In vitro and *in vivo* investigations for the determination of the antimicrobial, antiparasitic and antiviral mechanisms of natural antimicrobial mixtures

(SUMMARY OF Ph.D. THESIS)

PhD student: **Igori Balta**

Scientific coordinators:
Prof. Adriana Criste PhD
Prof. Nicolae Corcionivoschi PhD

(SUMMARY OF Ph.D. THESIS)

Introduction

Every year foodborne zoonotic illnesses affect approximately 226 million of people. Infections caused by zoonotic microorganisms such as *Campylobacter* spp., *Listeria* spp, *Salmonella* spp., and *Clostridium* spp., including parasitic and viral zoonoses which cause the porcine reproductive and respiratory syndrome, infectious bronchitis, Newcastle disease, avian influenza, porcine, bovine coronavirus infection, as well as other parasitic diseases, are associated with high morbidity rates worldwide in both humans and animals (HELLGREN, 2019).

Campylobacter genus constitutes a group of foodborne pathogens responsible for causing self-limited gastroenteritis in humans, which sometimes requires antimicrobial treatment due to the potential for serious complications (LOPES, 2021). In the European Union, campylobacteriosis was the most frequently reported zoonosis in humans, with many confirmed cases witnessed in all major animal categories, including small ruminants, pigs, broilers, cattle and companion pets (EFSA, 2022). The bacterium is uniquely adaptable to various niches, leading to complicated gastroenteritis and, in some cases, difficult to treat due to increased resistance to certain antibiotics.

Importantly, viral infections are increasingly frequent in humans and farm animals and because of their ability to suffer genetic modifications in a relatively short period of time there is a constant need to develop novel pharmaceuticals in order to increase the efficacy of treatment (FARHADI, 2019). In strong conjunction with the pathogens, the threat of antimicrobial resistance towards pathogenic microorganisms has been evolved due to excessive and unjustified use of antibacterial substances in human and animal health, along with inappropriate measures to prevent the spread of infectious illnesses.

Unicellular protozoa of the phylum Apicomplexa, such as *Eimeria* spp., cause severe infections in livestock (coccidiosis), particularly in poultry and cattle (MORGOGLIONE, 2020). On a global scale, avian coccidiosis alone is responsible for more than $3 billion in economic losses to the poultry industry (ABDULLAHI, 2020). While other *Eimeria* spp., cause disease significant consideration of *Eimeria bovis* is warranted because of its association with severe typhlocolitis in calves (LÓPEZ-OSORIO, 2018). Taken jointly, the abuse of antibiotics overuse and developed bacterial antimicrobial resistance urges the development of novel antimicrobial alternatives to alleviate the future peril and consequences. Apart from the long pervasive decline in natural product discovery and investigation, a void in developing new classes of antibiotics has concurrently occurred.

The fledgeling field of modern biotechnology suggests sustainable natural alternatives to antibiotics. Accumulating evidence suggests that naturally occurring plant metabolites are exerting antibacterial, antiviral, antioxidant and antiparasitic properties. Natural antimicrobial formulations enriched with organic acids and plant extracts are promising candidates for successful pathogen control on-farm practices and industrial instances. In addition, these substances can be used as novel feed additives in the animal diet to benefit intestinal health, fortify the immune system, advance weight gain and counteract pathogenic microorganisms. More interesting, natural antimicrobial mixtures can be utilized as sanitising solutions for the food industries instead of chemical synthetic sanitisers responsible for many negative effects. Modern studies have shown that the mixtures of natural antimicrobials containing plant extracts and organic acids can drop the colonization or infection of the epithelium in various hosts (e.g. monogastric, ruminants and crustaceans). Several of the newly reported anti-infectivity modes of actions of natural antimicrobial mixtures originates due to their potential to strengthen the integrity of the host gastrointestinal epithelium and induce the repression of many bacterial infectivity characteristics, including motility, polysaccharides production, efflux pumps, adhesion, invasion and membrane permeability. Reduction in the expression of such virulent factors can potentially attenuate the risk and severity of the disease triggered by pathogens. However, the vast majority of biological mechanisms of how natural antimicrobials can inactivate pathogenic virulence factors are not yet deciphered. Understanding the biological mechanism by which natural antimicrobials reduce bacterial infections and reduce epithelial inflammation is essential to establish best practices for end users, and ensure

