with true regard to the five domains of welfare and with respect for their life stage, have an **inherent capacity to resist infection and disease**, and to reach their potential.

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Use of herbal products' oral administration to control mammary gland infections in cows

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Mammary gland infections are a real issue in modern dairy farming since they affect both milk yield and milk quality with important economic and managerial implications (Halasa et al., 2007; Liang et al., 2017). Although the use of antimicrobial and other synthetic molecules continues to be a relevant part of mastitis control in dairy farms, other alternatives of "natural origin" have been proposed (Francoz et al., 2017). Among these, herbal products have been tested for mastitis in few studies, both *in vivo* and *in vitro*, with some interesting effects on biofilm formation from the bacteria (Mushtaq et al., 2018). Therefore, the use of herbal products could be part of a strategy aiming to reduce antibiotic usage.

Table 1. Relationship between somatic cells count (**SCC**) and occurrence of infection in the mammary gland is not linear, because a cow may have a high SCC without any bacterial infection or clinical sign. There are Mastitis characterised by only inflammation and not infection.

Definition	SCC	Bacteriology	Clinical
Healthy quarter	<100.000/ml	Negative	No
Sudden infection	< 100.000/ml	Positive	No
Inflammation Subclinical mastitis	100.000/ml- 200.000/ml >200.000/ml	Negative Positive o Negative	No No
Clinical mastitis	>200.000/ml	Positive o Negative	Yes
Chronical mastitis	>200.000/ml	Positive > cases	No, rarely

Moreover, current legal constrains were created to guarantee health products to the consumers (Hillerton and Berry, 2004) so the lowering of the somatic cell count (**SCC** - one of the main markers of mammary gland infections) has not only a clinical perspective (**Table 1**). In fact, the rise of **SCC** can also be exploited as a tool to diagnose the occurrence of initial infection and to monitor the effectiveness of the therapies adopted.

Considering the encouraging results obtained in other studies, an observational study was performed to evaluate the impact of an herbal product composed of many natural complexes on subclinical and clinical mastitis.

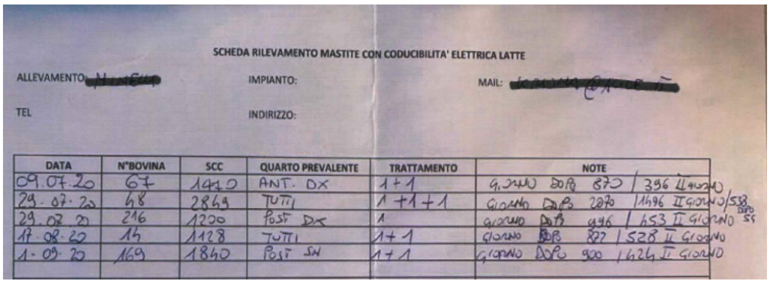
METHODOLOGY

Farms, product tested and experimental design

In order to test the effectiveness of this herbal product (**PFA**, Biotrade, Mirandola, Italy), some farmers were asked to register the data regarding the cows treated with the **PFA** and the variations in **SCC**. The form used for the data is shown the **Figure 1**. The PFA was evaluated in terms of effect on milk production, **SCC** and clinical signs. Among these, we also took into account ruminal activity, which can be affected by severe mastitis. The **PFA** was a phytoextract composed of many herbs with antioxidant (Carneiro de Siqueira Leite et al., 2018), ant-inflammatory and antibacterial properties (Wen et al., 2012; Silva Lopes et al., 2020).

Product administration, results evaluation and collection

The **PFA** was administered alone (single or double dose; second dose after maximum 24 hours from the first one) or in combination with other synthetic molecules in case of serious clinical signs, such as in mastitis caused by *E. coli*.


SCHEDA RILEVAMENTO MASTITE CON CODUCIBILITA' ELETTRICA LATTE

ALLEVAMENTO: [redacted] IMPIANTO: MAIL: [redacted]

TEL INDIRIZZO:

DATA	N°BOVINA	SCC	QUARTO PREVALENTE	TRATTAMENTO	NOTE
09.07.20	67	1410	ANT. DX	1+1	GIORNO DOPO 870 / 396 II GIORNO
29.07.20	48	2849	TUTTI	1+1+1	GIORNO DOPO 2070 / 1496 II GIORNO / 538 DOPO 5G
29.07.20	216	1200	POST DX	1	GIORNO DOPO 496 / 653 II GIORNO
17.08.20	14	1128	TUTTI	1+1	GIORNO DOPO 827 / 528 II GIORNO
1.09.20	169	1840	POST SN	1+1	GIORNO DOPO 900 / 624 II GIORNO

Figure 1. Data collection form, completed by the farmers

In farms enrolled, **PFA** was orally administered when clinical signs of inflammation were present at milking time and when an alarm was sent from the software linked to the milking robot. In fact, for data collection only farms with an automatic milking system (**AMS**, robot) were enrolled, because in these farms there is detailed information available for each cow on milk production, rumen activity time, locomotion time and some milk quality traits. Among these, the electrical conductivity of cow's milk is of great interest in diagnosing mastitis earlier, because it increases before clinical signs are manifested.

An example of an output from the robot referred to a single cow in which the electrical conductivity is for each single quarter is reported in **Figure 2**.

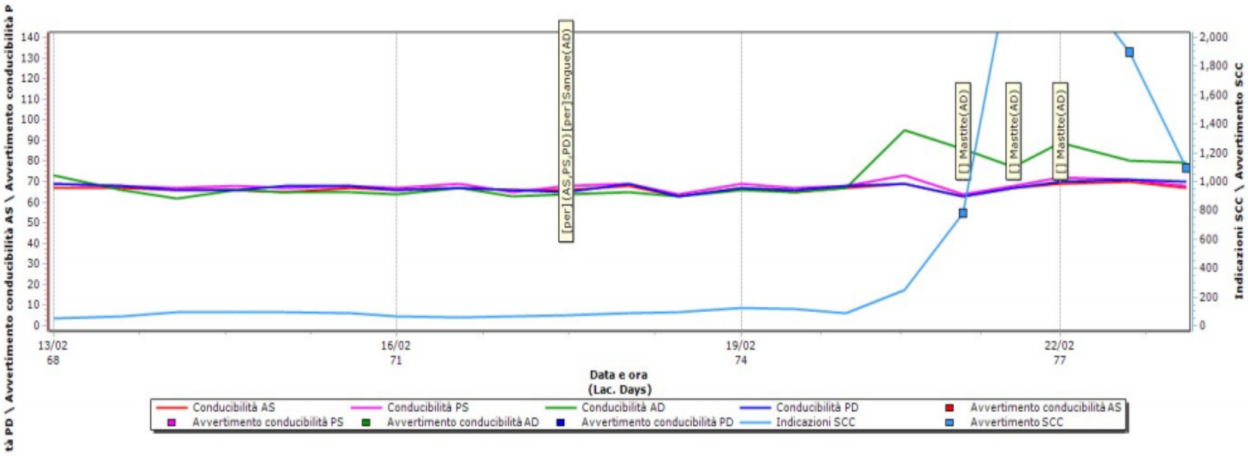


Figure 2. A peak of the electrical conductivity occurs earlier than the rise of the total somatic cell count. For the farmer, this is a precious information in order to decide which strategy is the best to prevent the clinical mastitis or at least to avoid that the scenario becomes worse.

MAIN RESULTS AND DISCUSSION

In an Italian dairy farm (of more than 200 lactating cows) located nearby Mantua (45.0190, 10.9059), the **PFA** was widely tested between February and August 2021, both alone and in combination with synthetic molecules in different cases of mastitis. After being administered orally, the **PFA** showed the capacity to reduce the **SCC** under the level of 400.000 cell/ml (legal limit in Italy) in a variable percentage of cows. Specifically, when **PFA** was administered alone, in case of **SCC** rise, 90% of cows showed a lower **SCC** after 10 days (**Table 2**). When **PFA** was associated, in a single or a double dose, with synthetic molecules, a positive effect on rumen activity time was observed in the majority of cows. Overall, these results indicate that the tested product, appropriately dosed, can be a valuable substitute for antibiotics, both in conventional and organic farms. Moreover, can help farmers in managing serious mastitis because the product has showed to positively influence rumen activity.

