Assessment of the antibacterial activity of chestnut (Castanea sativa) and cloves (Syzygium aromaticum) herbal extracts as an alternative to antibiotics use during post-hatching period of chicks

Rafał Kędzia[a,b], Marcin W. Lis[a,*]

[a] *Veterinary Clinic "AVI-MEDVET", ul Lelewela 16, 43-300, Bielsko-Biała, Poland*
[b] *Department of Zoology and Animal Welfare, University of Agriculture in Krakow, al. Mickiewicza 21, 31-120 Kraków, Poland*

Article history
Received: 12 December 2020
Received in revised form:
12 January 2021
Accepted: 14 January 2021
Available online:
16 January 2021

Abstract
Bacterial infections of newly hatched chicks are the most common cause of their death in the initial period of rearing. These infections are always treated with antibiotics.
The aim of the study was to investigate the antimicrobial activity of herbal extracts of chestnut (*Castanea sativa*) and clove (*Syzygium aromaticum*) against bacterial infections i.e. *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumonice* in comparison to antibiotics. The results of the microbiological analyses showed that the *Castanea sativa* and *Syzygium aromaticum* extracts had a slighter antibacterial activity in comparison to antibiotics. The diameter of zone inhibition of the culture's growth of gram-negative bacteria (i.e. *Escherichia coli* and *Klebsiella pneumoniae*) and gram-positive bacteria (i.e. *Staphylococcus aureus* and *Enterococcus faecalis*) was 6–13 mm for these extracts in comparison to 15–30 mm for antibiotics. However, some bacterial strains presented full resistance to the selected antibiotics, e.g., wild strains of *Enterobacteriaceae* to amoxicillin or *Staphylococcus aureus* and *Enterococcus faecalis* to florfenicol, colistin, and doxycycline.
In the second experiment, the effect of the herbal extract mixture added into drinking water on the growth and mortality of chicken broiler during the first rearing week was investigated. There was found that the use of herbal extracts improved the chickens' body weight (157.4 g; $P \leq 0.008$) and decreased mortality rate (2.4%) compared to the control group (144.1 g and 3.9%, respectively) but not to the group treated with antibiotic (161.5 and 0.6% respectively; $P \leq 0.009$).
In summary, the use of herbal extracts as a nutritional supplement for poultry seems to have a positive effect on weight gain of young birds, and to some extent reduce mortality in the first week of rearing.

Keywords: antibiotic resistance, herbal extracts, broiler chicken, mortality

Introduction

The quality and health of day-old chicks are crucial factors influencing the course of rearing and successful poultry production. Therefore, the high environmental and hygienic standards in parental flock and hatchery but also during chicks' transportation and brooding should be strictly required. Nevertheless, in poultry industry, inflammation of the umbilicus (*omphalitis*) and/or yolk sac infection remains the main cause of mortality for hatchlings during the first week of life [1, 2].

*Corresponding author: rzlis@cyfronet.pl

The etiological factor of these diseases are primarily bacteria *Escherichia coli*, but also *Enterococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Klebsiella* spp., but microbiological picture and domination of particular species of microbes may be varied in individual cases [3–5]. This high microbiological diversity leads to omphalitis and yolk sac infections being very difficult to treat and usually require involving antibiotics and/or chemotherapeutics [2, 5].

The widespread, sometimes indiscriminate, use of these drugs results in the selection of bacteria which are inherently resistant. Not only may these resistant bacteria become the predominant species in a population but they may also transfer genetic material to susceptible bacteria which then acquire resistance. The spread of antibiotic resistances through the food chain remains a relevant question for both scientists and public health operators [6–8]. Some poultry producers remain in favour of preventive treatment of setted chicks, especially since antibiotics and chemotherapeutics administration improves the growth and feed efficiency and decreases variability of flock [9]. Such proceeding should be unjustified because the prevalence of antibiotics in the food chain results in microbes' resistance to antibacterial drugs [6, 8]. In this situation, it seems necessary to develop an alternative therapy using, for example, phytobiotics [7, 10].

