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# THE EFFECT OF OREGANO ESSENTIAL OIL ON CHICKEN MEAT LIPID OXIDATION AND PEROXIDATION

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## ABSTRACT

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The study aimed to investigate and evaluate the oxidative stability of chicken thighs with skin stored in freezing conditions due to the effect of oregano essential oil for various times. The results were compared with a control group without the use of oregano essential oil. Samples of chicken thighs with skin were obtained from an experiment performed on a poultry farm in a deep litter breeding system. The results obtained from the application of oregano essential oil to chicken thighs with skin did not show a statistically significant difference (p > 0.05) in the dry matter content, fat content and acid value compared to the control group, where coccidiostats were used in starter and growth feed mixtures. A statistically significant difference was found in the peroxide value by applying oregano essential oil to chicken thighs with skin compared to a control group containing coccidiostats in starter and growth feed mixtures when stored for 1 day at room temperature ( $p \le 0.01$ ) and 12 months in freezing conditions at -18 °C ( $p \le 0.05$ ) and a statistically non-significant difference (p > 0.05) when thighs with skin were stored for 6 and 9 months in -18 °C freezing conditions. In the conclusion, it was stated that maintaining the oxidative stability of chicken meat means knowing the factors that affect it and prepare the conditions for its maintenance. Chicken meat is generally susceptible to oxidative damage because it is characterized by a high concentration of polyunsaturated fatty acids. With a sufficient amount of effective antioxidants, chicken meat could be a homoeostatic system, but it remains limited or free of oxidized compounds and reactive components. These questions are the subject of further research in the field of oxidative stability of chicken meat.

Keywords: oregano essential oil; chicken thigh with skin; freezing; storage; oxidative

# **INTRODUCTION**

In addition to microbial degradation, lipid oxidation is a major cause of quality degradation and one of the main factors limiting the quality and acceptability of meat and meat products. Products that result from the peroxidation of polyunsaturated fatty acids affect meat quality parameters and reduce shelf life. in addition, many of these peroxidation products, including malondialdehyde, are toxic, genotoxic, cause intracellular oxidative stress, and could increase the frequency of tumor and atherosclerosis. Consumption of foods containing malondialdehyde, therefore, poses a risk to human health. The use of antioxidants is one of the main strategies to prevent the oxidation of lipids in meat and meat products. Although most synthetic antioxidants show high efficacy at low concentrations and are classified as a grass, i.e. generally accepted as safe, the acceptable dosage of these substances is low and the potential health risk is limited by their widespread use. In addition, in recent years, consumer concerns about the relation between food composition and human health and the increasing demand for natural-based foods have resulted in the food industry's interest in alternative methods to prevent and slow down lipid oxidation. One of these alternatives is the use of plant extracts, especially essential oils (Moghrovyan et al., 2019).

Oxidation significantly reduces the shelf life of prepared meat products. The addition of antioxidants to foods can be an effective solution to reduce oxidation and maintain its properties such as softness, juiciness, palatability, and shelf life. Both natural and synthetic antioxidants are available. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), can effectively inhibit oxidation-induced adverse changes in meat products, but these antioxidants can have potential genotoxic effects. Therefore, the consumer prefers natural antioxidants (Feng et al., 2017).

Oregano essential oil, as well as thyme essential oil, is among the 10 most popular essential oils used as preservatives in food. Both are categorized as GRAS by the Food and Drug Administration. Due to the high content of phenolic compounds, these essential oils are effective antioxidants. Standardization of natural preservatives is needed (Boskovic et al., 2019).

Poultry meat is particularly susceptible to oxidative damage due to its high concentration of polyunsaturated fatty acids. The rate and extent of lipid oxidation in muscle tissue have been found to depend on the degree of muscle tissue damage during handling before the slaughter of broiler chickens, such as stress and physical injury, as well as after slaughter, such as early postmortem conditions, pH and carcass temperature.

Under practical conditions, synthetic antioxidants such as butylated hydroxytoluene or butylated hydroxyanisole are often used as antioxidants. However, there is a trend to look for compounds that allow the transition from synthetic to natural antioxidants. The activity of *Origanum vulgare* L. is mainly attributed to its main components carvacrol and thymol, substances that modify the permeability of the bacterial cell membrane and react with lipid and hydroxyl radicals, which convert them into stable products (Luna et al., 2010).

