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THE EFFECT OF ESSENTIAL OILS ON CHOLESTEROL CONTENT IN CHICKEN MEAT

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ABSTRACT
The study aimed to investigate cholesterol content in chicken breast and thigh muscles by the influence of feed supplements of various content of essential oils. The experiment was carried out under practical conditions in a poultry farm with broiler chickens of the Cobb 500 hybrid combination according to the feed supplement used thyme essential oil, cinnamon essential oil, commercial citrus fruit essential oil, and their combination. The control group was without the use of experimental feed supplements and commercial coccidiostats were used in their feed mixtures. The experiment lasted 40 days in welfare conditions. Broiler chickens were used for sample preparation of breast and thigh muscles with the skin and their analysis for dry matter, fat and cholesterol contents. Samples were analyzed using a Nicolet 6700 FT-IR Fourier transform infrared spectrometer. The results were processed by the SAS system program, version 8.2. The results, which were evaluated, indicated a tendency to reduce the cholesterol content in chicken breast muscle due to cinnamon essential oil and the combination of cinnamon essential oil with citrus fruit essential oil as well as thyme essential oil with citrus fruit essential. In the achieved results of dry matter, fat, and cholesterol content in breast and thigh muscles, the difference between the effects of the used feed supplements based on essential oils and concerning the control group were not statistically significant p >0.05. The correlation was a statistically significant strong linear relation only between dry matter content and fat content due to thyme and cinnamon essential oils. In conclusion, it was stated that the investigation of the feed supplement effect based on essential oils is an open question concerning the production of safe food of animal origin.

Keywords: essential oil; feed mixture; chicken thigh muscle; chicken breast muscle; cholesterol

INTRODUCTION
In recent years, epidemiological work and laboratory experiments have focused on the causes and modified factors associated with cardiovascular disease. It is generally accepted that the incidence of cardiovascular disease is closely related to the intake of cholesterol and saturated fatty acids in the diet. Therefore, great attention has been paid to the production of animal products and the modulation of animal products enriched with bioactive compounds, to improve their quality and protect consumer health against diseases **(Simitzis et al., 2011)**.

Achieving a better balance of fatty acids in the diet by reducing cholesterol and saturated fat intake is considered an effective and important strategy to reduce the incidence of cardiovascular diseases. Therefore, knowledge about the cholesterol content of foods is important. About a healthy diet, knowledge of the chemical composition of chicken and fish meat is important, because the consumption of these types of meat is increasing, even on the recommendations of nutritional experts **(Milićević et al., 2014)**.

An interesting focus of the research was realized by **Attia et al. (2017)** with broiler chickens. The results of their research showed that cholesterol metabolism in the brain and muscles are associated with the aggressive behavior of broiler chickens and with a reduction in meat quality. Liver cholesterol is an indicator of the chicken fatty liver syndrome. The fatty liver syndrome is a common metabolic disease that seriously affects the health and efficiency of broiler chicken production and causes major economic losses to the poultry industry.

Differences in cholesterol content between different muscles of the same chicken species and between the same muscles in different species are generally explained by changes in cholesterol absorption and biosynthesis, lipoprotein metabolism, distribution of muscle fiber types, genetic variations, subcutaneous and intramuscular fat **(Padre et al., 2006)**.

Meat and broiler chicken products are important worldwide in human nutrition as they address global food shortages due to their availability. In general, the energy value for chicken breast muscle is 435 kJ and for thigh muscle 481 kJ. It is similar to cholesterol. Its content is 62

Table 1 Design of a group feeding experiment on a poultry farm in the hall.

Grop	n	Pen area	Feed mixtures	Feed supplement	Content/proportion of active substance in feed and water
Control	20	1.5 m^2	Commercial: Starter	Coccidiostats: Maxiban G160	Narasin 80 mg per kg feed and Nicarbazin 80 mg per kg feed
			Growth	Coccidiostats: Sacox®	Salinomycin sodium 70 mg per kg feed
			Final	-	-
Thyme essential oil	20	1.5 m^2	Experimental: Starter	Thyme essential oil	0.05% in feed
			Growth	Thyme essential oil	0.05% in feed
			Final	Thyme essential oil	0.05% in feed
Cinnamon essential oil	20	1.5 m^2	Experimental: Starter	Cinnamon essential oil	0.05% in feed
			Growth	Cinnamon essential oil	0.05% in feed
			Final	Cinnamon essential oil	0.05% in feed
Citrus fruit essential oil	20	1.5 m^2	Experimental: Starter	Commercial: Citrus fruit essential oil	1.0 ml per L of water
			Growth	Citrus fruit essential oil	1.0 ml per L of water
			Final	Citrus fruit essential oil	1.0 ml per L of water
Thyme essential oil + Citrus fruit essential oil	20	1.5 m^2	Experimental: Starter	Thyme essential oil + Citrus fruit essential oil	0.05% in feed + 1.0 ml per L of water
			Growth	Thyme essential oil + Citrus fruit essential oil	0.05% in feed + 1.0 ml per L of water
			Final	Thyme essential oil + Citrus fruit essential oil	0.05% in feed + 1.0 ml per L of water
Cinnamon essential oil + Citrus fruit essential oil	20	1.5 m^2	Experimental: Starter	Cinnamon essential oil + Citrus fruit essential oil	0.05% in feed + 1.0 ml per L of water
			Growth	Cinnamon essential oil + Citrus fruit essential oil	0.05% in feed + 1.0 ml per L of water
			Final	Cinnamon essential oil + Citrus fruit essential oil	0.05% in feed + 1.0 ml per L of water

Note: n = number of broiler chickens in a group feeding experiment.

mg in breast muscle and 80 mg in thigh muscle **(Haščík et al., 2020)**.

Dehghani et al. (2018) reported that essential oils and some of their components can lower plasma cholesterol and triglyceride levels. This may be the result of the inhibition of the enzymes involved in the synthesis of these lipids by monoterpenes.

Essential oil extracts are a possible alternative to antibiotic growth stimulators because they are natural, readily available, non-toxic, and residue-free. Due to these properties, they are acceptable as natural feed additives for broiler chickens. Essential oils made from aromatic plants have become more interesting due to their potential effects as hypocholesterolemic, antioxidants, antimicrobials, antifungals, and digestive enzyme stimulants **(Abd El-Hack et al., 2020)**.

Jamroz et al. (2005) also demonstrated increased pancreatic and intestinal lipase activity in broilers fed diets with added plant extract.

The effect of the feed supplements with essential oils on the cholesterol content in chicken breast and tight muscles was evaluated.

Scientific hypothesis: feeding feed mixtures supplemented with thyme, cinnamon, and citrus fruit essential oils for broiler chickens lowers the cholesterol content in their meat.

MATERIAL AND METHODOLOGY

Technical implementation of the feeding experiment

The experiment will take place on a poultry farm. At the entrance gate to the hall, space was set aside for 6 groups of broiler chickens, 20 for each. Change pens small dimensions: 1.5 m wide and 1.0 m long for unrestricted movement of broiler chickens. Definitive Cobb 500 broiler chickens for fattening are used as experimental animals. The groups were marked according to the schemes (Table 1) depending on the active substance test. Commercial feed mixtures were used in control groups, commonly used in poultry farms based on wheat, corn, and soybean extracted meal. Coccidiostats, which are commonly used and form part of feed mixtures, have been used in starter and growth compound feeds. Control feed mixtures did not contain any essential oils. The same feed mixtures were used in the experimental groups as in the control group but without coccidiostats.

In the thyme group, the active substance thyme essential oil was used with a proportion of 0.05% in the feed. In the cinnamon group, the active ingredient cinnamon essential oil was used with a proportion of 0.05% in the feed. In the citrus fruit essential oil group, a commercial feed supplement made from citrus raw materials was used and applied to the water. In the experimental group thyme essential oil + citrus fruit essential oil, thyme essential oil with a proportion of 0.05% in the feed was used and citrus fruit essential oil was applied to the water of 1.0 ml.l^{-1}. In the experimental group cinnamon essential oil + citrus fruit essential oil, cinnamon silica with a proportion of 0.05% in the feed was used and citrus fruit essential oil was applied to water 1.0 ml.l^{-1}.