that their specific anti-bacterial effect can be consistently utilized. Exploration and characterization of natural antimicrobial complexes versus one distinct microbial agent or virus confer only a fragment of the puzzle.
For example the significance and prevalence of T6SS-positive *Campylobacter* strains responsible for gastroenteritis are commonly identified in immunocompromised patients. Afterwards, the demand to deactivate or impact the functionality of T6SS molecular machinery from animal products becomes obligatory. Alternatively, one of our recent studies concluded that antimicrobial activity of antimicrobial mixture (lactic and citric acid, citrus, oregano and grape seed extract) can disrupt the pathogenic patterns of T6SS positive *campylobacter* strains and may indicate a ulterior option as a practice to control campylobacteriosis. *Campylobacter jejuni*, *Salmonella enterica* and *Clostridium perfringens* are known for their potential to disrupt tight junctions, epithelial barriers and initiate epithelial generation and hydrogen peroxide release in both intracellular and extracellular spaces. The rise of hydrogen peroxide at the cytoplasmic level enables extracellular signal-regulated kinase (ERK) pathway accountable for the generation of pro-inflammatory cytokines. The ERK cascade is involved in pro-inflammatory episodes and is commonly interlinked with the initiation of bacterial infection. An antimicrobial mixture containing maltodextrin, citric acid, sodium citrate, malic acid, citrus and olive extract was concluded to inhibit bacterial internalisation via the restorative activity of the epithelial structures injured by the invasion of *Salmonella*, *Clostridium* and *Campylobacter* (BALTA, 2021). Moreover, the treatment of infected cells with low concentrations of novel antimicrobial mixtures induced a reduction in hydrogen peroxide production indicating an effect that preserves the intestinal redox balance after exposure to *Vibrio parahaemolyticus*, *C. jejuni, S. enterica and C. perfringens.* Similarly, antiparasitic effect was ascribed to a novel natural antimicrobial mixture which presented significant anti-cryptosporidial potential using *in vitro* approaches. The results revealed a notable decrease of *Cryptosporidium parvum* oocysts in an *ex vivo* model infection (STRATAKOS, 2017). Noteworthy, that the administration of antimicrobial mixture as a novel feed supplement has led to significantly reduced cryptosporidiosis manifestations in calves, by decreasing the expression of diarrheal incidences.
The identification of the active compounds with antiviral effects will lead to a better understanding on how natural antimicrobials inhibit viruses and diseases. Tangeretin is a polymethoxylated flavone found in citrus fruit peels

that inhibits viral entry into cells by blocking viral fusion, and citrus extracts are active against avian influenza virus (AIV), Newcastle disease virus (NDV), infectious bursal disease virus (IBDV) in different environments (KOMURA, 2019). Moreover, vaccines containing citrus derived molecules were more efficient in stimulating the immune system with fewer side effects (PENNISI, 2017). Maltodextrins are plant polysaccharides that are commonly used as food additives that when used as a vaccine nanoparticle increased activity against influenza virus (MIYAZAKI, 2017). Lactic acid also inhibited influenza A infection replication. Citric acid is another potent natural antimicrobial with anti-viral activity that inhibits the foot and mouth disease virus (FMDV) (HONG, 2015).

The solicitation for antibiotic-free food products is becoming ever more rigorous, requiring eliminating foodborne either zoonotic pathogens at different levels of the industrial food scale using interventions that keep away from antibiotics or chemical-derived products utilization. With the advances in multidisciplinary research, this could pave the way for new insights which involve studies combining different complexes of antimicrobial compounds producing a synergistic or additive action which may enhance antimicrobial potential towards pathogens, simultaneously making them more economically profitable. To clarify this hypothesis, the prevailing point of view in this field is usually accompanied by combining *in vitro* models' experiments followed by predictable *ex-vivo* and *in vivo* animal model studies for a more direct elucidation of intrinsic antimicrobial mechanisms.