Table 2. Results of using the herbal product tested (**PFA**) in 130 cases of cows with high somatic cells count (**SCC**) and mastitis.

	Total number of treatments	SINGLE PFA	PFA + synthetic molecule	Positive outcomes/total number of treatments
Robot alarm or SCC rising from milk analysis	50	30/50	20/50	**44/50**
	CURE RATE AND OR drop of SCC	90% (27/30)	85% (17/20)	**88%**
		PFA SINGLE + INTRAMAMMARY TREATMENT	DOUBLE PFA+ INTRAMMARY TREATMENT	
	60	50/60	10/60	**45**

Fever, milk abnormalities, inflammation	CURE RATE AND OR drop of SCC	82% (41) ↑ ruminal activity time by 12 hours in 48 cows over 50	40% (4) ↑ ruminal activity time by 12 hours in 8 cows over 10	**75%**
		Associated with fluido therapy	COWS RECOVERED TOTALLY AT MAMMARY LEVEL	
Serious mastitis from Coliforms	20	20	5	**25%**

PRACTICAL RESULTS FOR FARMERS

According to our results, phytoextracts could represent a viable solution in mastitis management for many reasons: no residues in the milk, their contribution to the fight against antimicrobial resistance and less use of antimicrobials which have a key role in human medicine. It is crucial that the knowledge about the mode of action of the single component of the phytoextract and the quality of raw materials be available on the market. In farms where data of single cows are available, phytoextract can be administered in advance, thus having the advantage of a prevention strategy.

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Looking into ethnoveterinary medicine to improve the animals' immune system

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Transition period is a critical phase for dairy cows that typically ranges from 3 weeks pre-partum to 3 weeks postpartum. During this period, cows usually experience a negative energy balance (**NEB**). A negative energy balance is when a trade-off lacks between input (energy and nutrients intake) and output (foetus growth, mammary tissue remodelling, and milk secretion). To overcome the **NEB**, cows mobilise body-lipids reserves, increasing several metabolites in blood and milk. Two of them are the non-esterified fatty acids (**NEFA**) and β-hydroxybutyrate (**BHB**), which is known as one of the ketone bodies produced. In addition, we can also see an important decrease in calcium plasmatic level due to calcium demands of lactation. The most frequent health problems related to the transition period are milk fever, ketosis, displaced abomasum, retained placenta, metritis, mastitis, and lameness. Therefore, it is interesting to find alternatives to improve peripartum immune system to face this delicate physiological period.

POTENTIAL OF ETHNOVETERINARY MEDICINE

There is an increasing interest in applying **ethnoveterinary medicine** to improve the animals' immune systems. **Ethnoveterinary medicine** is the scientific term that covers people's knowledge, skills, methods, practices and beliefs about traditional animal health care. However, there is still the need for scientific evidence to support the effectiveness of its application, because it could depend on the type of preparation (feeding complete herbs or the use of pure extract), interaction with other plants, animal species, etc.

In ruminants, some research has been conducted suing *Salix alba, Echinacea purpurea, Silybum marianum* and *Cynarae folium*. The use of herbal products as feed additives to boost the cows' immune systems could also impact milk quality.

Salix alba, known as white willow, is rich in bioactive compounds (**BC**) such as salicin, polyphenols and flavonoids. The bark and leaf extract of ***S. alba*** has strong antioxidant activity, similar to vitamin C (ascorbic acid). In poultry, the dietary supplementation with *S. alba* has been shown to improve the oxidative status due to promoting the protective activity of antioxidant enzymes against oxidation.

Echinacea purpurea is a medicinal plant from north America which has important immunostimulatory and anti-inflammatory properties. The immunoregulatory effects of ***E. purpurea*** are thought to involve stimulation of T-cell production, phagocytosis, lymphocytic activity, cellular respiration and inhibition of hyaluronidase enzyme secretion. The actives compounds in Echinacea are alkamides, glycoproteins, polysaccharides, phenolic compounds and cinnamic acids.

Silybum marianum, known as milk thistle, active extract is a mixture of flavonolignands, and polymeric and oxidised polyphenolic fraction. The polyphenolic fraction is call **silymarin** and it is used for its hepatoprotective effects. It also has cytoprotective effects due to its antioxidants and free radical scavenging properties. In dairy cows, the hepatoprotective effect can alleviate the severity of the peripartum fatty liver, reduce ketonuria and positively influences the metabolic profile of liver enzymes during the lactation onset.

Cynarae folium, known as artichoke, from the Mediterranean Basin and has antioxidant activity. Major **BC** are polyphenols and flavonoids. However, there are still limited trials in animals to support the antioxidant effect observed in the lab.

Fresh nutrient-rich herbal grass-clover ley fodder for organic cattle (Photo: Judith Conroy)

WHAT DID OTHERS FIND?

Cows supplemented with ***S. alba*** (white willow), quercetin extract and naringin extract during 70 days did not shown differences on milk yield and composition. On the other hand, dietary supplementation of Brown Swiss cows at late lactation with ***Salix babylonica*** increased milk production and decreased milk fat, maintaining the same level of milk protein and lactose.

The supplementation with dry whole-plant **Echinacea** meal during peripartum in cows did not impact on somatic cell count up to three months post-calving. In goats, two-week supplementation with ***E. purpurea*** extract did not impair milk yield, lactose and dairy matter content, but showed a small transient decrease in protein and increase in fat content.

In cows with subclinical fatty liver supplemented with **silymarin** during the peri-partum showed an increased milk yield and decreased milk fat and protein percentage. The supplementation with artichoke (***Cynara scolymus*** L.) by-products silage in goats' diets increased fat content when using artichoke bracts, and milk protein and Se content when using artichoke plant. The supplementation with artichoke plant also increased the polyunsaturated fatty acids milk content.

OUR STUDY IN DAIRY CATTLE

Three biodegradable herbal boluses containing ***Salix alba***, ***Echinacea purpurea***, ***Silybum marianum*** and ***Cynarae folium*** were administered to dairy cows during the post-partum period. From calving to 100 days post-calving, milk production and composition as well as fertility traits were monitored in 72 Holstein-Friesian cows.

Cows were enrolled in the study at calving and assigned into one of the two experimental groups, keeping them balanced:

Group Control: 32 animals – received placebo bolus

Group Supplemented: 40 animals – received the **herbal bolus**

The parameters we monitored were:

- Milk yield
- Milk chemical composition and pH
- Milk somatic cell score
- Main milk fatty acids
- Milk coagulation properties
- Fertility traits: days to first artificial insemination service, days open and calving-to-conception interval

MAIN RESULTS

The herbal treatment:

- Did not impair milk characteristics
- Did not increase somatic cell count
- Did not modify their metabolic profile

FINAL REMARKS

There is a huge interest from livestock producers and feed additive suppliers in using natural feed additives to ameliorate livestock health and performance. In particular, since 2006 when growth promoters were banned in the European Union. However, there are still very few studies conducted in animals, especially in ruminants.

Therefore, the research we did within the **Organic-PLUS** framework helps to increase the scientific knowledge on the potential use of **herbal products** as feed additives to boost the cows' immune systems and to improve milk quality.

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See them in the complete report D4.6 available at the **Organic-PLUS** website.

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How to reduce susceptibility to disease in beef cattle production

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Antibiotic use in organic production livestock should be avoided when possible, and it is forbidden as a growth promoter, as sometimes antibiotics can be used to hide poor management practices. Organic systems are more focussed on prevention, reducing animals' stocking density and allowing access to pasture. Within this framework, the use of natural bioactive compounds as feed additives have attracted the attention of producers and researchers to enhance animals' health, performance and product quality.

Micronutrients such as Selenium, vitamin E or yeast, which are rich in nutraceutical bioactive compounds, have already demonstrated the ability to improve animals' health and immune status. Recently, herbs from traditional Chinese medicine have captivated the attention of many researchers as possible sources of natural bioactive compounds.

Bioactive compounds are chemical molecules found in small amounts in plants that have actions in the body that may promote good health and are studied in the prevention of human and animal diseases.