Herbs are still an underappreciated potential source of anti-infective natural products in modern human and veterinary medicine. However, mankind has a long and rich history of medical traditions involving herbs in various traditional pharmacopoeia [11]. The abundance and diversity of biologically active compounds found in medicinal herbs affects their broad spectrum of antibacterial activity [12].

Some examples of herbs commonly used in traditional medicine and cuisine are: European chestnut (*Castanea sativa*) [13] and Cloves Java plum (jambolan, *Syzygium aromaticum*) [11, 14]. The extracts of different parts (leaves, seeds, buds) of these herbs demontstrate i.a. antioxidant, digestive, antihyperglycaemic, antihelminthic and antibacterial and antiviral effect. These properties are due to different chemical constituents, in particular, alkaloids, flavonoids, phenolics and polyphenolics (tannins) [11, 13–15]. Tannins and terpenes contained in these herbs exhibit a wide antimicrobial spectrum, including acting against pathogenic fungi, gram-positive (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae*) [16–19].

The various herbal extracts and essential oils as diet supplements and/or a replacement for antibiotics are increasingly used in the industry of poultry production [20–22]. The extracts of *Castanea sativa* and *Syzygium aromaticum* were also tested to improve eggs [23] and carcass quality [13, 24–25] and broiler gain [25].

Therefore, it was interesting to study the antimicrobial activity against most common infections in poultry of chestnut (*Castanea sativa*) and cloves (*Syzygium aromaticum*) extracts and their effect on mortality and body weight in the first week of chick rearing.

Material and Methods

Chemicals

The *Castanea sativa* (45%) and *Syzygium aromaticum* (37%) water extracts and their mixtures used in experiments were prepared by dr. Rafał Korzewski (Centaur Ltd, Poland).

Experiment 1. The in vitro test antimicrobial activity of Castanea sativa and Syzygium aromaticum water extracts

The antimicrobial test of *Castanea sativa* and *Syzygium aromaticum* extracts was performed according to the recommendations of the National Committee for Clinical Laboratory Standards (2013) and the guidelines of the Clinical and Laboratory Standard Institute (CLSI, M100-S23, 2013). American Type Collection Culture (ATCC) reference strains and the wild strains bacteria from collection of the Veterinary Clinic "AVI-MEDVET", Bielsko-Biała, POLAND were used, i.e.: 1) gram negative *Enterobacteriaceae* i.e.: *Escherichia coli* (one reference strain ATCC 25922 and two wild strains isolated from 3-day-old and 23-day-old chicken broilers) and *Klebsiella pneumoniae* (wild strain isolated from 3-day-old chicken broilers); and 2) gram positive bacteria: *Staphylococcus aureus* (AATCC 25923) and *Enterococcus faecalis* (ATCC 29212). All the strains were preserved under deep freeze before the experiments. The inoculations for the experiments were harvested from a few of the same colonies of each species, originating from fresh overnight cultures.

The tested bacteria cultures were cultivated on plates with medium Columbia agar + 5% sheep blood (Graso Biotech Ltd, Poland) for 24 h at 37° C. The types of cultivated colonies were confirmed with the following methods: *Enterobacteriaceae* bacteria by the analytical profile index (bio-Merieux Poland Ltd.); *Enterococcus spp*. by Wellcogen Strep B latex agglutination assay (Oxoid Ltd, UK) and next serotyped to *Enterococcus faecalis* and *Enterococcus faecium* with the use of diagnostic disc type EF (IBSS BIOMED JTC, POLAND); and Staphylococcus aureus by Staphaurex® Plus Rapid latex agglutination test (Oxoid Ltd, UK).