Research aims and objectives

Hence, this thesis aims to elucidate the biological mechanisms by which mixtures of novel antimicrobial substances attenuate the pathogenicity of foodborne, parasitic and viral pathogens by using *in vitro* cell culture infections, animal model infection *in vivo* experiments. The practical relevance of this thesis consists in the possibility of obtaining and evaluation of novel natural antimicrobial products to improve the safety and activity in the poultry and other farm sectors. These natural products derived from plant extracts blended with organic acids are mainly designed as feed additives that can have a positive impact either on animal and on farmer. Due to the potential to reduce the pathogen colonization of the animal microbiota with agents such as *Campylobacter, Salmonella, Clostridium* as well as viral and parasitic zoonotic agents that in recent decades have caused a series of foodborne illness outbreaks accompanied by severe gastrointestinal disorders. One of our study clearly showed that antimicrobial compounds and mixtures reduced viral pathogenicity *in vitro* and boosted the efficacy of vaccination *in vivo* by boosting the immune system through a mechanism that involves SCFA production and increased MnSOD expression. Subsequently, another study based on *in vitro* and *in vivo* results demonstrate that the natural antimicrobial mixture reduced parasitic infections through mechanisms that reduced pathogen virulence and attenuated host inflammatory events.

To achieve the purpose of the thesis research, several objectives have been defined:

O1 The initial objective was to carry out an exhaustive literature review on the main effects of the natural antimicrobials against *Campylobacter* species and their similarities to *Salmonella*, *Clostridium* spp, including viral and parasitic pathogens. I was also looking into the most recent developments, either using *in vitro* and *in vivo* models, focusing on the biological mechanisms by which natural antimicrobials express their anti-pathogenic effect.

O2 The second objective was to investigate the biological mechanisms underlying the antibacterial and anti-pathogenic activity of a mixture containing natural antimicrobial substances against *Campylobacter jejuni*, *Salmonella enterica*, and *Clostridium perfringens* in canine derived Madin–Darby Canine Kidney cells (MDCK). Further, we hypothesized that natural antimicrobials could prevent bacterially-induced oxidative stress and restore

cellular structure integrity (including TJ or ZO-1 and occludin) damaged after pathogen exposure.
O3 The third objective presented in this thesis was to test *in vitro* the ability of another antimicrobial mixture to inhibit the viruses that cause infectious bronchitis (B1648), Newcastle disease (ATCC, 699), avian influenza (H9N2, ATCC, VR-1642), porcine reproductive and respiratory syndrome (ATCC, VR-2386) and bovine coronavirus (ATCC, VR-874). Secondly, using an *in vivo* broiler model, the efficacy of the mixture by supplementing it via drinking water was tested against artificial induced infectious bronchitis.
O4 The fourth objective was to investigate was to investigate, *in vitro*, the antiparasitic effect of a mixture of organic acids, to prevent the invasion of MDBK and CLEC-213 cells and subsequently to examine the ability of *E. tenella* to colonise chicken caeca *in vivo*. Following our *in vitro* results, we have also examined the impact of mixed natural antimicrobials on *E. tenella* infections in chicken broilers using a mixture of citric acid, sodium citrate, silica, malic acid, citrus extract, and olive extract.

The literature analysis of this thesis was published in a review article in (Food Control journal, ISI indexed with IF 6.652) and the results were published in three original articles (two in the Scientific Reports, the ISI indexed journal with IF 4.379, another in Gut Pathogens, ISI indexed journal with IF 5.324 and one in the International Journal of Food Microbiology, ISI indexed journal with IF 5.911). Moreover, there are other articles submitted and are in the process of under review to ISI indexed journals as well as there are three articles that await the publication to BDI indexed journals.

The thesis is structured in two parts and the first part encompasses "INTRODUCTION" and "LITERATURE REVIEW", describing our incursion into the role of natural antimicrobials and their anti-pathogenic mechanistic insights against different pathogens. Most interestingly, the first part of the original research is based on our first published manuscript. The literature review describes the most recent developments in the area by looking at the new antimicrobial interventions aiming to combat the transmission and colonisation of *Campylobacter* spp. and its commonalities with other pathogens. In this part, we are also looking into the most recent developments, both *in vitro* and *in vivo*, focusing on the biological mechanisms by which natural antimicrobials express their anti-pathogenic effect. The second part of the thesis is composed of five chapters, "Chapter II,

Chapter III, Chapter IV, Chapter V and Chapter VI" and reflects my research work in the nexus with four separated manuscripts which shows and discuss the results obtained over the course of the doctoral study.