SCUTELLARIA BAICALENSIS SOURCE OF BIOACTIVE COMPOUNDS

Scutellaria baicalensis is an autochthonous flowering plant in several east Asian countries and has been cultivated in many European countries. It is listed officially in the Chinese Pharmacopoeia and their dried roots have been used in Chinese herbal medicine to treat liver and lung diseases, hypertension, acute respiratory infections, acute gastroenteritis, infantile diarrhoea and morning sickness.

Medicinal plant *Scutellaria baicalensis*: a) plant, b) dried roots and c) hand-drawn figure (extracted from Zhao et al. 2016)

The bioactive compounds isolated from this plant are flavonoids, terpenoids volatile, oils, and polysaccharides, which have exhibited many activities and effects on the immune system and liver protection. The four main flavonoids of ***Scutellaria baicalensis*** are baicalein, baicalin, wogonoside, and wogonin. These individual compounds have demonstrated antitumor, antibacterial, antiviral, antioxidant, antihypertensive, and hepato-protective effects, which suggests a potential beneficial effect of the whole plant on the animal organism.

USE OF *SCUTELLARIA BAICALENSIS* IN LIVESTOCK

Some scientific studies with ***Scutellaria baicalensis*** have been conducted in pigs and poultry to evaluate their antioxidant and anti-inflammatory properties. In pigs, the inclusion of a highly bioavailable form of ***Scutellaria baicalensis*** extract showed comparable effects to a synthetic antibiotic on growth performance, nutrient digestibility, faecal microbiota, faecal gas emission, blood haematology, improved average daily gain and final body weight. Another study in piglets showed that dietary supplementation with ***Scutellaria baicalensis*** decreased diarrhoea frequency and increased feed conversion ratio.

In broilers, the addition of ***Scutellaria baicalensis*** extract or root also increased the final body weight and feedstuff consumption without affecting meat quality and chemical composition. However, an excess of ***Scutellaria baicalensis*** root impaired the development of the immune organs in chickens.

So far, very few studies evaluated the effect of ***Scutellaria baicalensis*** in ruminants, and most of them are *in vitro* studies in the lab. These *in vitro* studies suggested that ***Scutellaria baicalensis*** could interact with rumen ecology altering rumen microbiome and which indicated a possible effect on diet digestibility.

Despite *in vitro* studies results were not also consistent, it seems that flavonoids, which are powerful antimicrobials, can increase ruminal volatile fatty acids production, while reducing ammonia and methane production. In dairy cattle, supplementation with ***Scutellaria baicalensis*** extract reduced somatic cell count and increased milk yield in early lactation. In beef cattle under heat stress, supplementation with a mix of herbs including ***Scutellaria baicalensis*** improved digestibility and growth performance.

OUR STUDY IN BEEF CATTLE

Based on the potential of ***Scutellaria baicalensis*** as a feed additive to improve animals' performance, within **Organic-PLUS** we decided to evaluate the effect of a mineral supplement containing vitamin E, organic selenium, yeast derivate and ***Scutellaria baicalensis*** extract during the fattening and finishing period (~4 months) of 143 Charolaise male cattle. The supplement was added as a top dressing.

Experimental design: Animals were housed in 12 pens and received an adaptation diet the month before starting the trial. The experimental groups were:

Group Control: 72 animals - Not supplemented

Group Supplemented: 71 animals - Supplemented with ***Scutellaria baicalensis*** (20 g/day per animal)

The parameters we look into were:

- Feed intake
- Chemical composition and mineral content of the diet
- Faeces composition
- Initial and final body weight
- Average daily gain
- Feed conversion ratio
- Plasmatic a-tocopherol (vitamin E)
- Plasmatic metabolic profile
- Carcass weight
- Carcass yield

MAIN RESULTS

The supplementation:

- Did not impair beef cattle intake and digestibility
- Did not have a detrimental or beneficial impact on growth performance
- Did not modify plasmatic vitamin E levels
- Did not modify their metabolic profile

FINAL REMARKS

There is a huge interest from livestock producers and feed additive suppliers in using natural feed additives to ameliorate livestock health and performance, particularly since 2006 when growth promoters were banned in the European Union. However, there are still very few studies conducted in animal, especially in ruminants.

Therefore, the research we did within the **Organic-PLUS** framework helps to increase the scientific knowledge on the inclusion of ***Scutellaria baicalensis*** as a source of bioactive compounds in beef cattle production.

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Use of plant feed additives as natural alternatives to synthetic antioxidant vitamins in ruminant production

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Plant extracts, essential oils and by-products of plants (e.g., from the grape or citrus industries) may contribute to finding alternatives to synthetic vitamins for livestock. These components present a potential effect on animal health and productivity and are known as natural **plant feed additives**.

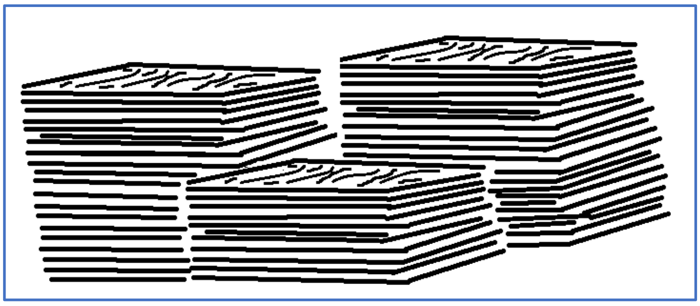
Plant feed additives can be freely fed to livestock reared under both conventional and organic conditions. As they are considered natural, harmless and residue free, they could meet both consumer expectations and the need of the feed industry to find valid alternatives to synthetic antioxidants.

In livestock such as pigs, dairy cattle and beef, the impact of **plant feed additives** on animals' oxidative status has been less well investigated than for poultry. In pigs, they have been evaluated more frequently as alternatives to antimicrobials rather than as antioxidants. In ruminants, research has been focused on essential oils as rumen modifiers. Very few studies have been carried-out in livestock to evaluate the antioxidant role of **plant feed additives** in comparison to synthetic molecules. However, it is worth mentioning that there is still controversy about the true effectiveness of **plant feed additives** when compared with vitamin E, probably because they have different efficacies and modes of action.

Therefore, we decided to review the existing literature on the antioxidant capacity of plant extracts, essential oils, and plant by-products as potential alternatives to synthetic antioxidant vitamins in ruminant production (lambs, goats, and dairy and beef cattle).

SELECTION OF THE ARTICLES TO BE INCLUDED

We did a systematic review of peer-reviewed studies published in specialised browsers (Pubmed, ISI Web of Science, Science Direct) performed covering a time-span of 20 years, from 2000 to 2020.



IMPACT ON FEED INTAKE, GROWTH AND OTHER PERFORMANCE TRAITS

In lambs, the addition of ethanolic saffron (*Crocus sativus* L.) petal extract or grape pomace did not impair growth, dry matter intake or feed-to-gain ratio.

Lactating cows showed a similar dry matter intake of an unsaturated fatty acid enriched diet when adding yerba mate compared to the control diet.

In goats, the supplementation with *Andrographis paniculata* or turmeric acid did not affect the average daily feed intake, but reduced the feed-to-gain ration. On the other hand, groups receiving *Andrographis paniculata* increased their feed efficiency despite growth performance was not affected.

IMPACT ON METABOLIC PARAMETERS AND OXIDATIVE STATUS

In lambs, the supplementation with ethanolic saffron petal extract decreased kidney lipid oxidation because it contains antioxidant compounds that scavenge free radicals and act as reductants. Moreover, when administered via subcutaneous, increased the GSH-Px activity in blood, which indicated a release of antioxidant enzymes, protecting the animal from a risk of oxidative stress. In addition, the supplementation with the flavonoid naringin reduced the degradation of fat and triglycerides concentration similarly than vitamin E. Goats receiving *Andrographis paniculata* and turmeric acid showed a similar reduction of lipid peroxidation in plasma than those receiving vitamin E.

In dairy cows, plants rich in polyphenols such as rosemary, grape, grapefruit and marigold decreased lipid peroxidation but did not affect lipid metabolic parameters (triglycerides, non-esterified fatty acids and phospholipids) nor the activity of the antioxidant enzymes in the liver.