The antibacterial activity of *Castanea sativa* and *Syzygium aromaticumtanin* water extracts was tested by the Kirby–Bauer disk diffusion test [26] and compared with bacterial antibiotic susceptibility determined with the use of the commercial diffusion disks type MASTDISCS™ (Mast Diagnostica Ltd., Germany) dedicated for antibiotics i.e.: amoxicillin (AMX), amoxicillin with clavulanic acid (AMC), enrofloxacin (ENR), florfenicol (FFC) colistin (Col), gentamicin (GM), trimethoprim with sulfamethoxazole (SXT) and doxycycline (D). The base extracts were diluted with sterile water (Aqua pro Injectione, Polpharma) 10-, 20-, 40-, 80-, 160- and 320-fold and 10 ml of each of the solutions was spotted on sterile diagnostic disc (Oxoid Ltd, UK).

All experimental and control discs (in 10 replications) were placed on Oxoid's Mueller-Hinton II Agar plates (Oxoid Ltd, UK) with bacterial colonies and incubated at 35°C for 24 h.

The antibacterial activity of the tested substance was evaluated based on the diameter of the transparent zone in the medium around the diffusion disc.

Experiment 2. The effect of Castanea sativa and Syzygium aromaticum on broiler chickens rearing in the first week of life

The broiler chicks (n = 2100 chicks) (ROSS 308, Aviagen) were bought from a commercial hatchery (ZWD Wolbrom, Poland). Chicks were randomly divided into three groups (n = 700 chicks per group, female and male mix) and placed in rearing boxes (n = 100 chicks/box; seven boxes per group). The chicks were housed on a wheat straw litter floor, *ad libitum* water and feed (Starter feed, De Haus) and environmental condition according with broiler line producer [27].

From the second to seventh day of rearing, the chicks were given, for inhibition of post-hatching infection: 1) mixture (one to one) of Centaur clove and horse chestnut extract at a dose of 5 ml/L water (i.e. 200-fold dilution); 10% enrofloxacin (according to the antibiogram, Tab.1 and 2) at a dose of 0.5 ml / L. The control group received pure water. On the seventh day of rearing, all chickens were weighed with an accuracy of one gram and the mortality rate was determined.

Table 1. Susceptibility gram negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) on *Castanea sativa* or *Syzygium aromaticum* in comparison to selected antibiotics, i.e., amoxicillin (AMX), amoxicillin with clavulanic acid (AMC), enrofloxacin (ENR), florfenicol (FFC); colistin (Col), gentamicin (GM), trimethoprim with sulfamethoxazole (SXT) and doxycycline (D). The results of antibacterial activity of the tested substance are shown as the diameter of the transparent zone in the medium around the diffusion disc [mm].

Bacteria	Antibiotic								Herbs extract	Extract diliution [-fold]					
	AMX	AMC	ENR	FFC	Col	GM	SXT	D		10	20	40	80	160	320
Escherichia coli (ATCC 25922)	18	20	30	25	12	21	25	19	*Castanea sativa*	6	6	6	6	6	6
									Syzygium aromaticumtaniny	6	6	6	6	6	6
Escherichia coli (wild – 6 day old chicken)	0	23	29	25	14	22	22	0	*Castanea sativa*	6	6	6	6	6	6
									Syzygium aromaticumtaniny	6	6	6	6	6	6
Escherichia coli (wild – 23 day old chicken)	0	6	12	22	15	23	25	0	*Castanea sativa*	6	6	6	6	6	6
									Syzygium aromaticumtaniny	6	6	6	6	6	6
Klebsiella pneumoniae (wild – 6 day old chicken)	0	20	10	0	11	22	23	15	*Castanea sativa*	6	6	6	6	6	6
									Syzygium aromaticumtaniny	6	6	6	6	6	6

Table 2. Susceptibility positive bacteria: *Staphylococcus aureus* and *Enterococcus faecalis* on *Castanea sativa* or *Syzygium aromaticum* in comparison to selected antibiotics, i.e., amoxicillin (AMX), amoxicillin with clavulanic acid (AMC), enrofloxacin (ENR), florfenicol (FFC) (Col), gentamicin (GM), trimethoprim with sulfamethoxazole (SXT) and doxycycline (D). The results antibacterial activity of the tested substance are shown as the diameter of the transparent zone in the medium around the diffusion disc [mm].