Essential oils were first mixed with vegetable oil, which was the feed material of the feed mixture to achieve the desired energy value of the feed mixture. Subsequently, the mixture of vegetable oil and essential oil was mixed with corn meal and then manually homogenized with other feed materials. The drinkers were cleaned daily and topped 2 to 3 times with fresh water enriched with citrus fruit essential oil. Feed feeds were made (mixed) in the feed company Biofeed, a.s. Kolárovo.

The hall, which included our experiment, was equipped with deep bedding of straw, automatic ventilation equipment in the ceiling, heat sources – radiators, and a light regime as recommended by the producer of fattening type broiler chickens. One-day-old broiler chickens were placed in the experiment. Feeding was provided from plate feeders and hat feeders until the age of 14 days. After 14 days, the experimental feeding and watering equipment was replaced by a tube feeder in each group of 1 piece and a bucket feeder in each group of 1 piece. The trial period was divided into 3 phases: starter: days 1 to 18 with the feeding of starter feed mixture, growth: days 15 to 33 with the feeding of the growth feed mixture, final: days 34 to 40 with the feeding of the final feed mixture. The feed mixtures used in the control and experimental groups differed from each other according to the use of the active substance (see scheme, Table 1). Essential oils were obtained from Calendula, a.s. Nová Ľubovňa, which supplied us with samples with an a-test, according to which it declared the safety and effectiveness of thyme and cinnamon essential oils. The citrus essential oil was obtained from a feed company.

Composition of the thyme essential oil, cinnamon essential oil, and a commercial feed supplement citrus fruit essential oil

Compound percentages of the essential oil of *Thymus vulgaris*: *α*-thujene 0.71%, *α*-pinène 0.49%, *cis*-sabinene hydrate 0.46%, camphene 0.14%, *β*-myrcene 0.71%, *α*-terpinene 0.58%, *β*-phelladrene 0.37%, *γ*-terpinene 1.92%, *ρ*-cymene 9.89%, carvacryl acetate 1.19%, *β*-linalool 1.51% **(Ben Jabeur et al., 2017)**.

Compound percentages of the essential oil of bark of *Cinnamomum zeylanicum:* styrene benzene, ethenyle- (CAS) 5.5%, *α*-pinene 0.50%, benzaldehyde 1.10%, cyclohexane, 1-methyl-3-(1-methylet) 0.52%, benzenepropanal (CAS) B-phenylpr 1.21%, L-borneol 0.46%, *trans*-cinnamaldehyde 1.07%, cinnamic aldehyde 52.30%, *α*-longipinene tricyclo (5.4) 0.70%, *α*-copaene 11.42%, sativene 0.48%, *trans*-caryophyllene bicyclo (7.2) 0.80%, ar-curcumene 0.55%, naphtalene, 1,2,3,4,4a,5,6,8a-octa 2.28%, *α*-muurolene naphtalene, 1 0.70% *δ*-cadinene 6.25%, *cis*-calamenene 3.61%, naphtalene, 1,2,3,4,4a,7-hexahydro 0.83%, *α*-calacorene 0.68%, naphtalene, 1,2,3,4,4a,7-hexahydro 0.78%, *t*-muurolol 1-naphtslenol 1,2,3 1.95% **(Kazemi and Mokhtariniya, 2016)**.

Compound percentages of the essential oil of *Citrus paradisi, Citrus reticulata Blanco*, *Citrus sinensis and Citrus aurantium* L. *subsp. bergamia:* *α*-pinene 0.27%, sabinene 0.08%, myrcene 3.03%, Z,Z,Z-1,4,6,9-nonadecatetraene 0.20%, limonene 50.42%, Z-β-ocimene 0.12%, *γ*-terpinene 0.05%, n-octanol 0.11%, linalool 0.45%, n-nonanal 0.17%, *trans*-para-mentha-2,8-dien-1-ol 0.77%, P-mentha-E-2,8(9)-dien-1-ol 0.73%, 3-(methoxy)-3-methyl-6-prop-1-en-2-ylcyclohexene 0.03%, *cis*-β-terpineol 0.04%, *α*-2,4-cyclohexadiene-1-methanol 0.08%, n-octanol 0.08%, (-)-terpinen-4-ol 0.45%, *trans*-isocarveol 0.13%, *α*-terpineol 1.19%, [1,1'-bicyclopentyl]-2-one 1.16%, decanal 1.28%, octyl-acetate 0.09%, *trans*-carveol 3.09%, carvomenthol 0.15%, 5-isoprorpenyl-2-methyl-2-cyclohexen-1-ol 0.81%, 2-methyl-5-(1-methylethene) 2-cyclohexen-1-one 0.50%, 3-methyl-6-(1-methylethene) 2-cyclohexen-1-one 0.07%, perillaldehyde 0.22%, undecanol 0.18%, limonen-10-ol 0.15%, perilla alcohol 0.04%, n-undecanal 0.12%, 4-vinyl- guaiacol 0.92%, *α*-a-cubebene 0.24%, 3,7-dimethyl 6-octen-1-ol 0.19%, *α*-copaene 1.49%, neryl acetate 0.06%, *β*-copaene 0.54%, *α*-, *trans*-bergamotene 0.06%, dodecanal 0.84%, (E)-caryophyllene 0.34%, *β*-copaene 0.06%, *α*-guaiene 0.07%, 6,10-dimethylundeca-5,9-dien-2-one 0.07%, *α*-humulene 1.23%, cadina-1,4-diene 0.07%, *γ*-muurolene 0.07%, *β*-copaene 1.30%, bicyclogermacrene 0.22%, *α*-muurolene 0.24%, (E,E)-, *α*-farnesene 1.67%, *γ*-cadinene 0.03%, δ-cadinene 2.53%, sesquisabinene 0.20%, *trans*-cadina-1,4-diene 0.07%, *α*-elemol 0.79%, (-)-spathulenol 0.54%, n-dodecanoic acid 1.28%, ethyl iso-allocholate 0.08%, epicubenol 0.22%, *γ*-eudesmol 0.31%, *α*-cadinol 0.08%, cadin-4-en-10-ol 0.99%, 3,7-dimethyl 6-octenal 0.15%, humulene 0.22%, *β*-sinensal 1.65%, 2,6,10-trimethyl 2,6,9,11-dodecatetraenal 0.05%, *α*-sinensal 3.14%, 2-pentyl-2-nonenal 0.19%, tetradecanoic acid 0.94%, nootkatone 0.28%, cryptomeridiol 0.05%, farnesyl acetone 0.05%, methyl-hexadecanoate 0.26%, 2-dodecen-1-yl(-) succinic anhydride 0.12%, n-hexadecanoic acid 5.65%, methyl ester 9,12-octadecadienoic acid 0.32%, linoleic acid 0.94%, methyl linoleate 0.19%, dodecenyl succinic anhydride 0.31% **(Goyal and Kaushal, 2018)**.

The results of the chemical composition of Citrus peel essential oil can slightly various in literature because it depending upon the age of the plant, harvesting time, geographical and ecological conditions **(Wu et al., 2013)**.

At the end of the feeding experiment, 6 broiler chickens were randomly selected from each group for chemical analysis. Selected broiler chickens were transported to the technological laboratory of the Department of Technology and Quality of Animal Products, where they were slaughtered humanely and technologically processed. The breast and thigh muscles with skin were separated from the carcass. These samples of breast and thigh muscles were transported in a portable refrigerator to the chemical laboratory of the Department of Food Hygiene and Safety, where the preparation of samples for chemical analysis was performed.