MEAT ANTIOXIDANT STATUS

In lambs, the inclusion of dried citrus pulp increased the concentration of vitamin E in liver, plasma and muscles, and prevented oxidation. The inclusion of dried tomato pomace increased concentrations of linoleic acid, vitamin E and vitamin A in lambs' meat. In fattening lambs, dietary supplementation with naringin reduced plasmatic byproducts of lipid peroxidation. Meat from lambs supplemented with rosemary diterpens presented better antimicrobial effects but did not prevent meat oxidation. Lambs receiving saffron petal extract showed a reduction in their plasmatic cholesterol level.

Conversely in lambs, the inclusion of red wine extracts did not improve the oxidative stability and linking sensory scores of *Longuissimus dorsi* muscle nor reduce the lipo-oxidation compounds. Grape seed extract or grape pomace supplementation were not able to show the same potential of vitamin E to reduce discoloration and lipid oxidation, and improve shelf-life.

In ewes, the addition of grape pomace to their diet did not affect the animal performance, carcass characteristics and meat quality of their suckling lambs.

The addition of plant extracts in cattle improved the oxidative stability of *Longissimus thoracis* and *semitendinosus* muscles.

Goat supplementation with *Andrographis paniculata* and turmeric acid improved the colour parameters of *infraspinatus* muscle and carcass traits.

MILK AND FATTY ACIDS PROFILE

In cows, extruded feed supplemented with plant extract rich in polyphenols modified the milk fatty acid profile: slightly increased caproic acid, stearic acid, arachidic acid and monounsaturated fatty acids and decreased palmitic acid, eicosapentaenoic (EPA) and saturated fatty acids proportion.

The simultaneous supplementation of extruded linseed-fed cows with plant extracts and vitamin E reduced the impact of vitamin E on the milk's fatty acids profile, although it had no effect on dairy performance.

Yerba mate reduced protein and solid milk percentage but increased the oxidative reducing power.

In ewes, the addition of grape pomace did not modify the milk fatty acid profile compared to those animals receiving vitamin E.

FINAL REMARKS

Plant feed additives and vitamin E have a similar effect in mitigating oxidative stress in ruminants under moderate stress conditions which are frequent in intensive livestock farming. Moreover, they can have a positive effect on the quality of the derived food products, with variations in their fatty acid content and oxidative status that generally improve the related traits (e.g., odour, colour, etc.). The effects of plant feed additives are variable but often similar to those of the synthetic antioxidant vitamin E, indicating their potential at least as a partial substitute of this vitamin in the diet.

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Read the full scientific review papers published in Antioxidants https://doi.org/10.3390/antiox10091461 and https://doi.org/10.3390/antiox10050780

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ORGANIC PLUS
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Innovation Centre for Organic Farming

tang.nu

THE VELUX FOUNDATIONS

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STØTTET AF
Fonden for økologisk landbrug

Do phenol-rich natural feed ingredients reduce diarrhoea in organic piglets?

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INTRODUCTION

Zinc is an essential nutrient (micromineral) and is very important in many biochemical and physiological processes in the body, especially for growing pigs. To assure fulfilling their requirements 100 mg zinc is given per FEsv (Danish feed unit). In addition to that, it is permitted to give higher levels of zinc – considered "medical zinc" or "veterinarian zinc", two weeks after weaning to prevent/avoid diarrhoea, which is a common practise when raising pigs. However, high inputs of zinc are considered a contentious input and therefore from mid-2022, the use of a high level of zinc (2,500 mg) from two weeks to weaned piglets to avoid diarrhoea will be banned by an EU regulation. This applies to both conventional and organic pig production. Thus, there is a need to identify new solutions so that healthy production without medical zinc – and without antibiotics – to avoid diarrhoea can be practised. The characteristics of organic production, such as later weaning, outdoor access and more space, are generally considered to be positive for animal health. However, the fact is that diarrhoea in organic piglets still occurs very often.

This work is based on the EU project Organic-PLUS, but in addition to this, two other projects are mentioned, because they also deal with the diarrhoea problem. The studies conducted within each project are:

- **Study 1.** Mapping the infection pressure in the farrowing outdoor pen (Project: Mapping infection pressure in organic pig production).
- **Study 2.** Test of p-phenol as a substitute for medical zinc to avoid diarrhoea (Project: Organic-PLUS).
- **Study 3.** Test of seaweed as a substitute for medical zinc to avoid diarrhoea (Project: Seaweed now).

METHODOLOGY

The experimental designs for each one of the studies conducted are described below.

Study 1: Mapping the infection pressure in the farrowing outdoor pen

During 2018, around 140 faeces samples from piglets with diarrhoea from 5 herds were analysed for e.g. *E. coli*, coccidia and intestinal worms. The age of the piglets was 0 – 7 weeks and a few weeks after weaning (7 weeks). Moreover, dead pigs during the trial were necropsied and analysed to identify the reason for death.

Study 2: Test of p-phenol as a substitute for medical zinc to avoid diarrhoea

During 2019 (January to November) a pilot test was conducted to test the feasibility of p-phenol as a substitute for medical zinc.

Test group: 5 groups of weaning pigs (approx.100 pigs/group). The dose 0.2% p-phenol added to the feed for lactating sows, suckling pigs and weaned pigs was the recommended dose by the manufacturer "Metodo".

Control group: 5 groups of weaning pigs (approx. 300 pigs/group) had normal feed with 2,500 mg zinc 2 weeks after weaning. The lactating sows and suckling pigs had normal feed.

Table 1. Feed for control and test groups

Control feed	Sows	Suckling pigs	Weaned pigs
Ordinary organic feed (100 mg zinc)	x	x	x
2,500 mg zinc two weeks after weaning			yes
Product with phenol-rich compounds	no	no	no

Test feed	Sows	Suckling pigs	Weaned pigs
Ordinary organic feed (100 mg zinc)	x	x	x
2,500 mg zinc two weeks after weaning			no
Product with phenol-rich compounds	yes	yes	yes

Study 3: Test of seaweed as a substitute for medical zinc to avoid diarrhoea

During 2019, a study was conducted with 90 piglets divided into 5 groups with 18 piglets in each to test three different kinds of seaweed: *Saccharina latissimi*, *Ulva lactuca* and *Ascophyllum nodosum* as a substitute for medical zinc. Groups were:

- Control group without medical zinc.
- Control group with medical zinc.
- Test group with 5% of seaweed *Saccharina latissimi*.
- Test group with 5% of seaweed *Ulva lactuca*.
- Test group with 5% of seaweed *Ascophyllum nodosum*.

RESULTS

Study 1: Mapping the infection pressure in the farrowing outdoor pen

During testing, it became obvious that it is very difficult to perform diagnostics in outdoor conditions because the diarrhoea faeces disappear very easily. 2018 was a very dry year with both frost and sunshine which contributed to destroying *E. coli* bacteria as confirmed by the faeces analyses. But a wide range of other infection agents were found such as types of *E. coli*, other than F4 and F18, *Clostridium perfringens*, different lung infections, coccidia and intestinal worms.

Study 2: Test of p-phenol as a substitute for medical zinc to avoid diarrhoea

With regard to diarrhoea, all 5 test groups developed serious diarrhoea on day 6-7 after weaning and we were forced to give them medical zinc again. Group 5 was given a double dose of p-phenol (0.4% in the feed) but with no effect. For the pigs in the test groups, the mortality rate reached 10% compared to 0% in the control group.

Study 3: Test of seaweed as a substitute for medical zinc to avoid diarrhoea

Results did not show a consistent effect on gut health after feeding seaweed meal or medical zinc. Upcoming results on short chain fatty acids in intestinal digesta may show changes in fermentation profile from feeding seaweeds. For more details, contact Marleen Elise van der Heide, Aarhus University, who is responsible for study 3.


Sow and suckling pigs are kept outdoors

Weaned piglets in stable – indoor area

Weaned piglets in stable – outdoor area

CONCLUSIONS

In the Organic-PLUS test (study 2), p-phenol did not show enough effect as to avoid diarrhoea compared to the use of medical zinc. Likewise, seaweed in study 3 showed only a tendency to decrease post-weaning diarrhoea. As demonstrated in study 1, infection pressure is high and varies very much when in the farrowing outdoor pen. Even if the infection is not directly related to diarrhoea (e.g. in the case of lung disease), it can cause weak health, which in turn may be partly responsible for the diarrhoea.