Bacteria	Antibiotic								Herbs extract	Extract diliution [-fold]					
	AMX	AMC	ENR	FFC	Col	GM	SXT	D		10	20	40	80	160	320
Enterococcus faecalis (ATCC 29212)	18	0	0	0	0	15	0	0	*Castanea sativa*	6	6	6	6	6	6
									Syzygium aromaticumtaniny	6	6	6	6	6	6
Staphylococcus aureus (ATCC 25923)	28	30	25	0	0	23	28	0	*Castanea sativa*	13	9	6	6	6	6
									Syzygium aromaticumtaniny	6	6	6	6	6	6

Table 3. Body weight of chicken broiler Ross 308 (mean ± SD) at 7th day of rearing, which were applicated for inhibition of post-hatching infection, by: 1) pure water (control); 2) herbal extract of *Castanea sativa* or Syzygium aromaticum (mixture in proportion one to one) at a dose of 5 ml/L water; 3) antibiotic 10% enrofloxacin (according to the antibiogram, Tab. 1 and 2) at a dose of 0.5 ml/L.

Repetition	Pure water			Herbal extract			Antibiotic		
	N	chicken body weight [g]		N	chicken body weight [g]		N	chicken body weight [g]	
1	97	142.0 ± 11.30		98	141.1 ± 28.16		100	145.4 ± 28.01	
2	93	145.5 ± 18.15	A	98	175.1 ± 20.22	B	99	169.1 ± 21.94	B
3	98	145.0 ± 21.27	A	98	155.5 ± 20.09	B	100	176.1 ± 22.43	C
4	95	146.0 ± 18.20	A	98	150.0 ± 21.31	A	99	154.9 ± 25.18	B
5	98	145.5 ± 19.04	A	97	154.6 ± 15.85	B	100	166.2 ± 23.99	C
6	96	143.2 ± 22.14	A	97	167.7 ± 15.30	B	98	171.1 ± 18.52	B
7	96	141.8 ± 18.83	A	98	157.8 ± 18.69	B	100	147.7 ± 15.97	A
Total	**673**	**144.1 ± 18.69**	**A**	**684**	**157.4 ± 22.76**	**B**	**696**	**161.5 ± 25.16**	C

ABC –values marked with the various capital letters differ highly significantly (P ≤ 0.01)

Table 4. Mortality rate of chicken broiler Ross 308 (mean ± SD) at 7th day of rearing, which were applicated for inhibition of post-hatching infection, by: 1) pure water (control); 2) herbal extract of Castanea sativa or Syzygium aromaticum (mixture in proportion one to one) at a dose of 5 ml/L water; 3) antibiotic 10% enrofloxacin (according to the antibiogram, Tab. 1 and 2) at a dose of 0.5 ml/L.

Repetition	Control group (pure water)				Herbal extract				Enrofloxacyne			
	Group size	Dead chicken			Group size	Dead chicken			Group size	Dead chicken		
	N	N	[%]		n	N	[%]		N	n	[%]	
1	100	3	3.00		100	2	2.00		100	0	0.00	
2	100	7	7.00		100	2	2.00		100	1	1.00	
3	100	2	2.00		100	2	2.00		100	0	0.00	
4	100	5	5.00		100	2	2.00		100	1	1.00	
5	100	2	2.00		100	3	3.00		100	0	0.00	
6	100	4	4.00		100	3	3.00		100	2	2.00	
7	100	4	4.00		100	2	2.00		100	0	0.00	
Total	**700**	**27**	**3.86**	**Bc**	**700**	**16**	**2.29**	**b**	**700**	**4**	**0.57**	Aa

ABC – values marked with the various capital letters differ highly significantly (P ≤ 0.01)
abc – values marked with the various small letters differ highly significantly (P ≤ 0.01)