Preparation of breast and thigh muscle samples with skin for chemical analysis

The thighs were boned and labeled with the TEO, STO, B, TEO + B, and SEO + B experimental groups, and in each group with a sample number from 1 to 6. Breast muscle samples were similarly labeled. Samples of the control group were marked K and with sample numbers from 1 to 6. Each sample was mixed in a Grindomix 200 laboratory grinder and weighed 50.0 g. Samples of breast and thigh muscles with skin were transferred to laboratory flasks with ground glass. Weighing of breast and thigh muscle samples was performed on an automatic scale of the Kern 440-49N type with an accuracy of d = 0.01 g.

Chemical analysis of breast and thigh muscle samples

Chemical analysis of breast and thigh muscle samples with skin was performed using a Nicolet 6700 FT-IR Fourier Transform Infrared Spectrometer. The samples were measured by the attenuated total reflectance method, which uses a diamond crystal attachment. The device works fully automatically, using OMNIC computer software. Infrared spectroscopy is a method whose principle is the ability of substances to absorb radiation from the infrared region, i.e. 800 – 106 nm of the electromagnetic spectrum. The sample was placed on the surface and in contact with the diamond crystal. Subsequently, a total reflection was formed at the interface of the crystal, which represents an optically denser environment, with the sample, which represents an optically denser environment. In this type of reflection, part of the radiation penetrates slightly into the sample and spectral information is obtained. The analytical output is the infrared spectrum. This spectrum graphically shows the functional dependence of the energy expressed as a percentage of the transmittance, i.e. transmittance (T) or absorbance units (A) at the wavelength of the incident radiation. Chemical analyzes were performed at the Department of Special Zootechnics. Samples of breast and thigh muscles with skin were measured for moisture (calculated on the dry matter), fat, and cholesterol contents using a Nicolet 6700 FT-IR Fourier transform infrared spectrometer.

It is more advantageous to use Fourier transform spectrometers today, which have dispersive elements replaced by an interferometer, which prevents the decomposition of radiation and allows folding and recombination (**Challa Kumar, 2011; Čech Barabaszová, 2012**).

Statistical Analysis

The statistically evaluated results are reported in the study as arithmetic mean (*M*), standard deviation (*SD*), which are commonly used in descriptive statistics. The F test was used to test the difference in the observed indicator between the groups, followed by the Scheffe test, which was set to test at a significance level of $p = 0.05$.

The relation between the two variables in each group was tested using Pearson's correlation coefficient (*r*). The value of the coefficient is between –1 and +1. If its value is –1, it means a high negative linear relationship between two variables, +1 means a high positive linear relation between two variables. If the correlation coefficient reaches 0, it means that there is no linear relation between the two variables. The procedure according to Cohen (1988) was chosen to evaluate the results: below 0.1 trivial relations (simple, easy), 0.1 – 0.3 weak relation, 0.3 – 0.5 medium relation, above 0.5 strong relations (0.7 – 0.9 is often reported as very strong and 0.9 – 1.0 as almost perfect). The results of the correlation coefficient were based on a statistically significant at the significance level $\alpha = 0.05$, $\alpha = 0.01$, $\alpha = 0.001$. The SAS system program, version 8.2 was used for statistical analysis of the results.

RESULTS AND DISCUSSION

Dry matter of the chicken breast and thigh muscles with skin

Dry matter of the chicken breast muscle with skin

The average dry matter content of the chicken breast muscle with skin and the statistical evaluation of the results are given in Table 2.

The average dry matter content of breast muscle with skin in the experimental groups ranged from 26.73 g.100 g^{-1} with citrus fruit essential oil, followed by an average dry matter content of 26.98 g.100 g^{-1} with thyme essential oil, 27.07 g.100 g^{-1} with thyme essential oil + citrus fruit essential oil, 27.12 g.100 g^{-1} with cinnamon essential oil nd 27.89 g.100 g^{-1} with cinnamon essential oil + citrus fruit essential oil. These values of the average dry matter content in the breast muscle with the skin are lower compared to the average dry matter content in the breast muscle with the skin of the control group. The difference in the dry matter content of the pectoral muscle with the skin was not statistically significant among the groups ($p > 0.05$). Statistical evaluation of the measured breast muscle dry matter content data expressed as the standard deviation revealed that the smallest variation was in the thyme essential oil experimental group and the highest in the thyme essential oil + citrus fruit essential oil experimental group, i.e. *SD* = 0.98 versus *SD* = 1.56.

Mosca et al. (2018) in their study report breast muscle dry matter content in broiler chicken hens and cocks with an average value of 28.21 g.100 g^{-1}, which is higher compared to our results. **Evaris et al**. **(2021)** report a dry matter content of breast muscle of only 23.47 g.100 g^{-1} and point out that the addition of essential oil to feed reduces the dry matter content of breast muscle broiler chickens.

Dry matter of the chicken thigh muscle with skin

The average dry matter content of the chicken thigh muscle with skin and the statistical evaluation of the results are given in Table 3. The average dry matter content of the thigh muscle with skin in the experimental groups ranged from 27.33 g.100 g^{-1} with cinnamon essential oil, followed by an average dry matter content of 27.42 g.100 g^{-1} with thyme essential oil, 28.07 g .100 g^{-1} with cinnamon essential oil + citrus fruit essential oil, 28.09 g.100 g^{-1} with citrus fruit essential oil and 28.14 g.100 g^{-1} with thyme essential oil + citrus fruit essential oil. These values of the average dry matter content of the skin thigh muscle are lower compared to the average dry matter content of the skin of the control group.

The difference in the dry matter content of the thigh muscle with the skin was not statistically significant among the groups ($p > 0.05$).

Table 2 The average dry matter content of the chicken breast muscle with skin, g.100 g^{-1}, and the statistical evaluation of the results.

Group	n	*M*	*SD*	Thyme EO	Cinnamon EO	Citrus fruit EO	Thyme EO + Citrus fruit EO	Cinnamon EO + Citrus fruit EO
				F test: 1.326^{-}				
Control	6	28.61	1.49	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Thyme EO	6	26.98	0.98		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Cinnamon EO	6	27.12	1.32			$p > 0.05$	$p > 0.05$	$p > 0.05$
Citrus fruit EO	6	26.73	1.24				$p > 0.05$	$p > 0.05$
Thyme EO + Citrus fruit EO	6	27.09	1.56					$p > 0.05$
Cinnamon EO + Citrus fruit EO	6	27.89	1.23					

Note: EO = essential oil, n = number of samples obtained from 6 broiler chickens, *M* = mean, *SD* = standard deviation, $p > 0.05$: no statistically significant difference among groups.

Table 3 The average dry matter content of the chicken thigh muscle with skin, g.100 g^{-1}, and the statistical evaluation of the results.

Group	n	*M*	*SD*	Thyme EO	Cinnamon EO	Citrus fruit EO	Thyme EO + Citrus fruit EO	Cinnamon EO + Citrus fruit EO
				F test: 1.326^{-}				
Control	6	28.66	1.22	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Thyme EO	6	27.42	0.96		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Cinnamon EO	6	27.33	1.44			$p > 0.05$	$p > 0.05$	$p > 0.05$
Citrus fruit EO	6	28.09	1.19				$p > 0.05$	$p > 0.05$
Thyme EO + Citrus fruit EO	6	28.14	1.28					$p > 0.05$
Cinnamon EO + Citrus fruit EO	6	28.07	1.36					

Note: EO = essential oil, n = number of samples obtained from 6 broiler chickens, *M* = mean, *SD* = standard deviation, $p > 0.05$: no statistically significant difference among groups.

Table 4 The average fat content of the chicken breast muscle with skin, g.100 g^{-1}, and the statistical evaluation of the results.

Group	n	*M*	*SD*	Thyme EO	Cinnamon EO	Citrus fruit EO	Thyme EO + Citrus fruit EO	Cinnamon EO + Citrus fruit EO
				F test: 1.952^{-}				
Control	6	1.32	0.14	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Thyme EO	6	1.78	0.11		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Cinnamon EO	6	1.81	0.13			$p > 0.05$	$p > 0.05$	$p > 0.05$
Citrus fruit EO	6	1.56	0.16				$p > 0.05$	$p > 0.05$
Thyme EO + Citrus fruit EO	6	1.68	0.17					$p > 0.05$
Cinnamon EO + Citrus fruit EO	6	1.49	0.16					

Note: EO = essential oil, n = number of samples obtained from 6 broiler chickens, *M* = mean, *SD* = standard deviation, $p > 0.05$: no statistically significant difference among groups.