A 7-week-old piglet must have a generally high level of health in order to manage the weaning period. In many cases, the piglets looked very fit before weaning but they quickly developed serious health problems. Because there are so many different infection agents, it is not realistic to expect one specific additive to be the solution to all problems.

We conclude that so far, no equivalent substitute for medical zinc to prevent diarrhoea has been found. The alternatives we tested showed not enough effect or only a tendency to decrease diarrhoea. A direct input substitution is therefore not, or not yet possible.

However, organic farmers in Denmark associated to Organic-PLUS have performed their own experiments and have gained experience in how to manage without the contentious input medical zinc to prevent diarrhoea and any hence 'input substitution' with a less contentious input. Their practical farm experiences, which could be described as a 'system solution', are summarised below.

Examples of good management of piglets to provide a system solution to medical Zinc:

Before weaning:

- Be aware of an updated vaccination program - both for sows and piglets.
- Screen for coccidiosis and make plans for treatment.
- Make sure the sows are in good condition.
- Only use nursing sows when absolutely necessary.
- Only move piglets between sows when absolutely necessary.
- Arrange for enough feeding stations for piglets in the suckling period.
- Provide a high level of hygiene.
- Extend the suckling period by 1-2 weeks.

Weaning process:

- Avoid any kind of stress.
- Provide easy access to water and feed.
- Use the same feed as the piglets had in the suckling period.
- Provide optimal temperature/regulation of temperature.

After weaning:

- Arrange for small group size.
- Keep the weaned piglets in the outdoor system.
- See to it that the piglets have access to soil. This is very important.
- Provide access to high quality roughage. This is important.

In general:

- Have qualified staff.
- Analyse problem pigs.
- Exchange knowledge with advisers and vets.
- Accept a little lower productivity.

PRACTICAL RESULTS FOR FARMERS

- No additives can replace good animal husbandry management.
- The trial results regarding phasing out high contentious inputs of zinc showed that both p-phenol and seaweed only have a tendency to decrease post-weaning diarrhoea.
- There is a need for further studies on how to produce strong and healthy pigs. This can be done by focusing on management and combining it with healthy feed.
- Using medical zinc requires a lot of administrative work!

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This Work was also carried out as part of the project "Kortlægning af smittepres i økologisk svineproduktion" (Mapping of the infection pressure in organic pig production), that has received funding by "Fonden for Økologisk Landbrug" (The Fund for Organic Farming). The project has financed the P-phenol for the on-farm test, retrieval, coordination and publishing of results from the project on mapping of the infection pressure in the farrowing outdoor pen in organic pig production.

Proof reading by P. Pennington (Landbrug & Fødevarer F.m.b.A.; SEGES), Judith Conroy and Ulrich Schmutz (Coventry University) and C.L. Manuelian (University of Padova).

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Does *Artemisia absinthium* have an effect on intestinal worms in weaned organic piglets?

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INTRODUCTION

Intestinal worms such as *Ascaris suum* and *Oesophagostomum dentatum* are common in organic pig production. This is difficult to avoid because production takes place outdoors and worm eggs can survive in the soil for many years. Sows build up immunity against the worms, but piglets ingest the eggs and worms can easily develop. There is no clear conclusion about the negative effect (daily growth and feed conversion) but from an animal welfare and an ethical point of view, we want to minimise intestinal worms in organic piglets. Veterinary treatment may risk the development of resistance and also it takes quite a lot of administration work to handle prescriptions, perform analyses that confirm the need, and the treatment itself and may leave a rest for destruction.

Therefore, the aim was to evaluate ***Artemisia absinthium*** (wormwood) to prevent worms' infestation in weaned organic piglet.

METHODOLOGY

The experimental design for the on-farm test of ***Artemisia absinthium*** isdescribed below.

The pigs were born in outdoor huts and were weaned at 10 weeks of age at a weight of approx. 30 kg. An analysis was initially performed for worm eggs in the manure to ensure that the herd had an infection pressure. The results showed moderate infection pressure. For weaning, the pigs were put in housing units in cleaned pens that had been disinfected/dried by liming. The housing system is deep litter and each batch size was 80 pigs. The pigs stayed in this housing until reaching a weight of approx. 60 kg, i.e. approx. 30 kg gain over 35 days. Manure samples were taken to analyse for worm eggs at penning and before transfer. Treatment for intestinal worms took place 5 - 7 days after penning. Artemisia absinthium was given at a dose of 10 g per day per pig over 10 days. The dose of traditional deworming treatment (Pigfen) was given according to the manufacturer's instructions. Despite the fact that Artemisia absinthium is very bitter, no refusal to eat was observed. Daily growth was calculated. No feed consumption was registered.

Test design: There were 3 groups of 80 pigs each and 3 repetitions. Each repetition looked like this:

Group 1: 80 pigs - No treatment for intestinal worms
Group 2: 80 pigs - Treatment with ***Artemisia absinthium***
Group 3: 80 pigs - Treatment with traditional deworming, Pigfen (vet. medicine)

There were 3 weeks between each recurrence with first transfer from field to stable in week 5, second in week 8 and last insertion in week 11, 2021.

Presence of worm eggs (FEC, faecal egg count) in the manure was divided into levels (**Table 1**).

Table 1. Levels of presence of worm eggs (FEC, faecal egg count) in the manure

FEC	*Ascaris suum*	*Oesophagostomum dentatum*
None	0	0
Low	< 500	< 200
Moderate	500 - 5000	200 2000
Low	500	2000

RESULTS

In general, the incidence of worm eggs was low. Pigfen had a good effect, but when the statistical analysis was performed, there was not enough difference between the treatments to be able to highlight one over another.

Weaned piglets in pen – indoor area

Table 2 shows the results of the 3rd repetition at penning/weaning (10 weeks and approx. 30 kg versus transfer at approx. 15 weeks and 60 kg).

Table 2. Number of eggs for *Ascaris suum* and *Oesophagostomum dentatum* from the 3rd repetition at weaning and transfer, and gain (g)

	Treatment											
	None				**Pigfen**				***Artemisia absinthium***			
	Ascaris suum											
	eggs	low	mod	no eggs	eggs	low	mod	no eggs	eggs	low	mod	no eggs
3rd Repetition Weaning	0			x	3250		x		100	x		
	0			x	0			x	100	x		
	0			x	0			x	600		x	
	550		x		0			x	150	x		
	Oesophagostomum dentatum											
	0			x	0			x	0			
	0			x	0			x	0			x
	0			x	0			x	300		x	
	0			x	300		x		0			x
	Treatment											
	None				**Pigfen**				***Artemisia absinthium***			
	Ascaris suum											
	eggs	low	mod	no eggs	eggs	low	mod	no eggs	eggs	low	mod	no eggs
3rd Repetition Transfer	100	x			0			x	300	x		
	350	x			0			x	250	x		
	50	x			0			x	650		x	
	50	x			0			x	0			x
	Oesophagostomum dentatum											
	50	x			0			x	50	x		
	0			x	0			x	150	x		
	0			x	0			x	300		x	
	0			x	0			x	50	x		
Gain	1000				968				1085			

It is also interesting to look at growth (**Table 3**).

Table 3. Overview of growth (g) in each repetition

Treatment	None	Pigfen	*Artemisia absinthium*
Repetition 1	917	820	847
Repetition 2	822	904	871
Repetition 3	1000	968	1085

The results show a generally higher growth rate in repetition 3 compared to repetitions 1 and 2, with the highest growth in the ***Artemisia absinthium*** group, in which the difference corresponds to a daily gain of approx. 225 g. The difference is not statistically significant, but the alternative treatment ***Artemisia absinthium*** did not reduce growth, if anything it was higher.

Conclusion

The excretion of worm eggs in manure was generally low. This result does not allow one treatment to be highlighted over another. However, the result may give rise to considerations regarding the need for any deworming in general, unless infection pressure is much higher.