Statistical analysis

Statistical analysis was performed by One Way ANOVA and Tukey post hoc test, using a program SigmaStat 3.5. (Systat Software, Inc USA)

Results and Discussion

The results of the microbiological analyses showed that the *Castanea sativa* and *Syzygium aromaticum* extracts had a slighter antibacterial activity in comparison to antibiotics. The diameter of zone inhibition of the culture's growth of gram-negative bacteria (i.e. *Escherichia coli* and *Klebsiella pneumonia,* table 1) and gram-positive bacteria (i.e. *Staphylococcus aureus* and *Enterococcus faecalis,* table 2) was 6–13 mm for these extracts in comparison to 15–30 mm for antibiotics. However, some bacterial strains presented full resistance to the selected antibiotics, e.g., wild strains of *Enterobacteriaceae* to amoxicillin (Tab. 1) or Staphylococcus aureus and *Enterococcus faecalis* to florfenicol, colistin, and doxycycline (Tab. 2)

Diverse antibacterial influence of clove oil or chestnut extract on *Escherichia coli* was obtained by many authors [19, 28–30]. The observed resistance of Gram-negative bacteria to the action of tannins may be related to the presence of liposaccharide compounds in the cell walls [31–32]. However, gram positive bacteria seem more sensitive to the action of herbal extracts. The cell walls of this bacteria type consists mostly of peptidoglycans, which allow hydrophobic molecules to penetrate it easily. Therefore, the phenolic compounds contained in herbal extracts can interfere with the activity of enzymes involved in energy production at lower concentrations, or lead to protein denaturation at higher concentrations [33].

The use of herbal extracts in the mixture of *Castanea sativa* and *Syzygium aromaticum* in drinking water during the first week of chick rearing allowed to achieve chicken body weight higher by 13.3 g (9.2%; $P \leq 0.008$) compared to the control group (but lower 4.1 g (2.6%; $P \leq 0.005$) than antibiotic treated ones (Tab. 3). Similar results were obtained by Schiavone [24] for horse chestnut extract and by Hajati et al. [34] for clove. The mechanisms by which antimicrobial substances improve chicken growth are not fully understood. They are probably influenced by the reduction of: pathogenic bacteria present in the intestines, e.g. *Escherichia coli* and *Clostridium perfingers*, subclinical infections and toxins. Moreover, herbal extracts can enrich a feed aroma and taste, which is attractive to animals and strengthens the appetite. The exogenous enzymes contained in the extracts improve the digestibility of nutrients. The active ingredients of the extracts stabilize the gastrointestinal microflora through the growth of probiotic bacteria, e.g., lactic acid bacteria, and consequently affects the intestinal environment and the development of the immune system [7, 35–36]. Moreover, they influence the increased secretion of mucus by the intestinal epithelium, hindering the adhesion of pathogens [10, 35, 37].

It our study, herbal extracts administrated to drinking water significantly reduced, by 41% (1.57 pp), chicken mortality rate in the first week of rearing compared to the control group ($P \leq 0.034$). However, this index was still significantly higher (four-fold) than in the groups treated with antibiotics ($P \leq 0.009$). In both cases, the reduction of chicken death cases was associated with the decrease of after-hatching infections, the main cause of which are pathogenic strains of *Escherichia coli* [38]. As could be expected, the antibiotic enrofloxacin, used in accordance with the antibiotic profile, successfully inhibited the development of pathogens. On the other hand, the positive effect of herbal extracts on the survival of chickens was related to the natural stabilization of the bacterial flora and the increase of general immunity [39], which was described above. Similar *in vivo* reduction of post-hatching infections caused by *Escherichia coli* with the use of *Castanea sativa* extract was confirmed by Bole-Hribovsek et al. [21].