Statistical evaluation of the measured data of the dry matter content of the thigh muscle with skin expressed by the standard deviation revealed that the smallest fluctuation of values was in the experimental group with thyme essential oil and the highest in the experimental group with cinnamon essential oil, i.e. *SD* = 0.96 vs. *SD* = 1.44.

Haščík et al. (2012) report higher values of dry matter content in the thigh muscle as are our data. They achieved results of 31.51 g.100 g^{-1} in the control group with coccidiostats, and 30.21 g.100 g^{-1} or 29.88 g.100 g^{-1} in the experimental groups with pollen extract. They experimented with a hybrid combination of the Ross 308 broiler chickens. **Mosca et al. (2018)** report the dry matter content in the thigh muscle of broiler chickens without taking into account sex 26.3 g.100 g^{-1}, which is a lower value compared to our results. The stated lower dry matter value in the thigh muscle may be due to another hybrid combination of broiler chickens or the feeding of feed mixtures of a different composition than our feed mixtures. **Evaris et al. (2021)** report a dry matter content of the thigh muscle of only 24.14 g.100 g^{-1}.

Fat of the chicken breast and thigh muscles with skin

Fat of the chicken breast muscle with skin

The average fat content of the chicken breast muscle with skin and the statistical evaluation of the results are given in Table 4.

The average fat content of breast muscle with skin in the experimental groups ranged from 1.49 g.100 g^{-1} with cinnamon essential oil + citrus fruit essential oil, followed by an average fat content of 1.56 g.100 g^{-1} with citrus fruit essential oil, 1.68 g.100 g^{-1} with thyme essential oil + citrus fruit essential oil, 1.78 g.100 g^{-1} with thyme essential oil and 1.81 g.100 g^{-1} with cinnamon essential oil. These values of the average fat content of the breast muscle with the skin are higher compared to the average fat content of the breast muscle with the skin of the control group.

The difference in the fat content of the breast muscle with the skin was not statistically significant among the groups (p >0.05). Statistical evaluation of the measured data of fat content in the breast muscle with skin expressed by the standard deviation revealed that the smallest fluctuation of values were in the experimental group with thyme essential oil and the highest in the experimental group with cinnamon essential oil + citrus fruit essential oil, i.e. *SD* = 0.11 vs. *SD* = 0.16.

A study by **Zotte et al.** (**2020**) reports a breast fat content of 1.21 g.100 g^{-1}, which is lower than our results, which may indicate that the addition of essential oils to feed for broiler chickens has the effect of increasing the fat content as in our results. In another study, **Zampiga et al.** (**2019**) report a fat content in breast muscle of 1.71 g.100 g^{-1}, which is lower compared to the results of our study after feeding of feed mixtures with thyme and cinnamon essential oils and higher compared to the group, where cinnamon essential oil + citrus fruit essential oil, thyme essential oil + citrus fruit essential oil and citrus fruit essential oil were used. These results may indicate that feeding of thyme and cinnamon essential oils tends to increase fat storage.

Kim et al. (**2020**) recorded a fat content of chicken breast muscle of 1.10 to 1.44%, who examined this indicator in terms of the impact of conventional farming systems and welfare systems with the application of welfare principles. Our results of the fat content of the control group and cinnamon essential oil + citrus fruit essential oil are the closest to their highest value of fat content in breast muscle.

Fat of the chicken thigh muscle with skin

The average fat content of the chicken thigh muscle with skin and the statistical evaluation of the results are given in Table 5.

The average fat content of the thigh muscle with skin in the experimental groups ranged from 2.59 g.100 g^{-1} with cinnamon essential oil + citrus fruit essential oil, followed by an average fat content of 2.71 g.100 g^{-1} with thyme essential oil, 2.83 g.100 g^{-1} with cinnamon essential oil, 2.91 g.100 g^{-1} with thyme essential oil + citrus fruit essential oil of 2.93 g.100 g^{-1} with citrus fruit essential oil. These values of the average fat content of the thigh muscle with the skin are higher compared to the average fat content of the thigh muscle with the skin of the control group. The difference in the fat content of the original thigh muscle mass with the skin among the groups was not statistically significant (p >0.05). Statistical evaluation of the measured data of the fat content of the thigh muscle with skin expressed by the standard deviation revealed that the smallest fluctuation of values was in the experimental group with thyme essential oil and the highest in the experimental group with cinnamon essential oil, i.e. *SD* = 0.96 vs. *SD* = 1.44.

Evaris et al. (**2021**) report a fat content in the thigh muscle of 9.66 g.100 g^{-1}, which is a significant difference compared to our results.

The cholesterol of the chicken breast and thigh muscles with skin

The cholesterol of the chicken breast muscle with skin

The average cholesterol content of the chicken breast muscle with skin and the statistical evaluation of the results are given in Table 6.

The average cholesterol content of the breast muscle with skin in the experimental groups ranged from 0.028 g.100 g^{-1} with cinnamon essential oil, cinnamon essential oil + citrus fruit essential oil, and thyme essential oil + citrus fruit essential oil, followed by average cholesterol content of 0.034 g .100 g^{-1} with citrus fruit essential oil and 0.042 g.100 g^{-1} with thyme essential oil. These values of the average cholesterol content of the breast muscle with the skin are lower compared to the control group (when using a cinnamon essential oil, cinnamon essential oil + citrus fruit essential oil, thyme essential oil + citrus fruit essential oil and when using citrus fruit essential oil) or higher (when using thyme essential oil). The difference in the cholesterol content of the breast muscle with the skin between the groups was not statistically significant (p >0.05).

Table 5 The average fat content of the chicken thigh muscle with skin, g.100 g^{-1}, and the statistical evaluation of the results.

Group	n	*M*	*SD*	Thyme EO	Cinnamon EO	Citrus fruit EO	Thyme EO + Citrus fruit EO	Cinnamon EO + Citrus fruit EO
				F test: 1.952^{-}				
Control	6	2.64	1.22	*p* >0.05	*p* >0.05	*p* >0.05	*p* >0.05	*p* >0.05
Thyme EO	6	2.71	0.96		*p* >0.05	*p* >0.05	*p* >0.05	*p* >0.05
Cinnamon EO	6	2.83	1.44			*p* >0.05	*p* >0.05	*p* >0.05
Citrus fruit EO	6	2.93	1.19				*p* >0.05	*p* >0.05
Thyme EO + Citrus fruit EO	6	2.91	1.28					*p* >0.05
Cinnamon EO + Citrus fruit EO	6	2.59	1.36					

Note: EO = essential oil, n = number of samples obtained from 6 broiler chickens, *M* = mean, *SD* = standard deviation, *p* >0.05: no statistically significant difference among groups.

Table 6 The average cholesterol content of the chicken breast muscle with skin, g.100 g^{-1}, and the statistical evaluation of the results.

Group	n	*M*	*SD*	Thyme EO	Cinnamon EO	Citrus fruit EO	Thyme EO + Citrus fruit EO	Cinnamon EO + Citrus fruit EO
				F test: 0.146^{-}				
Control	6	0.035	0.003	*p* >0.05	*p* >0.05	*p* >0.05	*p* >0.05	*p* >0.05
Thyme EO	6	0.042	0.005		*p* >0.05	*p* >0.05	*p* >0.05	*p* >0.05
Cinnamon EO	6	0.028	0.003			*p* >0.05	*p* >0.05	*p* >0.05
Citrus fruit EO	6	0.034	0.001				*p* >0.05	*p* >0.05
Thyme EO + Citrus fruit EO	6	0.028	0.003					*p* >0.05
Cinnamon EO + Citrus fruit EO	6	0.028	0.002					

Note: EO = essential oil, n = number of samples obtained from 6 broiler chickens, *M* = mean, *SD* = standard deviation, *p* >0.05: no statistically significant difference among groups.