**Scientific hypothesis**: Oregano essential oil supports the oxidative stability of chicken thigh muscle fat.

# MATERIAL AND METHODOLOGY

#### Technique carried out of the experiment

The feeding experiment was carried out under practical conditions on a broiler chicken farm. A hybrid combination of Cobb 500 was used as the final fattening type of broiler chickens. A feeding experiment was performed to obtain samples for chemical analyzes. Each box had dimensions of 2 m wide and 3.8 m long, which is following the principle of unrestricted movement of broiler chickens, i.e. 33.0 kg live weight per m<sup>2</sup> when removed from storage. 100 one-day-old chickens were placed in each group. The experiment in practical conditions lasted 40 days. Broiler chickens were fed from plate feeders and sucked water from hat feeders until the age of 14 days, and later until the end of the experiment, they received a feed from tube feeders and drank water from bucket feeders. Feed mixtures starter, growth, and final were used for feeding, which were the same in the basic raw materials in both the control and experimental groups. The difference in feed mixtures between the control and experimental groups was in the active substance added by the feed supplement in the control group and the additive applied to the thighs after the slaughter of the broiler chickens in the experimental group. The control group used a commercially produced feed mixture starter in chickens aged 1 to 14 days with the feed supplement coccidiostat Maxiban G160, a growth feed mixture in chickens aged 15 to 33 days with a feed supplement coccidiostat Sacox and a final feed mixture aged 34 to 40 days. In the experimental group, the feed mixtures were the same as in the control group, but without coccidiostats. Feed mixtures were manufactured by Biofeed, a.s., Kolárovo, where they were manually mixed from feed materials 4 times in a row.

#### Sample preparation for laboratory measurements

Broiler chickens were randomly selected at the end of the feeding experiment in the number of 24 pcs from the control group (which means obtaining 48 pcs of thighs) and 24 pcs from the experimental group (i.e. 48 pcs of thighs). Broiler chickens were killed humanely and

technologically processed. The thighs were separated from the carcass in a total of 48 pcs in each group. Each thigh was packed in a microtene bag and labeled with K (control group) and P (experimental group). All thigh samples with the skin from the control and experimental groups were boned. The thigh samples with the skin of the control group were packed in a microtene bag and marked K (control group) and further marked 1, 6, 9, and 12, which means 12 thighs for analysis depending on the different storage time (1 means 1 day after slaughter the broiler chickens and kept at room temperature, 6, 9 and 12 means 6, 9 and 12 months after the slaughter of broiler chickens and kept under freezing storage conditions at -18 °C.

#### Application of oregano essential oil to the chicken thighs in the experimental group and their storage

Oregano essential oil was applied to each thigh sample with the skin of the experimental group. A thigh sample was placed in a porcelain mortar and 4.0 mL of oregano essential oil was applied to the surface of the entire thigh. Oregano essential oil was applied with a syringe and handthoroughly spread over the entire thigh surface using surgical gloves.

Thigh samples with the skin of the experimental group were packed in a microtene bag and marked P (experimental group) and another designation 1, 6, 9, and 12, which means 12 thighs for analysis depending on different storage time (1 means 1 day after slaughter broiler chickens and application of oregano essential oil and stored at room temperature, 6, 9 and 12 means 6, 9 and 12 months after the slaughter of broiler chickens and application of oregano essential oil and stored in freezing conditions -18 °C.

Each thigh with skin wrapped in a microtene bag and labeled was stored under the specified conditions according to the scheme in Table 1.

Oregano essential oil was obtained as a commercial additive from Calendula, a. s., Nová Ľubovňa. Oregano essential oil was accompanied by an A-test. The main components of oregano essential oil according to Özkan et al. (2017) are carvacrol (63.97%), p-cymene (12.63%), linalool (3.67%),  $\alpha$ -terpineol (2.54%), and (-)- terpinen-4-ol (2.24%).