Conclusions

In summary, the use of herbal extracts as nutritional supplements for poultry seems to have a positive effect on weight gain of young birds, and to some extent on reducing mortality in the first week of rearing. Further research should be continued to describe their effect on the gastrointestinal microflora and the pharmacokinetics and pharmacodynamics of active compounds in the body of birds, thus limiting the use of antibiotics.

Acknowledgements

This research was supported by the Ministry of Science and Higher Education of the Republic of Poland (Subject no 215-DZ06, University of Agriculture in Krakow). Acknowledgments to Ms. Katarzyna Cullen for professional British English proofreading of the manuscript.

References

1. Deeming DC, Clyburn V, Williams K, Dixon RA. In ovo microbial contamination of the yolk sac of unhatched broiler and pheasant embryos. Avian Poultry Biology Reviews. 2002;13(4):240–241.
2. Lister SA, Barrow P. Yolk sac infection. In: Pattison M, McMullin PF, Bradbury JM, Alexander DJ, editors. Poultry disease [= Choroby drobiu]. 6th ed. 2008. 1 st Polish ed. Gajewski M, Łukuć P, translators. Wrocław: Elsevier Urban & Partner. 2011.
3. Rosario CC, Téllez IG, López CC, Villaseca FJM, Anderson RC, Eslava CC. Bacterial isolation rate from fertile eggs, hatching eggs, and neonatal broilers with yolk sac infection. Revista Latinoamericana de Microbiología. 2004;(46(1-2):12–16.
4. Ulmer Franco AM, Yolk sac infections in broiler chicks: studies on Escherichia coli, chick acquired immunity and barn microbiology [doctoral dissertation]. Alberta: University of Alberta Edmonton; 2011. doi: https://doi.org/10.7939/R3WH37.
5. Jahantigh M, Rashki A, Najimi M. A study on bacterial flora and antibacterial resistance of yolk sac infection in Japanese quail (Coturnix japonica). Comparative Clinical Pathology. 2012;22:645–648. doi: https://doi.org/10.1007/s00580-012-1459-9.