Table 7 The average cholesterol content of the chicken thigh muscle with skin, g.100 g^{-1}, and the statistical evaluation of the results.

Group	n	*M*	*SD*	Thyme EO	Cinnamon EO	Citrus fruit EO	Thyme EO + Citrus fruit EO	Cinnamon EO + Citrus fruit EO
				F test: 0.146^{-}				
Control	6	0.037	0.003	*p* >0.05	*p* >0.05	*p* >0.05	*p* >0.05	*p* >0.05
Thyme EO	6	0.034	0.002		*p* >0.05	*p* >0.05	*p* >0.05	*p* >0.05
Cinnamon EO	6	0.037	0.002			*p* >0.05	*p* >0.05	*p* >0.05
Citrus fruit EO	6	0.044	0.004				*p* >0.05	*p* >0.05
Thyme EO + Citrus fruit EO	6	0.045	0.005					*p* >0.05
Cinnamon EO + Citrus fruit EO	6	0.039	0.003					

Note: EO = essential oil, n = number of samples obtained from 6 broiler chickens, *M* = mean, *SD* = standard deviation, *p* >0.05: no statistically significant difference among groups.

Statistical evaluation of the measured cholesterol content data in the breast muscle with the skin expressed by the standard deviation revealed that the smallest fluctuation of values was in the experimental group with citrus fruit essential oil and the highest in the experimental group with thyme essential oil, i.e. *SD* = 0.001 vs. *SD* = 0.005.

In general, raw poultry meat has approximately 27 to 90 mg of cholesterol.100 g^{-1} (**Bragagnolo, 2009**). **Shang et al.** (**2020**) report a cholesterol content in the breast muscle of 0.054 g.100 g^{-1}, which is a higher value of the cholesterol content in the breast muscle compared to our results. **Krauze et al.** (**2021**) note that broiler chickens fed cinnamon essential oil in feed showed better growth performance due to the beneficial effect of the product on the small intestinal microbiome, metabolism. Their study states that HDL cholesterol level was increased and levels of total cholesterol and non-esterified fatty acids as well as triacylglycerols were decreased. Their opinion on the total cholesterol also confirmed our results in an experiment with broiler chickens, which was used only cinnamon oil or cinnamon essential oil also combined with citrus fruit essential oil. **Lin et al.** (**2003**) also state cinnamon essential oil has, among other things, strong hypocholesterolemic effects. **Koochaksaraie et al.** (**2011**) believe that cinnamon aldehyde found in cinnamon essential oil is primarily responsible for the hypocholesterolemic effect of stimulating the excretion of cholesterol from the body.

In the context of citrus fruit essential oil, it is known that the active compound in sweet oranges such as tannins, saponins, flavonoids, and essential oil can inhibit the absorption of cholesterol in the intestine. The presence of tannins will stick or lining the intestine membrane thus inhibiting the absorption of cholesterol (**Oluremi et al., 2007**). In our experiment, the cholesterol content was reduced mainly when using citrus fruit essential oil in combination with thymus and cinnamon essential oils. Some studies have shown that bioactive compounds from plant materials, including sterols, polysaccharides, and polyphenols, exhibit cholesterol lowering effects both *in vitro* and *in vivo*. Condensed tannins belong to the family of flavonoids, which occur in plants as mixtures of flavan-3-ol oligomers or polymers (**Desrues et al., 2017**). To determine the potential cholesterol-lowering activities of polyphenols, researchers usually investigate their inhibitory effects on the cholesterol solubility in mixed micelles *in vitro* at first. It is believed that reduction in cholesterol micellar solubility by phenolic compounds may inhibit the cholesterol absorption in the intestinal lumen and is attributed to their interactions with phosphatidylcholine or bile acid (**Ogawa et al., 2015**). In recent years, some research teams put forward that the direct interactions of plant functional components with cholesterol may be another possible mechanism of their hypocholesterolemic effects. For instance, β-sitosterol can be co-crystallized with cholesterol to form insoluble mixed crystals to further reduce the absorption of cholesterol in the small intestine (**Christiansen et al., 2003**).

There is also a study by **Kriaa et al.** (**2019**), who report that bacteria found in the intestinal microbiome use cholesterol from the intestinal contents to form their cell walls and thereby reduce the amount of exogenous cholesterol in the body. In their study, **Koeth et al.** (**2013**) showed that dietary carnitine and direct supplementation with trimethylamine-N-oxide both reduced reverse cholesterol transport ($p < 0.05$) in mice only in case of the intact gut microbiome.

The main issue regards the molecular mechanisms, which link microflora and trimethylamine-N-oxide synthesis to reverse cholesterol transport reduction. In the intestines, trimethylamine-N-oxide is referred to diminish cholesterol uptake by reducing Niemann-Pick C1-like 1, which takes cholesterol from the lumen into the enterocytes (**Altmann et al., 2004**). However, the intestinal metabolism of the cholesterol cannot justify the decrease of reverse cholesterol transport. Of note, in the liver, trimethylamine-N-oxide has been referred to reduce the expression of Cyp7a1, a fundamental bile enzyme in the metabolism, and transport of cholesterol (**Bronzato and Durante, 2017**).

Chicken meat is recognized for several health benefits due to its high nutritional value and, in addition to its high protein content, also its low cholesterol content, low energy value, and low-fat content (**Sujiwo et al., 2018**).

The cholesterol of the chicken thigh muscle with skin

The average cholesterol content of the chicken thigh muscle with skin and the statistical evaluation of the results are given in Table 7.

The average cholesterol content of the thigh muscle with skin in the experimental groups ranged from 0.034 g.100 g^{-1} with thyme essential oil, followed by an average cholesterol content of 0.037 g.100 g^{-1} with cinnamon essential oil, 0.039 g.100 g^{-1} with cinnamon essential oil + citrus fruit essential oil, 0.044 g.100 g^{-1} with citrus fruit essential oil up to 0.045 g.100 g^{-1} with thyme essential oil + citrus fruit essential oil.

These values of average cholesterol content in the thigh muscle with skin are either lower (when using thyme essential oil), the same (when using cinnamon essential oil), or higher (when using citrus fruit essential oil, thyme essential oil + citrus fruit essential oil or cinnamon essential oil + citrus fruit essential oil).

The difference in the cholesterol content of the thigh muscle with the skin between the groups was not statistically significant ($p > 0.05$).

Statistical evaluation of the measured data of the cholesterol content of the thigh muscle with skin expressed by the standard deviation revealed that the smallest fluctuation of values was in the experimental group with cinnamon essential oil and the highest in the experimental group with thyme essential oil + citrus fruit essential oil, i.e. *SD* = 0.002 vs. *SD* = 0.005.

Shang et al. (**2020**) report a cholesterol content in the thigh muscle of 0.060 g.100 g^{-1}, which is a higher value of cholesterol content compared to our results. The cholesterol content of raw and cooked meat and poultry products ranges from 40 to 90 mg.100 g^{-1} (**Honikel, 2009**).

Phytobiotics are products of plant origin extracted from medicinal plants, spices, or even fungi. They have a high impact on the body due to the high content of bioactive compounds provided by secondary metabolism. Numerous studies performed on various livestock, including broiler chickens, have confirmed the multidirectional effects of phytobiotics in animal nutrition on improving health and productivity.

Table 8 Correlation between dry matter and fat, dry matter and cholesterol, and between fat and cholesterol in the breast muscle with skin by groups.