There is a current lack of knowledge to define an acceptable infection pressure, whether the number of worm eggs is the best indicator of a strain and what measures the farmer can take to reduce the infection pressure when it comes to outdoor herds. Differences in growth figures the repetitions in between cannot be immediately linked to the treatments.

Upon examination of details of the test, it became clear that the only change between repetitions 1 and 2 held against repetition 3, occurred in the farrowing pen. In repetitions 1 and 2, farrowing took place in pens that have been in use for almost a year. In repetition 3, farrowing took place in 'new' pens that had been "pig-free" for a minimum of 12 months. A difference of approx. 225 g of daily gain in the period from 30 to 60 kg is surprisingly high. Experiences from herds housed in new pens for each farrowing confirm that it provides better health/robustness and improved productivity. There is no knowledge of data from practice showing growth in the weight range that was discussed in the test, but production results generally testify a significantly positive effect of offering 'new' pens for each farrowing. 'New' is how farmers describe it, meaning that it was cleaned and rested for 12 months . The use of a biocidal disinfectant with the active ingredient of the potassium salt of peroxymonosulfuric acid (e.g. Virkon S) is not approved for organic pigs in Denmark.

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How different is organic from conventional milk?

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In the last 10 years, the share of organic cow milk production has doubled. This is related to an increased interest from consumers for this type of product. Consumers perceive organic production to be more respectful of animal welfare and more sustainable. Moreover, they associate organic food with being healthier and of better quality than conventional foods. To support these beliefs around organic foods, scientific research in this area is also increasing.

In Europe, there is scarce information on the health, robustness, and characteristics of native breeds for dairy production. Moreover, neither the rules for organic production nor the literature clarify which breeds should be considered. Although high-milk-production cow breeds – such as Holstein-Friesian – are usually considered not in-line with the organic system philosophy, the truth is that Holstein-Friesians have been selected under different conditions. Farmers have been selecting them under different conditions to better fit their production system, including grazing systems. Therefore, in some areas, Holstein-Friesians are raised under organic conditions because they are well adapted to the local characteristics and needs. Therefore, it is interesting to evaluate organic milk's chemical composition from Holstein-Friesian cows.

WHAT DID OTHERS FIND?

A review on the topic revealed that **organic** and conventional milk had similar concentrations of saturated fatty acids (**SFA**) and monounsaturated fatty acids (**MUFA**), but **organic** milk had higher total polyunsaturated fatty acids (**PUFA**), omega-3 PUFA, and a-tocopherol content. Nevertheless, milk fatty acid composition in **organic** and conventional pasture-based retail milk purchased in supermarkets differs in most of the individual and groups of fatty acids. In addition, lower concentrations of cobalt, chromium, copper, iodine, selenium, and zinc as well as higher arsenic levels have been reported in **organic**.

However, it should be taken into account that other factors have a huge impact on milk composition such as breed and diet. For example, higher fat concentration in milk has been reported in **organic** systems, which could be related to a preference for breeds other than Holstein-Friesian in **organic** herds and a lower farming intensity. That means that breed, pasture-feeding and intensity interplay when comparing **organic** and conventional systems. The lack of a control in most published studies of variables that affect milk composition make it difficult to really extrapolate results

Holstein-Friesian cows in the rotary milking parlour (Photo: Claudia De Lorenzi)

Impact of farming system on milk gross composition, vitamin E and amino acids

While some studies did not find differences in fat and protein content between both farming systems, others reported greater total milk fat in **organic** retail milk. To the best of our knowledge, there are no published studies focusing on the evaluation of amino acid composition, comparing **organic** and conventional systems, and very few have investigated vitamin E milk content. A greater content of a-tocopherol has been reported in **organic** than conventional milk, but not other vitamins. Some studies have reported a higher somatic cell count in **organic** than conventional herds, which has been linked with the use of alternative treatments instead of antibiotics, without using teat dipping in the milking routine, and with lower milk production.

Impact of farming system on milk mineral and fatty acid profile

Very few studies have evaluated differences in milk mineral profile. It has been reported that there is a higher iron and a lower iodine and selenium content in **organic** than conventional milk. Other studies revealed more differences between both farming systems, with lower concentrations of cobalt, copper, selenium, zinc, iodine and chrome. Moreover, the lower iodine content of **organic** milk could be related to a lower use of iodine-containing teat dipping.

Fatty acids profile is one of the most studied parameters in **organic** milk. Usually studies reported differences in several individual and fatty acid groups. However, other factors that are known to affect milk fatty acid profile usually are not considered, such as the breed or feeding systems. In fact, a study with a milk retailer showed that differences between **organic** and conventional milk diminish when the non-organic milk system is pasture-based.

OUR STUDY IN DAIRY CATTLE

We designed a study to enable us to compare the same herd breed composition and similar levels of intensity. This could be described as input-substitution **organic**, where mainly the feed (**organic** feed), stocking density, and veterinary treatments change while all other inputs and management remain unchanged.

We focused on detecting differences and similarities in milk composition in bulk milk from Holstein-Friesian cows. The farms were located in northern Italy and showed a similar annual average milk production per cow and day. Moreover, the diet of lactating animals included corn (meal and/or silage). Although all organic farms included a period of time on pasture, only 7 out of 12 **organic** farms grazed on pasture during lactation.

Group Conventional: 12 farms

Group Organic: 12 farms

The parameters we monitored were:

- Milk yield
- Milk chemical composition, pH and somatic cell score
- Milk vitamin E
- Milk mineral, amino acid and fatty acid profile

MAIN RESULTS

In **organic** systems:

- Total fat, lactose, vitamin E, and amino acids did not differ
- Total protein and casein content was lower
- Somatic cell count was greater
- Among minerals, differences were observed for Fe, K, Mg, and S in some months, being usually lower
- Among fatty acids, **PUFA** and **Omega-3** tended to be greater, and palmitoleic acid was lower

FINAL REMARKS

There are still some differences between **organic** and conventional milk even if selected farms were quite similar in terms of: production level, the Holstein-Friesian breed and in their generally high levels of. However, other aspects related to the production system and indicators of animal health and welfare should be considered in order to have a more complete overview.

For instance, the expected better welfare, which includes outdoor access and lower stocking density, in **organic** farming systems is often assumed by **organic** consumers but is not demonstrated by its intrinsic milk quality.

REFERENCES

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ACKNOWLEDGEMENTS

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Can plant feed additives be valuable substitutes for synthetic antioxidant vitamins in organic broiler production?

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INTRODUCTION

Plant feed additive (**PFA**, including plant tissues, plant extracts, essential oils, and plant by-products) can be a viable alternative to synthetic antioxidant vitamins in poultry nutrition. They contain biologically active compounds that can improve animal productivity. Some examples of positive effects are in the following (**Table 1**):

Table 1. Positive effects of plant feed additive on broiler product quality (from Pitino et al., 2021, modified).

Plant Feed Additive	Biological effect	Practical effect
Grape pomace Oregano essential oil Oregano oil Hesperidin	Reduce lipid peroxidation Prevent vitamin E drop in tissues during preservation	Increased product vitamin content and shelf-life
Rosemary oil Rosemary plant	Reduce Malondialdehyde (MDA) Inhibit microbial growth	Increased product shelf-life Reduced microbial contamination of the product
Tea catechins Rosemary leaves Orange peel Thyme oil	Reduce Thiobarbituric Acid Reactive Substances (TBARS)	Increased product oxidative stability and shelf-life
Coneflower Thyme Sage Marigold extract Sweet chestnut extract	Increase long-chain polyunsaturated fatty acids (PUFA) of breast muscle	Increased product characteristics related to human health
Sweet chestnut wood extract	Improve meat colour	Increased consumer's desirability of the product
Oregano aqueous extract	Improve organoleptic meat traits	Increased consumer's acceptability of the product

Considering the encouraging results obtained in other studies, a study was performed to evaluate the impact of the use of green tea combined with grape skin extract and of a commercial product composed of hydrolysed polyphenols from wood, as substitutes for synthetic vitamin E on poultry meat characteristics.