The third chapter (Chapter III.) depicts the *in vitro* anti-infectivity and anti-inflammatory effects of an antimicrobial mixture containing maltodextrin, sodium citrate, citric acid, malic acid, citrus extract and olive extract (Auraguard) against the infections caused by *Campylobacter jejuni*, *Salmonella enterica* and *Clostridium perfringens*. Chapter III. is written in is divided into another 6 sub-chapters that covers a section of introduction and aim (3.1.), material and methods (3.2.), results (3.3.), discussion (3.4.) and conclusions (3.5.). Subchapters material and methods (3.2.) are divided into ten sub-sections (from 3.2.1. to 3.2.10.) and the results section is segregated into another six sub-chapters (from 3.4.1. to 3.4.6.). Subsequently this chapter was connected with my third published manuscript. It was established that the minimum sub-inhibitory concentrations were determined for *Campylobacter jejuni* (0.25%), *Salmonella enterica* (0.50%) and *Clostridium perfringens* (0.50%) required for the *in vitro* infection assays with MDCK cells. The antimicrobial mixture significantly reduced the virulence of all three pathogens towards MDCK cells and restored the integrity of cellular tight junctions through increased transepithelial resistance (TEER) and higher expression levels of ZO-1 (zonula occludens 1) and occludin. This study also identified the ERK (external regulated kinase) signalling pathway as a key mechanism in blocking the pro-inflammatory cytokine production (IL-1β, IL-6, IL-8, TNF-α) in infected cells. The reduction in hydrogen peroxide (H_2O_2) production and release by infected MDCK cells, in the presence of the antimicrobial mixture, was also associated with less tetrathionate formed by oxidation of thiosulphate ($p < 0.0001$). The present study describes for the first time that mixtures of natural antimicrobials can prevent the formation of substrates used by bacterial pathogens to grow and survive in anaerobic environments (e.g. tetrathionate). Moreover, we provide further insights into pathogen invasion mechanisms through the restoration of cellular structures and describe their ability to block the ERK–MAPK kinase pathway responsible for inflammatory cytokine release.

The next chapter (Chapter IV.) describes the antiviral activity of a novel mixture (AuraShield) of natural antimicrobials by using *in vitro*, and in a chicken infection model *in vivo*. Similar to the previous chapters, chapter IV

is divided into another six sub-chapters that covers a section of introduction and aim (4.1.), material and methods (4.2.), results (4.3.), discussion (4.4.) and conclusions (4.5.). Subchapters material and methods (4.2.) are divided into eleven sub-sections (from 4.2.1. to 4.2.11.) and the results section is split into another four sub-chapters (from 4.3.1. to 4.3.4.), respectively. Chapter four included the data from the fourth manuscript and have shown *in vitro*, that these antimicrobials had expressed antiviral activity against all five viruses through all phases of the infection process of the host cells. *In vivo*, the antimicrobial mixture reduced the virus load in the tracheal and lung tissue and significantly reduced the clinical signs of infection and the mortality rate in the experimental group E2 receiving AuraShield. All these effects were accompanied by a significant reduction in the levels of pro-inflammatory cytokines and an increase in IgA levels and short-chain fatty acids (SCFAs) in both the trachea and lungs. Our study demonstrated that mixtures of natural antimicrobials, such AuraShield, can prevent *in vitro* viral infection of cell cultures. Secondly, *in vivo*, the efficiency of vaccination was improved by preventing secondary viral infections through a mechanism involving significant increases in SCFA production and increased IgA levels. As a consequence, the clinical signs of secondary infections were significantly reduced resulting in recovered production performance and lower mortality rates in the experimental group E2. Suggestively this study clearly shows that antimicrobial compounds and mixtures, such as AuraShield, can reduce viral pathogenicity *in vitro* and can boost the efficacy of vaccination *in vivo* by boosting the immune system through a mechanism that involves SCFA production and increased MnSOD expression.

In the last chapter (Chapter V.) of the thesis, I describe the anti-Eimeria efficacy of the mixture (Auraguard) containing natural antimicrobials such as maltodextrin, sodium chloride, citric acid, sodium citrate, silica, malic acid, citrus extract and olive extract individually, *in vitro* and in combination, *in vivo*. Also, Chapter V. is divided into six sub-chapters that covers the section of introduction and aim (5.1.), material and methods (5.2.), results (5.3.), discussion (5.4.) and conclusions (5.5.). In addition, subchapters material and methods (5.2.) are divided into ten sub-sections (from 5.2.1. to 5.2.10.) and the results in another five sub-chapters (from 5.3.1. to 5.3.5.). Moreover, this chapter was interlinked with my last manuscript and demonstrated that antimicrobials reduced ($p < 0.05$), both singly and in combination (AG), the ability of *E. tenella* and *E. bovis* to infect MDBK and CLEC-213 epithelial cells, and the virulence reduction was similar to that of the anti-coccidial drug