Indicator	Fat	Cholesterol
Group	Control	
Dry matter	0.71	0.38
	0.14^{-}	0.31^{-}
Fat		0.34
		0.29^{-}
Group	Thyme EO	
Dry matter	0.42	0.18
	0.83^{-}	0.87^{-}
Fat		0.57
		0.39^{-}
Group	Cinnamon EO	
Dry matter	0.51	0.47
	0.49^{-}	0.99^{-}
Fat		0.38
		0.53^{-}
Group	Citrus fruit EO	
Dry matter	0.74	0.17
	0.11^{-}	0.69^{-}
Fat		0.09
		0.98^{-}
Group	Thyme EO + Citrus fruit EO	
Dry matter	0.66	0.33
	0.91^{-}	0.55^{-}
Fat		-0.29
		0.63^{-}
Group	Cinnamon EO + Citrus fruit EO	
Dry matter	0.41	0.36
	0.56^{-}	0.49^{-}
Fat		-0.57
		0.66^{-}

Note: in each column, the 1st number in the row is the value of the Pearson correlation coefficient (r) and the 2nd number in the row is the *p-value*; numeric value marked with a superscript –: no statistically significant difference of the linear relation between 2 variables (p >0.05); EO = essential oil.

In addition, a positive effect on the nutritional and dietary value of products of animal origin has been revealed (**Al-Yasiry et al., 2017; Kiczorowska et al., 2017**). **Ciftci et al.** (**2010**) state that after feeding a diet with cinnamon essential oil, the level of cholesterol in the blood serum is reduced, as well as in the breasts and thighs of broiler chickens. These authors also confirmed that this essential oil manifests hypocholesterolemic properties. But their opinion was not confirmed in our experiment on the thigh muscle. It has been reported that the thymol and carvacrol content of thyme slows down the activity of the cholesterol-synthesizing enzyme hydroxymethyl-glutaryl coenzyme A reductase, and thereby, decreases cholesterol levels (**Elson, 1995**). In agreement with this finding, it has also been reported that thyme significantly decreases cholesterol and triglyceride levels in quails (**Khaksar et al., 2012**) and also in broiler chickens (**Safa and Al-Beitawi, 2009**). The reduction of cholesterol content in the thigh muscle due to thyme essential oil was also found in our experiment.

Correlation between dry matter and fat, dry matter and cholesterol, and between fat and cholesterol in the breast and thigh muscles with skin by groups

Correlation between dry matter and fat, dry matter and cholesterol, and between fat and cholesterol in the breast muscle with skin by groups

The correlation between dry matter and fat, dry matter and cholesterol, and between fat and cholesterol in the breast muscle with skin by groups are shown in Table 8.

Based on the evaluation of the correlation relationship between the two variables, i.e. different results were found between dry matter and fat, between dry matter and cholesterol, and between fat and cholesterol of breast muscle with skin. Differences in the linear relation between the observed variables were not statistically significant (p >0.05).

A strong positive linear relationship with an r value above 0.5 was found between dry matter and fat in the control group, in the cinnamon essential oil experimental group, in the citrus fruit essential oil experimental group, and the thyme essential oil + citrus fruit essential oil experimental group.

Table 9 Correlation between dry matter and fat, dry matter and cholesterol, and between fat and cholesterol in the thigh muscle with skin by groups.

Indicator	Fat	Cholesterol
Group	Control	
Dry matter	0.58	0.37
	0.27^{-}	0.43^{-}
Fat		0.78
		0.17^{-}
Group	Thyme EO	
Dry matter	0.78	0.49
	0.04^{+}	0.37^{-}
Fat		0.64
		0.14^{-}
Group	Cinnamon EO	
Dry matter	0.83	0.17
	0.02^{+}	0.77^{-}
Fat		0.21
		0.86^{-}
Group	Citrus fruit EO	
Dry matter	0.75	0.82
	0.09^{-}	0.73^{-}
Fat		0.26
		0.63^{-}
Group	Thyme EO + Citrus fruit EO	
Dry matter	0.76	0.89
	0.65^{-}	0.84^{-}
Fat		0.41
		0.43^{-}
Group	Cinnamon EO + Citrus EO	
Dry matter	0.95	0.27
	0.73^{-}	0.10^{-}
Fat		-0.17
		0.81^{-}

Note: in each column, the 1st number in the row is the value of the Pearson correlation coefficient (*r*) and the 2nd number in the row is the *p-value*; numeric value marked with a superscript –: no statistically significant difference of the linear relation between 2 variables ($p > 0.05$); numeric value marked with a superscript +: statistically significant difference of the linear relation between 2 variables ($p \leq 0.05$); EO = essential oil.

Furthermore, a strong positive linear relationship with an *r* value above 0.5 was found between fat and cholesterol in the experimental group with thyme essential oil and a strong negative linear relation with a value above 0.5 between fat and cholesterol in the experimental group with cinnamon essential oil + citrus fruit essential oil.

An interesting result of the correlation relation was recorded in the experimental group with citrus fruit essential oil between fat and cholesterol, which manifested itself only in a trivial positive linear relation with a value below 0.1.

Other values of the linear relation between dry matter and fat, between dry matter and cholesterol, and between fat and cholesterol showed a mean value, either positive or negative.

Correlation between dry matter and fat, dry matter and cholesterol, and between fat and cholesterol in the thigh muscle with skin by groups

The correlation between dry matter and fat, dry matter and cholesterol, and between fat and cholesterol in the thigh muscle with skin by groups are shown in Table 9.

Based on the evaluation of the correlation relationship between the two variables, i.e. between dry matter and fat, between dry matter and cholesterol, and between fat and cholesterol of the thigh muscle with the skin, different results were found, which in some relation, only the effect of thyme and cinnamon essential oils were statistically significant ($p \leq 0.05$).

A strong positive linear relationship with an r value above 0.5 was found between dry matter and fat in the control group, which was not statistically significant ($p > 0.05$), in the experimental group with thyme essential oil, which was statistically significant ($p \leq 0.05$), in the experimental group with cinnamon essential oil, which was statistically significant ($p \leq 0.05$), in the experimental group with citrus fruit essential oil, which was not statistically significant ($p > 0.05$), in the experimental group with thyme essential oil + citrus fruit essential oil, which was not statistically significant ($p > 0.05$), and in the experimental group with cinnamon essential oil + citrus fruit essential oil, which was not statistically significant ($p > 0.05$).

Furthermore, a strong positive linear relationship with an *r* value above 0.5 was found between dry matter and cholesterol in the citrus fruit essential oil experimental group, which was not statistically significant ($p > 0.05$) and in the thyme essential oil + citrus fruit essential oil experimental group, which was not statistically significant ($p > 0.05$).

Furthermore, a strong positive linear relationship with an *r* value above 0.5 was found between fat and cholesterol in the control group, which was not statistically significant ($p > 0.05$), and in the experimental group with thyme essential oil, which was not statistically significant ($p > 0.05$).

Other values of linear dependence between dry matter and fat, between dry matter and cholesterol, and between fat and cholesterol showed a mean value, either positive or negative, the difference of which was not statistically significant ($p > 0.05$).

Lipid and cholesterol contents of meat and poultry have been of great research interest for decades. This topic is repeatedly studied and reviewed **(Dinh et al., 2011)**.

Dinh et al. **(2008)** reported a positive correlation between intramuscular fat and cholesterol content.

Sweeten et al. (1990) further investigated the distribution of cholesterol in adipose tissues of both muscle (intramuscular adipose tissues) and subcutaneous fat (subcutaneous adipose tissues).

The intramuscular adipose tissues had similar cholesterol contents to that of the subcutaneous adipose tissues; however, only 54% of the cholesterol in the intramuscular tissues was from the cytoplasm (cholesterol in storage lipid), compared with a 90% proportion from the cytoplasm in subcutaneous adipose tissues.

Unless muscle type, muscle lipid, and their interactions with each other and with other contributory factors cause a dramatic change in muscle structure and composition, their effects on the cholesterol content of muscle might be limited **(Dinh et al., 2011)**.