METHODOLOGY

Animals and experimental design

For the study performed at the Department of Veterinary Science of Parma, 252 one-day-old ROSS 308 broiler chickens were divided into 3 replicated groups and fed diets containing vitamin E (**Vit E**, 0.05 g/kg), a mixture of green tea and grape extract (**GT+GE** at 0.74 g/kg) or a mix of wood hydrolysed polyphenols (**OXI**, 3.65 g/kg) as antioxidant additives (**Figure 1** and **2**). The doses of the different antioxidants were calculated to obtain a similar "gross antioxidant capacity" of the diet (see protocol for alternative vitamin use D4.11). Chickens were fed starter (0-21 days of age), grower (22-42 days of age) and finisher (43-84 days of age) feedstuffs and environmental temperature and humidity were recorded daily. Feed and water intake were measured daily, and chickens were periodically weighed. Half of the animals were slaughtered at 42 and the remaining at 84 days of age.

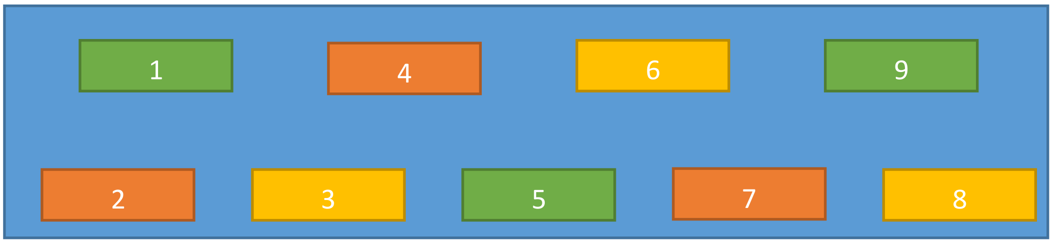


Figure 1. Graphical representation of the experimental unit setting. Treatments were: **Vitamin E** in boxes 1, 5 and 9; **Green tea + Grape** extract in boxes 2-4-7 and **wood hydrolysed polyphenols** in boxes 3-6-8.

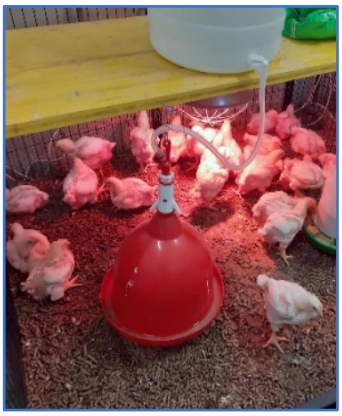


Figure 2. Some pictures of the replicates of the 3 groups of chickens enrolled in the experiment.

Processing of the chickens, chemical and physical analysis

After slaughtering, the whole coelomic content, was removed and weighed. The poultry carcasses were then transported to the laboratory of DAFNAE of the University of Padova (Italy) where they were weighed and then deboned, skinned and chilled. After head, neck and feet removal, the eviscerated carcasses were weighed and then dissected to separate the main cuts (thighs, wings and breast). Moisture, fat and protein (%) content were determined by near-infrared spectroscopy. Moreover, pH, colour and shear force were measured. Cooking losses were also calculated.

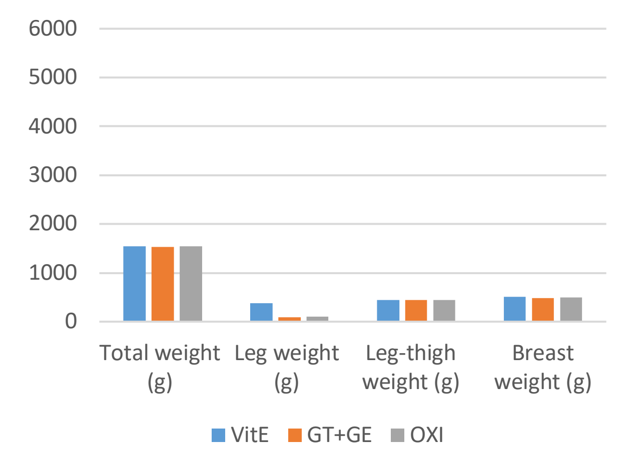
MAIN RESULTS AND DISCUSSION

The tested PFA did not affect organ weight and development, neither at 42 nor at 84 days (**Figure 3**), with similar results to those obtained with the **Vit E** control group.

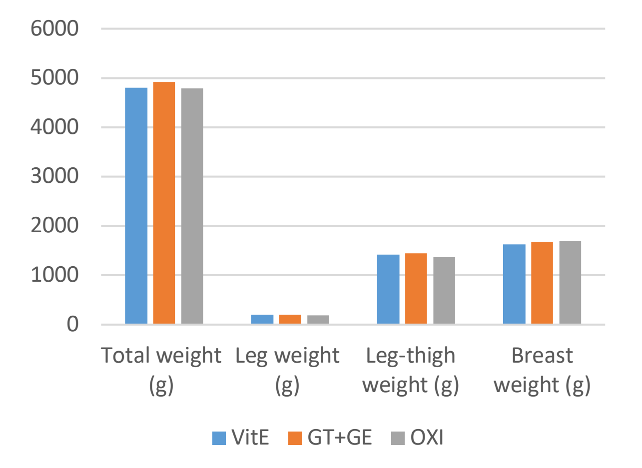
Consistently, carcass weight, total coelomic content weight and carcass yield parameters were similar between groups. Concerning meat quality and characteristics at the two intervals considered, only yellowness at 42 days was greater for **GT+GE** and lower for **OXI**; in any case, both results were similar to those obtained using **Vit E**.

Organ weights and meat pH values are within the literature ranges in particular results at 42 days of age. Colorimetric data shown at 84 days of age are in accordance with findings from other authors who reported that lightness is negatively correlated with the live weight of the chickens, indicating a darker colour in older birds. However, those authors specify that colour variation is related to a complex interaction between weather season of slaughter (winter vs summer), ante-mortem temperature, stress, pH values and water holding capacity, so these results cannot be directly attributable to the antioxidant additive employed. Overall, these results indicate that the tested PFA, appropriately dosed, can be a valuable substitute for synthetic antioxidant vitamins (**Vit E**) in organic broiler production.

Meat characteristics at 42 days



Meat characteristics 84 days



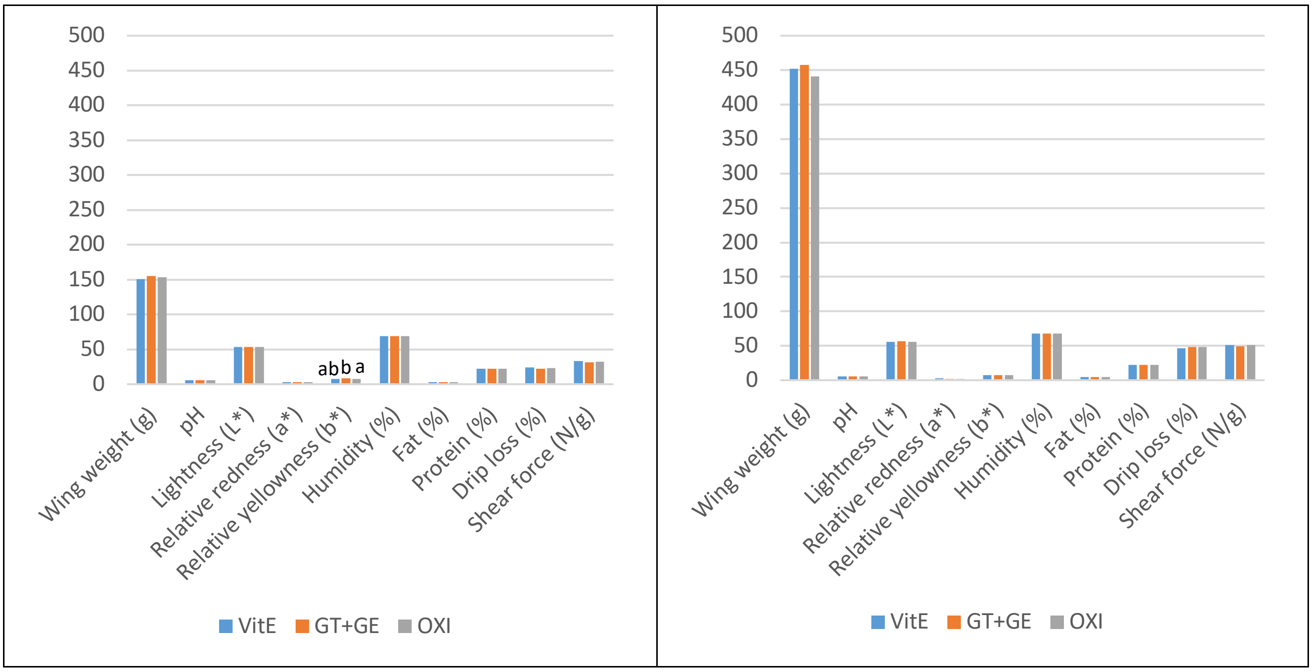


Figure 3: Meat characteristics at 42 and 84 days. ^{a,b,} indicated significant differences

PRACTICAL RESULTS FOR FARMERS

The substitution of **Vitamin E** with **Green tea + Grape Extract** or with a mix of **hydrolysed polyphenols** is possible, without impairing the animals' development or the chicken meat characteristics.