6. Nhung NT, Chansiripornchai N, Carrique-Mas JJ. Antimicrobial resistance in bacterial poultry pathogens: a review. Frontiers in Veterinary Science. 2017;4:126. doi: https://doi.org/10.3389/fvets.2017.00126.
7. Siwek M, Slawinska A, Stadnicka K, Bogucka J, Dunislawska A, Bednarczyk M. Prebiotics and synbiotics – in ovo delivery for improved lifespan condition in chicken. BMC Veterinary Research. 2018;14(1):402. doi: https://doi.org/10.1186/s12917-018-1738-z.
8. Velhner M, Todorović D, Grego E, Cerar Kišek T, Ljubojević D, Cvitković Špik V, Pajić M, Kozoderović G. Characterisation of multidrug resistant Escherichia coli from poultry litter and poultry carrying virulence genes for evaluation of poultry farm management. European Poultry Science. 2020;84. doi: https://doi.org/10.1399/eps.2020.296.
9. Parks CW, Grimes JL, Ferret PR. Effects of virginiamycin and a mannanoligosaccharide-virginiamycin shuttle program on the growth and performance of large white female turkeys. Poultry Science. 2005;84(12):1967–1973. doi: https://doi.org/10.1093/ps/84.12.1967.
10. Windisch W, Schedle K, Plitzner C, Kroismayr A. Use of phytogenic products as feed additives for swine and poultry. Journal of Animal Science. 2008;86(14 Suppl.):E140–148. doi: https://doi.org/10.2527/jas.2007-0459.
11. Quave CL, Lyles JT, Kavanaugh JS, Nelson K, Parlet CP, Crosby HA, Heilmann KP, Horswill AR. Castanea sativa (European chestnut) leaf extracts rich in ursene and oleanene derivatives block Staphylococcus aureus virulence and pathogenesis without detectable resistance. PLoS ONE. 2015;10:e0136486. doi: https://doi.org/10.1371/journal.pone.0136486.
12. Adamczak A, Ożarowski M, Karpiński TM. Antibacterial activity of some flavonoids and organic acids widely distributed in plants. Journal of Clinical Medicine. 2019;9(1):109. doi: https://doi.org/10.3390/jcm9010109.
13. De Vasconcelos, MCBM, Bennett RN, Rosa EAS, Ferreira-Cordoso JV. Composition of European chestnut (Castanea sativa Mill.) and association with health effects: fresh and processed products. Journal of the Science of Food and Agriculture. 2010;90(10):1578–1589. doi: https://doi.org/10.1002/jsfa.4016.
14. Srivastava S, Chandra D. Pharmacological potentials of Syzygium cumini: a review. Journal of the Science of Foodand Agriculture. 2013;93(9):2084–2093. doi: https://doi.org/10.1002/jsfa.6111.
15. Lupini C, Cecchinato M, Scagliarini A, Graziani R, Catelli E. In vitro antiviral activity of chestnut and quebracho woods extracts against avian reovirus and metapneumovirus. Research in Veterinary Science. 2009;87(3):482–487. doi: https://doi.org/10.1016/j.rvsc.2009.04.007.
16. Redondo LM, Chacana A, Dominguez JE, Fernandez Miyakawa ME. Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. Frontiers in Microbiology. 2014;5:118. doi: https://doi.org/10.3389/fmicb.2014.00118.
17. Semeniuc CA, Pop CR, Rotar AM. Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. Journal of Food and Drugs Analysis. 2017;25(2):403-408. doi: https://doi.org/10.1016/j.jfda.2016.06.002.
18. Kaczmarek B. Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials – a minireview. Materials. 2020;13(14):3224. doi: https://doi.org/10.3390/ma13143224.
19. Štumpf S, Hostnik G, Primožič M, Leitgeb M, Salminen J-P, Bren U. The effect of growth medium strength on minimum inhibitory concentrations of tannins and tannin extracts against E. coli. Molecules. 2020;25(12):2947. doi: https://doi.org/10.3390/molecules25122947.
20. Mueller-Harvey I, 2006: Unravelling the conundrum of tannins in animal nutrition and health. Journal of Science of Food and Agriculture. 2006;86(13):2010–2037. doi: https://doi.org/10.1002/jsfa.2577.
21. Bole-Hribovsek V, Drobnic-Kosorek M, Ponebsek J, Moran D, Hooge DM. 2012: Minimum inhibitory concentrations of Farmatan® dried all-natural tannic acid (flavoring) feed additive on pathogenic strains of Clostridium perfringens and Escherichia coli plus isolates of four Salmonella species in vitro. Poultry Science Association Annual Meeting. Athens, GA, 2012 July 9–12, P375.
22. Erfan AM, Marouf S. Cinnamon oil downregulates virulence genes of poultry respiratory bacterial agents and revealed significant bacterial inhibition: an in vitro perspective. Veterinary World. 2019;12(11),1707–1715. doi: https://doi.org/10.14202/vetworld.2019.1707-1715.
23. Minieri S, Buccioni A, Serra A, Galigani I, Pezzati A, Rapaccini S, Antongiovanni M. Nutritional characteristics and quality of eggs from laying hens fed on a diet supplemented with chestnut tannin extract (Castanea sativa Miller). British Poultry Science. 2016;57(6):824–833. doi: https://doi.org/10.1080/00071668.2016.1216944.
24. Schiavone A, Guo K, Tassone S, Gasco L, Hernandez E, Denti R, Zoccarato I. Effects of a natural extract of chestnut wood on digestibility, performance traits, and nitrogen

balance of broiler chicks. Poultry Science. 2008;87(3):521–527. doi: https://doi.org/10.3382/ps.2007-00113.