CONCLUSION

We can state from the evaluation and analysis of the results on the effect of thyme, cinnamon, and citrus fruit essential oils and their combinations after feeding in the broiler chickens that:

a) the dry matter content in the breast muscle ranged from 26.73 to 27.89 $g.100\ g^{-1}$ compared to 28.61 $g.100\ g^{-1}$ of the control group with no statistically significant differences, and in the thigh muscle in the ranged from 27.33 to 28.14 $g.100\ g^{-1}$ compared to 28.66 $g.100\ g^{-1}$ of the control group with no statistically significant differences,

b) the fat content in the breast muscle ranged from 1.56 to 1.81 $g.100\ g^{-1}$ compared to 1.32 $g.100\ g^{-1}$ in the control group without statistically significant differences and in the thigh muscle in the range from 2.58 to 2.93 $g.100\ g^{-1}$ versus 2.64 $g.100\ g^{-1}$ of the control group with no statistically significant differences,

c) the cholesterol content in the breast muscle ranged from 0.028 to 0.042 $g.100\ g^{-1}$ compared to 0.035 $g.100\ g^{-1}$ of the control group with no statistically significant differences, and in the thigh muscle from 0.034 to 0.045 $g.100\ g^{-1}$ vs. 0.037 $g.\ 100\ g^{-1}$ control group with no statistically significant differences,

d) in correlation, a strong positive linear relation between dry matter content and fat content tended to be indicated in the breast muscle due to cinnamon essential oil, citrus fruit essential oil, thyme essential oil in combination with citrus fruit essential oil and in the control group, a strong positive linear relation between fat and cholesterol due to thyme essential oil and a strong negative linear relation between fat and cholesterol due to cinnamon essential oil in combination with citrus fruit essential oil;

in the thigh muscle, a strong positive linear relation between dry matter content and fat content was statistically confirmed by thyme and cinnamon essential oils and tended to be indicated by thyme essential oil in combination with citrus fruit essential oil, cinnamon essential oil in combination with citrus fruit essential oil and in the control group, there was a tendency to indicate a strong positive linear relation between fat content and cholesterol content due to citrus fruit essential oil.

We can conclude that essential oils are one of the alternatives to feed antibiotics. Therefore, a thorough knowledge of their effect and mechanism remains an open question for future research.

REFERENCES

Abd El-Hack, M. E., Alagawany, M., Abdel-Moneim, A. M. E., Mohammed, N. G., Khafaga, A. F., Bin-Jumah, M., Othman, S. I., Allam, A. A., Elnesr, S. S. 2020. Cinnamon (*Cinnamomum zeylanicum)* oil as a potential alternative to antibiotics in poultry. *Antibiotics,* vol. 9, p. 75-79. https://doi.org/10.3390/antibiotics9050210

Altmann, S. W., Davis, H. R., Jr., Zhu, L. J., Yao, X., Hoos, L. M., Tetzloff, G., Prasad, S., Iyer, N. 2004. Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. *Science*, vol. 303, p. 1201-1204. https://doi.org/10.1126/science.1093131

Al-Yasiry, A. R. M., Kiczorowska, B., Samolinska, W. 2017. The nutritional value and content of mineral elements in the meat of broiler chicken feed diets supplemented with *Boswellia serrata*. *Journal of Elementology*, vol. 22, no. 3, p. 1027-1037. https://doi.org/10.5601/jelem.2017.22.1.1294

Attia, Y. A., Al-Harthi, M. A., Korish, M. A., Shiboob, M. M. 2017. Fatty acid and cholesterol profiles, hypocholesterolemic, atherogenic, and thrombogenic indices of broiler meat in the retail market. *Lipids in Health and Disease*, vol. 16, no. 40. https://doi.org/10.1186/s12944-017-0423-8

Ben Jabeur, M., Somai-Jemmali, L., Hamada, W. 2017. Thyme essential oil as an alternative mechanism: biofungicide-causing sensitivity of Mycosphaerella graminicola. *Journal of Applied Microbiology*, vol. 122, p. 932-939.

Bronzato, S., Durante, A. A. 2017. Contemporary Review of the Relationship between Red Meat Consumption and Cardiovascular Risk. *International Journal of Preventive Medicine*, vol. 8. https://doi.org/10.4103/ijpvm.IJPVM_206_16

Challa Kumar, C. S. S. R. 2011. *Carbon nanomaterials*. Chichester : John Wiley. 482 p. ISBN 978-3-527-32169-8.

Christiansen, L., Karjalainen, M., Seppänen-Laakso, T., Hiltunen, R., Yliruusi, J. 2003. Effect of β-sitosterol on precipitation of cholesterol from non-aqueous and aqueoussolutions. *International Journal of Pharmaceutics*, vol. 254, no. 2, p. 155-166. https://doi.org/10.1016/s0378-5173(03)00007-3

Ciftci, M., Simsek, U. G., Yuce, A., Yilmaz, O., Dalkilic, B. 2010. Effects of Dietary Antibiotic and Cinnamon Oil Supplementation on Anioxidant Enzyme Activities, Cholesterol Levels and Fatty Acid Compositions of Serum and Meat in Broiler Chickens. *Acta Veterinaria*, vol. 79, p. 33-40. https://doi.org/10.2754/avb201079010033

Cohen, J. 1988. *Statistical power analysis for the behavioral sciences*, 2nd ed. New York: Academic Press, 1988. 590 p. ISBN-13: 978-0805802832.

Čech Barabaszová, K. 2012. *Vybrané instrumentální metody analýzy materiálů a nanomateriálů, vyd. 1 (Selected instrumental methods of materials and nanomaterials analysis, ed. the 1st)*. Brno : *Academic Publishing House CERM*. 194 p. ISBN 978-80-7204-810-6 (In Czech).

Dehghani, N., Afsharmanesh, M., Salarmoini, M., Ebrahimnejad, H., Bitaraf, A. 2018. Effect of pennyroyal, savory and thyme essential oils on Japanese quail physiology. *Heliyon*, vol. 4, no. 10. https://doi.org/10.1016/j.heliyon.2018.e00881

Desrues, O., Mueller-Harvey, I., Pellikaan, W. F., Enemark, H. L., Thamsborg, S. M. 2017. Condensed tannins in the gastrointestinal tract of cattle after sainfoin (*Onobrychis viciifolia*) intake and their possible relationship with anthelmintic effects. *Journal of Agricultural and Food Chemistry*, vol. 65, no. 7, p. 1420-1427. https://doi.org/10.1021/acs.jafc.6b05830

Dinh, T. T. N., Blanton, J. R Jr., Brooks, J. C., Miller, M. F., Thompson, L. D. 2008. A simplified method for cholesterol determination in meat and meat products. *Journal of Food Composition and Analysis*, vol. 21, no. 4, p. 306-314. https://doi.org/10.1016/j.jfca.2008.02.001

Dinh, T. T. N., Thompson, L. D., Galyean, M. L., Patterson, K. Y., Boylan, L. M. 2011. Cholesterol content and methods for cholesterol determination in meat and poultry. *Comprehensive Reviews in Food Science and Food Safety*, vol. 10, 5, p. 269-289. https://doi.org/10.1111/j.1541-4337.2011.00158.x

Evaris, E. F., Sarmiento-Franco, L., Sandoval-Castro, C. 2021. Meat and bone quality of slow-growing male chickens raised with outdoor access in tropical climate. *Journal of Food Composition and Analysis*, vol. 98 https://doi.org/10.1016/j.jfca.2021.103802

Goyal, L., Kaushal, S. 2018. Evaluation of Chemical Composition and Antioxidant Potential of Essential Oil from Citrus reticulata Fruit Peels. *Advances in Research*, vol. 15, no. 2, p. 1-9. https://doi.org/10.9734/AIR/2018/41981

Haščík, P., Eliman, I. O. E., Garlík, J., Kačániová, M., Bobko, M., Kňazovická, V., Vavrišinová, K., Arpášová, H., Bučko, O. 2012. Chemical composition of muscle after pollen application in nutrition of broiler chickens. *Potravinarstvo*, vol. 6, no. 2, p. 26-32. https://doi.org/10.5219/190

Haščík, P., Čech, M., Pavelková, A., Čuboň, J., Kačániová, M., Bobko, M. 2020. Porovnanie chemického zloženia vybraných druhov mäsa (Comparison of chemical composition of selected types of meat). In Proceeding on New Trends in Gastronomy, Hospitality and Tourism. Brno : College of Business and Hotel Management Ltd. p. 39-47. ISBN 978-80-7627-006-0 (In Slovak).