Robenidine. Secondly, using an *in vivo* broiler infection model, we demonstrated that Auraguard reduced ($p = 0.001$) *E. tenella* levels in the caeca and excreted faeces, reduced inflammatory oxidative stress, improved the immune response through reduced ROS, increased Mn-SOD and SCFA levels. Levels of IgA and IgM were significantly increased in caecal tissues of broilers that received 0.5% Auraguard and were associated with improved ($p < 0.0001$) tissue lesion scores. A prophylactic approach increased the anti-parasitic effect *in vivo*, and results indicated that administration from days 0, 5, and 10 post-hatch reduced tissue lesion scores ($p < 0.0001$) and parasite excretion levels ($p = 0.002$).

General conclusions and recommendations

1. The demand for antibiotic-free food products is becoming ever more increased requiring elimination of foodborne zoonotic pathogens at different stages of food production using interventions that exclude the usage of antibiotic based products.
2. The first study describes for the first time that mixtures of natural antimicrobials can prevent the formation of substrates used by bacterial pathogens to grow and survive in anaerobic environments (e.g. tetrathionate). Moreover, we provide further insights into pathogen invasion mechanisms through restoration of cellular structures and describe their ability to block the ERK–MAPK kinase pathway responsible for inflammatory cytokine release.
3. The current study elucidates the biological mechanism that underlies the anti-pathogenic and anti-inflammatory effects of Auraguard, a mixture of natural antimicrobials. We found that this type of natural antimicrobial mixtures inhibited bacterial internalisation through the restoration of epithelial structures damaged by invasion. Secondly, they act via an anti-inflammatory mechanism involving the deactivation of the ERK signalling pathway through de-phosphorylation. We also show that these mechanisms are similar across pathogenic species as the effects were observed in the case of *C. jejuni*, *S. enterica* and *C. perfringens* infections.
4. Our study demonstrated that mixtures of natural antimicrobials, such as AuraShield can prevent *in vitro* viral infection of cell cultures.
5. Secondly, *in vivo*, the efficiency of vaccination was improved by preventing secondary viral infections through a mechanism involving significant increases in SCFA production and increased IgA levels. As a consequence the clinical signs of secondary infections were significantly reduced resulting in recovered production performance and lower mortality rates in the experimental group E2.
6. The present study demonstrated that mixtures of organic acids reduced the *in vitro* sporulation of *E. tenella* and *E. bovis*. Results suggest that mixtures of natural antimicrobials can: modulate the host immune response, reduce parasite-induced host oxidative events, and alleviate clinical signs and growth inhibition associated with coccidiosis.
7. Thus, these data indicate that antimicrobial mixtures can potentially have a significant impact on the immune responses of broilers and could be considered as an efficient intervention at the farm level, but that each phytochemical may have a unique mode of action.