25. Basmacioğlu-Malayoğlu H, Özdemir P, Bağriyanik HA. Influence of an organic acid blend and essential oil blend, individually or in combination, on growth performance, carcass parameters, apparent digestibility, intestinal microflora and intestinal morphology of broilers. British Poultry Science. 2016:57(2):227–234. doi: https://doi.org/10.1080/00071668.2016.1141171.
26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology; 2009. https://www.asmscience.org/content/education/protocol/protocol.3189?crawler=true.
27. Aviagen. Ross broiler management handbook 2018. Aviagen [Internet]. 2018 [cited 2020 August 22]. Available from: *https://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerHandbook2018-EN.pdf.*
28. Nuñez L, D'Aquino M. Microbicide activity of clove essential oil (Eugenia caryophyllata). Brazilian Journal of Microbiology. 2012;43(4):1255–1260. doi: https://doi.org/10.1590/S1517-83822012000400003.
29. Hu Q, Zhou M, Wei S. Progress on the antimicrobial activity research of clove oil and eugenol in the food antisepsis field. Journal of Food Science. 2018;83(6):1476–1483. doi: https://doi.org/10.1111/1750-3841.14180.
30. Kunicka-Styczyńska A, Tyfa A, Laskowski D, Plucińska A, Rajkowska K, Kowal K. Clove oil (Syzygium aromaticum L.) Activity against Alicyclobacillus acidoterrestris biofilm on technical surfaces. Molecules. 2020;25(15):3334. doi: https://doi.org/10.3390/molecules25153334.
31. Ikigai H, Nakae T, Hara Y, Shimamura T. Bactericidal catechins damage the lipid bilayer. Biochimica et Biophysica Acta (BBA) – Biomembranes. 1993;1147(1):132–136. doi: https://doi.org/10.1016/0005-2736(93)90323-R.
32. Caturla N, Vera-Samper E, Villalaín J, Mateo CR, Micol V. The relationship between the antioxidant and the antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. Free Radical Biology and Medicine. 2003;34(6):648–662. doi: https://doi.org/10.1016/s0891-5849(02)01366-7.
33. Yang C, Chowdhury MAK, Hou Y, Gong J. Phytogenic compounds as alternatives to in-feed antibiotics: potentials and challenges in application. Pathogens. 2015;4(1):137–156. doi: https://doi.org/10.3390/pathogens4010137.
34. Hajati H, Hassanabadi A, Ahmadian F. Application of medicinal plants in poultry nutrition. Journal of Medicinal Plants and By-products. 2014;3(1):1–12. doi: https://doi.org/10.22092/jmpb.2014.108597.
35. Jamroz D, Wiliczkiewicz A, Skorupińska J, Orda J, Kuryszko J, Tschirch H. Effect of sweet chestnut tannin (SCT) on the performance, microbial status of intestine and histological characteristics of intestine wall in chickens. British Poultry Science. 2009;50(6):687–699. https://doi.org/10.1080/00071660903191059.
36. Redondo, LM, Chacana, PA, Dominguez, JE, Fernandez Miyakawa, MED. Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. Frontiers in Microbiology, 2014;5:118. https://doi.org/10.3389/fmicb.2014.00118.
37. Takó M, Kerekes EB, Zambrano C, Kotogán A, Papp T, Krisch J, Vágvölgyi C. Plant phenolics and phenolic-enriched extracts as antimicrobial agents against food-contaminating microorganisms. Antioxidants. 2020;9(2):165. doi: https://doi.org/10.3390/antiox9020165.
38. Sharma I, Yaiphathoi S, Hazarika P. Pathogenic Escherichia coli: virulence factors and their antimicrobial resistance. In: Siddhardha B, Dyavaiah M, Syed A, editors. Model organisms for microbial pathogenesis, biofilm formation and antimicrobial drug discovery. Singapore: Springer; 2020. p. 159–173. doi: https://doi.org/10.1007/978-981-15-1695-5_10.
39. Wallace RJ, Oleszek W, Franz C, Hahn I, Baser KHC, Mathe A, Teichmann K. Dietary plant bioactives for poultry health and productivity. British Poultry Science. 2010;51(4):461–487. doi: https://doi.org/10.1080/00071668.2010.506908.