Kim, H. J., Kim, H. J., Jeon, J. J., Nam, K. C., Shim, K. S., Jung, J. H., Kim, K. S., Choi, Y., Kim, S. H., Jang, A. 2020. Comparison of the quality characteristics of chicken breast meat from conventional and animal welfare farms under refrigerated storage. *Poultry Science*, vol. 99, no. 3, p. 1788-1796. https://doi.org/10.1016/j.psj.2019.12.009

Jamroz, D., Wiliczkiewicz, A., Wertelecki T., Orda, J., Sukorupinska, J. 2005. Use of active substances of plant origininchicken diets based on m aize and locally grown cereals. *British Poultry Science*, vol. 46, no. 4, p. 485-493. https://doi.org/10.1080/00071660500191056

Kazemi, M., Mokhtariniya, S. 2016. Essential Oil Composition of Bark of *Cinnamomum zeylanicum*. *Journal of Essential Oil Bearing Plants*, vol. 19, no. 3, p. 786-789. https://doi.org/10.1080/0972060X.2016.1165151

Khaksar, V., Krimpen, M., Hashemipour, H., Pilevar, M. 2012. Effects of Thyme Essential Oil on Performance, Some Blood Parameters and Ileal Microflora of Japanese Quail. *The Journal of Poultry Science*, vol. 49, p. 106-110. https://doi.org/10.2141/jpsa.011089

Kiczorowska, B., Samolinska, W., Al-Yasiry, A. R. M., Kiczorowski, P., Winiarska-Mieczan, A. 2017. The natural feed additives as immunostimulants in monogastric animal nutrition – a review. *Annals of Animal Science*, vol. 17, p. 605-625. https://doi.org/10.1515/aoas-2016-0076

Koeth, R. A., Wang, Z., Levison, B. S., Buffa, J. A., Org, E., Sheehy, B.T. et al. 2013. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nature medicine,* vol. 19, p. 576-585. https://doi.org/10.1038/nm.3145

Koochaksaraie, R. R., Irani, M., Gharavysi, S. 2011. The effect of cinnamon powder feeding on some blood metabolites in broiler chicks. *Revista Brasileira de Ciencia Avicola*, vol. 13, no. 3, p. 197-201. https://doi.org/10.1590/S1516-635X2011000300006

Krauze, M., Cendrowska-Pinkosz, M., Matusevicius, P., Stepniowska, A., Jurczak, P., Ognik, K. 2021. The Effect of Asministration of a Phytobiotic Containing Cinnamon Oil and Citric Acid on the Metabolism, Immunity, and Growth Performance of Broiler Chickens. *Animals,* vol. 11, no. 2, p. 399. https://doi.org/10.3390/ani11020399

Kriaa, A., Bourgin, M., Potiron, A., Mkaouar, H., Jablaoui, A., Gerard, P., Maguin, E., Rhimi, M. 2019. Microbial impact on cholesterol and bile acid metabolism: Current status and future prospects. *Journal of Lipid Research*, vol. 60, p. 323-332. https://doi.org/10.1194/jlr.R088989

Lin, C. C., Wu, S. J., Chang, C. H., Ng, L. T. 2003. Antioxidant activity ofCinnamomum cassia. *Phytotherapy Research,* vol. 17, no. 7, p. 726-730. https://doi.org/10.1002/ptr.1190

Milićević, D., Vranić, D., Mašić, Z., Parunović, N., Trbović, D., Nedeljković-Trailović, J., Petrović, Z. 2014. The role of total fats, saturated/unsaturated fatty acids and cholesterol content in chicken meat as cardiovascular risk factors. *Lipids in Health and Disease*, vol. 13, p. 42-50. https://doi.org/10.1186/1476-511X-13-42

Mosca, F., Zaniboni, L., Stella, S., Kuster, C. A., Iaffaldano, N., Cerolini, S. 2018. Slaughter performance and meat quality of Milanino chickens reared according to a specific free-range program. *Poultry Science*, vol. 97, p. 1148-1154. https://doi.org/10.3382/ps/pex439

Ogawa, K., Hirose, S., Nagaoka, S., Yanase, E. 2015. Interaction between tea polyphenols and bile acid inhibits micellar cholesterol solubility. *Journalof Agriculturaland Food Chemistry*, vol. 64, no. 1, p. 204-209. https://doi.org/10.1021/acs.jafc.5b05088

Oluremi, O. I. A., Ngi, J., Andrew, I. A. 2007. Phytonutriens in citrus fruit peel meal and nutritional implication for livestock production. *Livestock Research for Rural Development*, vol. 19, no. 7. http://www.lrrd.org/lrrd19/7/olur19089.htm

Padre, R. G., Aricetti, J. A., Moreira, F. B., MizubutI, I. Y., Prado, I. N., Visentainer, J. V., Souza, N. E., Matsushita, M. 2006. Fatty acid profile, chemical composition of longissimus muscle of bovine steers and bulls finished in pasture system. *Meat Science*, vol. 74, p. 242-248. https://doi.org/10.1016/j.meatsci.2006.02.012

Safa, S. E. G., Al-Beitawi, N. A. 2009. The effect of feeding of crushed thyme (Thymus valgaris) on growth, blood constituents, gastrointestinal tract and carcass characteristics of broiler chickens. *Journal of Poultry Science*, vol. 4, p. 100-104.

Shang, H. M., Zhao, J. C., Guo, Y., Zhang, H. X., Song, H. 2020. Effect of supplementing feed with fermentation concentrate of *Hericium caput-medusae* (Bull.:Fr.) Pers. On cholesterol deposition in broiler chickens. *Livestock Science*, vol. 235. https://doi.org/10.1016/j.livsci.2020.104009

Simitzis, P. E., Symeon, G. K., Charismiadou, M. A., Ayoutanti, A. G., Deligeorgis, S. G. 2011. The effects of dietary hesperidin supplementation on broiler performance and chicken meat characteristics. *Canadian Journal of Animal Science*, vol. 91, p. 275-282. https://doi.org/10.4141/cjas10094

Sujiwo, J., Kim, D., Jang, A. 2018. Relation among quality traits ofchicken breast meat during cold storage: correlations betweenfreshness traits and torrymeter values. *Poultry Science*, vol. 97, p. 2887-2894. https://doi.org/10.3382/ps/pey138

Sweeten, M. K., Cross, H. R., Smith, G. C., Smith, S. B. 1990. Subcellular distribution and composition of lipids in muscle and adipose tissues. *Journal of Food Science*, vol. 55, no. 1, p. 43-45. https://doi.org/10.1111/j.1365-2621.1990.tb06012.x

Wu. Z., Li, H., Yang, Y., Zhan, Y., Tu, D. 2013. Variation in the components and antioxidant activity of Citrus medica L. var. sarcodactylis essential oils at different stages of maturity. *Industrial Crops and Products*, vol. 46, p. 311-316. https://doi.org/10.1016/j.indcrop.2013.02.015

Zampiga, M., Soglia, F., Petracci, M., Meluzzi, A., Sirri, F. 2019. Effect of different arginine-to-lysine ratios in broiler chicken diets on the occurrence of breast myopathies and meat quality attributes. *Poultry Science*, vol. 98, p. 2691-2697. https://doi.org/10.3382/ps/pey608

Zotte, A. D., Ricci, R., Cullere, M., Serva, L., Tenti, S., Marchesini, G. 2020. Research Note: Effect of chicken genotype and white striping-wooden breast condition on breast meat proximate composition and amino acid profile. *Poultry Science*, vol. 99, no. 3, p. 1797-1803. https://doi.org/10.1016/j.psj.2019.10.066

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