References

1. Abdullahi, A. Y., et al., Effects of Dietary Supplement of Organic Acids Induced Protective Immunity against Coccidiosis. Iranian Journal of Applied Animal Science, (2020), 10(1), 119-129.
2. Agnetti, J., et al., Clinical impact of the type VI secretion system on virulence of Campylobacter species during infection. BMC Infect Dis, 2019. 19(1): p. 237.
3. Balta, I., et al., Antiviral activity of a novel mixture of natural antimicrobials, in vitro, and in a chicken infection model in vivo. Scientific Reports, 2020. 10(1): p. 16631.
4. Balta, I., et al., Mixtures of natural antimicrobials can reduce Campylobacter jejuni, Salmonella enterica and Clostridium perfringens infections and cellular inflammatory response in MDCK cells. Gut Pathogens, 2021. 13(1): p. 37.
5. Balta, I., et al., The effect of natural antimicrobials against Campylobacter spp. and its similarities to Salmonella spp, Listeria spp., Escherichia coli, Vibrio spp., Clostridium spp. and Staphylococcus spp. Food Control, 2021. 121: p. 107745.
6. Balta, I., et al., The effect of natural antimicrobials on the Campylobacter coli T6SS+/− during in vitro infection assays and on their ability to adhere to chicken skin and carcasses. International Journal of Food Microbiology, 2021. 338: p. 108998.
7. Balta, I., et al., The in vitro and in vivo anti-virulent effect of organic acid mixtures against *Eimeria tenella* and *Eimeria bovis*. Scientific Reports, 2021. 11: 16202.
8. Ch Stratakos, A., et al., The in vitro and ex vivo effect of Auranta 3001 in preventing Cryptosporidium hominis and Cryptosporidium parvum infection. Gut Pathogens, 2017. 9(1).
9. Corcionivoschi, N., et al., Mucosal Reactive Oxygen Species Decrease Virulence by Disrupting Campylobacter jejuni Phosphotyrosine Signaling. Cell Host & Microbe, 2012. 12(1): p. 47-59.
10. Durand, G.A., et al., Antibiotic discovery: history, methods and perspectives. Int J Antimicrob Agents, 2019. 53(4): p. 371-382.
11. EFSA. (2022). The European Union One Health 2021 Zoonoses Report. EFSA Journal, 20(12), e07666.
12. Farhadi, F., et al., Antibacterial activity of flavonoids and their structure–activity relationship: An update review. Phytotherapy Research, 2019. 33(1): p. 13-40.
13. Hellgren, J., et al., Occurrence of Salmonella, Campylobacter, Clostridium and Enterobacteriaceae in raw meat-based diets for dogs. Veterinary Record, 2019. 184(14).
14. Hong, J.-K., et al., Inactivation of Foot-and-Mouth Disease Virus by Citric Acid and Sodium Carbonate with Deicers. Applied and Environmental Microbiology, 81(21), 2015, 7610-7614. https://doi.org/10.1128/AEM.01673-15
15. Kelly, C., et al., The In Vitro and In Vivo Effect of Carvacrol in Preventing Campylobacter Infection, Colonization and in Improving Productivity of Chicken Broilers. Foodborne Pathogens and Disease, 2017. 14(6): p. 341-349.

16. Komura, M., et al., Inhibitory effect of grapefruit seed extract (GSE) on avian pathogens. Journal of Veterinary Medical Science, 2019.
17. Lopes, G. V., et, Virulence factors of foodborne pathogen Campylobacter jejuni. Microbial Pathogenesis, 2021, 161, 105265.
18. López-Osorio, S., et al., Concomitant in vitro development of Eimeria zuernii- and Eimeria bovis-macromeronts in primary host endothelial cells. Parasitology International, 67(6), 2020, 742-750. https://doi.org/https://doi.org/10.1016/j.parint.2018.07.009
19. Miyazaki, T., Protective effects of lactic acid bacteria on influenza A virus infection. AIMS Allergy and Immunology, 2017. 1: p. 138-142.
20. Miyazato, S., et al., Continuous intake of resistant maltodextrin enhanced intestinal immune response through changes in the intestinal environment in mice. Bioscience of Microbiota, Food and Health, 2015.
21. Morgoglione, M. E., et al., A 10-Year Surveillance of Eimeria spp. in Cattle and Buffaloes in a Mediterranean Area [Original Research]. Frontiers in veterinary science, 7(410). https://doi.org/10.3389/fvets.2020.00410
22. Pennisi, M., et al., Combining agent based-models and virtual screening techniques to predict the best citrus-derived vaccine adjuvants against human papilloma virus. BMC Bioinformatics, 18(16), 2017, 544. https://doi.org/10.1186/s12859-017-1961-9
23. Pinkerton, L., et al., Attenuation of Vibrio parahaemolyticus Virulence Factors by a Mixture of Natural Antimicrobials. Microorganisms, 2019. 7(12): p. 679.
24. Sima, F., et al., A Novel Natural Antimicrobial Can Reduce the in vitro and in vivo Pathogenicity of T6SS Positive Campylobacter jejuni and Campylobacter coli Chicken Isolates. Frontiers in Microbiology, 2018. 9(2139).
25. Sima, F., et al., The effect of an antimicrobial mixture on Cryptosporidium. AgroLife Scientific Journal, 2019. 8(1): p. 227-232.
26. Stratakos, A.C., et al., In vitro and in vivo characterisation of Listeria monocytogenes outbreak isolates. Food Control, 2020. 107: p. 106784.
27. Stratakos, A.C., et al., The Antimicrobial Effect of a Commercial Mixture of Natural Antimicrobials Against Escherichia coli O157:H7. Foodborne Pathogens and Disease, 2018. 16(2): p. 119-129.
28. Tang, K., et al., Tangeretin, an extract from Citrus peels, blocks cellular entry of arenaviruses that cause viral hemorrhagic fever. Antiviral Research, 2018. 160: p. 87-93.
29. World Health, O., WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. 2015, Geneva: World Health Organization.