# **Examined indicators**

A feeding experiment was performed to obtain samples for chemical analysis. Chemical analysis of thigh samples was aimed at determining:

(a) the dry matter of thigh muscle with skin depending on the storage period,

(b) the fat of thigh muscle with skin depending on the storage period,

(c) the acid value of thigh muscle with skin depending on the storage period,

(d) the peroxide value of the thigh muscle with the skin depending on the storage period.

# The procedure of investigation established indicators *Weighing of samples*

All samples and laboratory equipment requiring weighing for chemical analyzes of the thigh were performed on automatic scales of the Kern 440-49N type with an accuracy of d = 0.01 g.

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Group	n (broilers)	Thigh samples prepared for storage	n (thighs)	Weight of grinded thigh samples prepared for chemical analysis, g
Control	24	1 day	12	70
		6 months	12	70
		9 months	12	70
		12 months	12	70
Experimental	24	1 day	12	70
1		6 months	12	70
		9 months	12	70
		12 months	12	70

Note: thigh sample: boned thigh with skin and in the experimental group with oregano essential oil application,

1 day after slaughter of broiler chickens and in the experimental group after application of oregano essential oil, stored samples at room temperature,

6 months after the slaughter of broiler chickens and in the experimental group after application of oregano essential oil, stored samples at under freezing conditions -18 °C,

9 months after the slaughter of broiler chickens and in the experimental group after application of oregano essential oil, stored samples at under freezing conditions -18 °C,

12 months after the slaughter of broiler chickens and in the experimental group after application of oregano essential oil, stored samples at under freezing conditions -18 °C,

weight of sample for chemical analysis – weighed 70 g from each mixed thigh with skin.

#### Grinding and homogenization of samples

The AOAC Official Method 983.18 which is Codex-Adopted - AOAC Method especially for Meat and Meat Products was used for the samples preparation.

Before each chemical analysis, each sample was individually ground and homogenized in a Grindomix 200 laboratory mixer and quantitatively transferred to a glass laboratory beaker. 70 g of the homogenized fresh mass of each thigh sample was weighed.

#### The procedure for analyzed sample drying

Sea sand was poured to the bottom of the petri dish. A petri dish with sea sand was weighed. The homogenized sample was transferred from the mixer to sea sand of the petri dish and the sample thus prepared was weighed. The prepared samples with Petri dish and sea sand for chemical analysis were dried in an oven of type HS 62A at a temperature of  $103 \pm 2$  °C for 12 hours to constant weight. The dried samples together with the petri dish and sand were transferred to a desiccator using laboratory tongs. The samples in the desiccator were allowed to cool. Cooled samples with a petri dish and sand were weighed. The difference between the weight of the dried sample with the petri dish together with the sea sand and the weight of the petri dish together with the sea sand represented the sample for chemical analysis. The weight of each dried thigh sample with skin ranged on average 19.3 g.

#### Dry matter per fresh mass

The standard reference method (AOAC Method 950.46) for measurements of moisture in meat was used.

Analyzed samples of thigh muscle of 5.0 g were placed in the pre-weighed aluminum dishes  $\geq 50$  mm diameter and  $\leq$ 40 mm deep then placed in the drying oven (type: HS 62A) at temperature 103  $\pm 2$  °C for 4 hours to a constant weight. Samples in aluminum dishes were partially dried covered. Samples with covered aluminum bowls after drying were

transferred to the desiccator to cool. Then the dishes and their dried samples were reweighed.

Calculation of dry matter per fresh mass of thigh muscle with skin and dry matter of analyzed sample:

$$Dry mater = ----- x \ 100 \tag{1}$$

where:

dry matter per fresh mass and dry matter of the analyzed sample (same calculation procedure), %,

a = weight of the dried sample, g,

я

n = sample weight, g.

#### The procedure for analyzed sample extraction

The fat content of the samples was determined 1 day after the slaughter of the broiler chickens, 3, 6, 9, and 12 months after the slaughter of the broiler chickens. The samples were stored for 1 day at room temperature, 6, 9, and 12 months in freezing conditions at -18 °C. The analyzed sample (dried) was extracted with boiling petroleum ether, the fat is determined by evaporation of the extraction system by mass.