REFERENCES

See them in the complete report D4.6 and D4.7 available at the **Organic-PLUS** website.

ACKNOWLEDGEMENTS

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Alternative bedding materials in organic broiler production

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INTRODUCTION

Bedding materials can be categorised as organic (wood-based or plant-based) or inorganic (clay, sand, bentonite) and are characterised by different absorbance, comfort, cost and availability. The bedding quality in poultry production can affect poultry behaviour, performance and health status, especially focusing on foot and respiratory system health (Figure 1). Furthermore, the physical properties of each bedding material can affect thermal and humidity regulation, microbial development and ammonia release.

Figure 1. Plantar lesion and feather dirtiness derived from inadequate bedding (Photo by Marica Simoni and Federico Righi)

Wood shavings are the most used conventional bedding material but the broiler industry is expanding worldwide. Therefore, researchers are encouraged to search for and test alternative bedding materials. In this context, the increase of agroforestry and forest thinning material could provide, after appropriate treatments (e.g. pelleting or chipping), local material for bedding.

In the frame of the **Organic-PLUS** project, we performed a trial to test the impact of bedding material alternatives to wood shavings on broiler productivity.

METHODOLOGY

Animals and experimental design

For the study, 252 one-day-old ROSS 308 broiler chickens were divided into three homogeneous replicate groups (**Figure 2**) and bedded on wood shavings (**WS**), which was considered the control, poplar pellet (**PP**) or vineyards pellet (**VP**; **Table 1**) at the Department of Veterinary Science of the University of Parma (Italy).

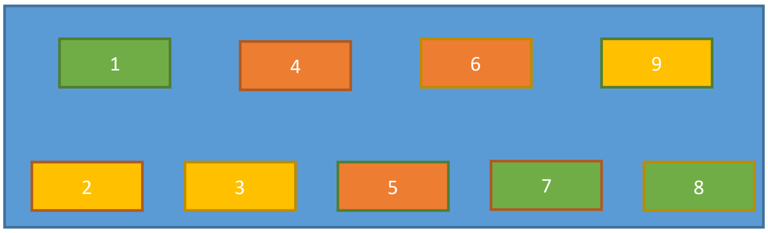


Figure 2. Graphical representation of the experimental unit setting. Beddings were: **Vineyard Pellet** in boxes 1, 7 and 8; **Poplar Pellet** in boxes 4-6-5 and **Wood Shavings** in boxes 2-3-9.

Chickens were fed starter (0-21 days of age), grower (22-42 days of age) and finisher (43-84 days of age) feedstuffs. Half of the animals were slaughtered at 42 days of age and the remaining at 84 days of age.

Table 1. Some characteristics of the bedding materials tested.

	Wood shavings	Poplar pellet	Vineyard pellet
Dry Matter (%)	83.6	84.6	85.3
Ash (% DM)	1.9	3.2	3.3
aNDFom (% DM)	87.2	89.5	82.1
Lignin(sa) (% DM)	19.3	20.2	20.3
Bulk density g/100 mL	7.43	51.63	58.30

aNDFom = ash free neutral detergent fibre

Processing of the chickens, chemical and physical analysis

After slaughter, the whole coelomic content was isolated and its weight determined. The poultry carcasses were then transferred to the meat laboratory of DAFNAE of the University of Padova (Italy) where they were weighed, deboned, skinned and chilled (**Figure 3**). After head, neck and feet removal, eviscerated carcasses were weighed and then dissected to separate the main cuts (thighs, wings and breast).

Figure 3. Processing of the animals at the meat laboratory of DAFNAE of the University of Padova (Italy; Photo by Massimo De Marchi).

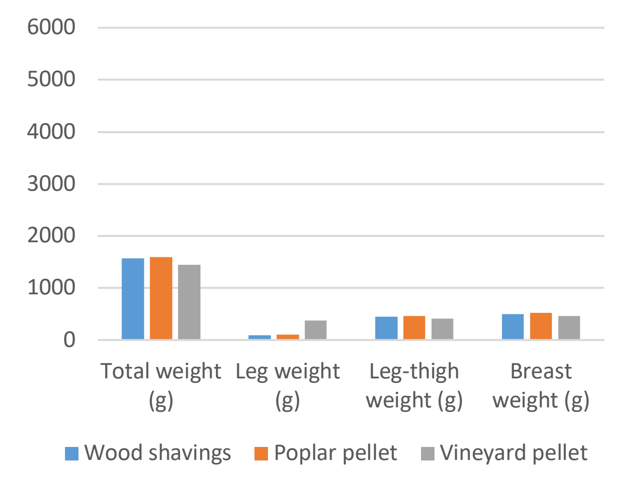
Moisture, fat and protein (%) content were determined by near-infrared spectroscopy. Moreover, pH, colour and shear force were measured. Cooking losses were also calculated.

MAIN RESULTS AND DISCUSSION

Animals raised on **WS** bedding had the highest whole coelomic content weight and hot carcass weight at 42 days (**Figure 4**). These animals in fact consume bedding material and this offered them health benefits by increasing gizzard size and activity. No differences were detected at 84 days for these two parameters, while **PP** had a higher carcass weight compared to **WS** (**Figure 4**).

No differences were observed in the meat quality characteristics at 42 days of age (**Figure 4**) with the exception of dry matter content which was higher in the **WS** group. At 84 days of age, the meat of **PP** and **VP** chickens was softener and richer in protein than for **WS**. Additionally, the **WS** poultry carcasses had a higher fat content than the **VP** (**Figure 4**).

Meat characteristics at 42 days



Meat characteristics 84 days

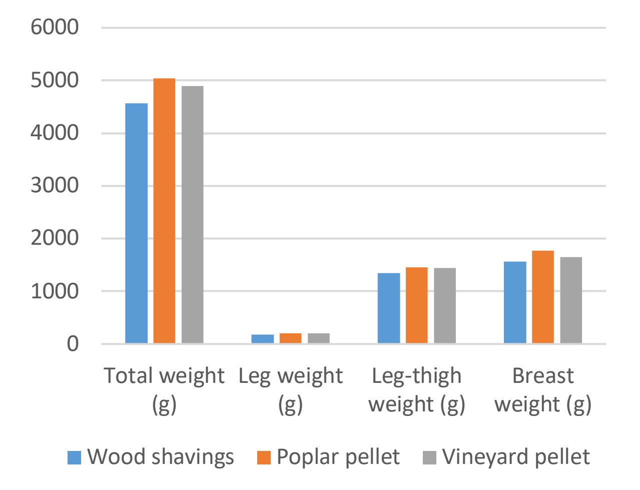


Figure 4: Meat characteristics at 42 and 84 days.[a,b], $P<0.05$.

PRACTICAL RESULTS FOR FARMERS

- Compared to wood shaving bedding, the poplar pellet bedding can potentially give a higher carcass weight.

- The meat of animals reared on pelleted bedding (poplar and vineyard pellets) can be softener and richer in protein.

REFERENCES

See them in the complete report D4.6 and D4.7 available at the **Organic-PLUS** website.

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