The AOAC Official Method 991.36 for Fat (Crude) in Meat and Meat Products was used. In this experiment were used petroleum ether as a solvent and Det-gras N apparatus, Model 4002842 - capable of simultaneous extraction of 6 test portions.

#### Procedure for determining the fat of the analyzed sample

Empty aluminum cartridges were weighed, and 6 pcs were needed for one analysis. Each assay sample was first mixed in a Grindomix 200 laboratory mixer from which 12 g was weighed. Each weighed sample was transferred to an extraction cellulose cartridge, which was sealed with

cotton wool. The filled sample cartridge was placed in an aluminum cartridge and connected to a Detras N-type extractor. The operating temperature on the apparatus was 120 °C and the fat extraction itself took 60 minutes. The fat extraction from the analysis sample was into the aluminum cartridge of the instrument. After the fat extraction was complete, the remaining petroleum ether was evaporated from the fat sample and each aluminum fat cartridge was weighed. The difference between the weight of the aluminum grease cartridge and the weight of the empty aluminum cartridge was considered to be the fat of the analysis sample. The weight of each fat analysis sample ranged from 8.55 to 9.97 g.

Calculation of the fat for an analysed sample of the thigh muscle with skin:

$$Fat = ---- x 100$$
b
where:
(2)

where:

fat of the analysed sample, %,

a = weight of fat extracted, g,

b = weight of the analysed sample, g.

#### Acid value

The acid value was determined in fat samples performed 1 day after the slaughter of the broiler chickens, 6, 9, and 12 months after the slaughter of the broiler chickens. The samples were stored for 1 day at room temperature, 6, 9, and 12 months in freezing conditions at -18 °C. The acid value expresses the quantity of potassium hydroxide needed to neutralize the free fatty acids present in 1.0 g of fat (mg KOH.g<sup>-1</sup>). The acid number of the fat expresses the amount of potassium hydroxide (KOH) in mg needed to neutralize the fatty acids in 1 g of the sample.

The acid value is the quantity of potassium hydroxide (KOH) in mg necessary to neutralize the fatty acids in 1 g of sample.

 $RCOOH + KOH \rightarrow RCOOK + H_2O$ 

Often, the acid value is converted to a free fatty acid (FFA) content by multiplying the acid value with a factor that equals the molecular weight of the fatty acid concerned (usually oleic acid, MW = 282.4) divided by ten times the molecular weight of the potassium hydroxide (56.1). This factor ten stems from the fact that the acid value is expressed as mg.g-1, whereas the free fatty acid content is expressed as a percentage. When the free fatty acid content is expressed as 'wt% oleic acid,' this factor, therefore, equals 0.50. When examining the risk from food fat, the acid value expresses the degree of hydrolysis of fats. It is an indicator for evaluating the condition of processed food raw material and its state in the storage conditions.

#### Principle of determining the acid value

The principle of determining the acid value is to dissolve the fat in the extract with an ethanol-diethyl ether mixture using alkalimetric titration in the presence of phenolphthalein.

#### Procedure for determining the acid number of fat

First, 25.0 mL burettes and extraction flasks were prepared. 2.5 g of fat was weighed into each extraction flask, and a 1:1 mixture of ethanol-diethyl ether in a volume of 25 mL was added. Before titration, 2 drops of phenolphthalein indicator were added to the mixture in the extraction beaker. Each extraction beaker was slightly heated with a laboratory gas burner. The burette was made up to 25.0 mL with potassium hydroxide solution before each measurement of the sample. The titration was completed when the sample in the extraction flask acquired a pale pink color, which had a shelf life of 30 seconds. Calculation:

V

$$AV = \frac{1}{n} x T$$
(4)

where:

 $AV = acid value, mg KOH.g^{-},^{1}$ 

V = quantity of standard KOH solution during titration, mL

n = weight of extracted fat, g, i.e. 2.5 g,

T = concentration of KOH in the measuring solution,  $mg.mL^{-1}$  (at the concentration of the solution exactly c (KOH) = 0.05 mol.L<sup>-1</sup> is T = 2.8055 mg.mL<sup>-1</sup>).

The weight of extracted fat was determined based on the results of the assumed acid value in the thighs of broiler chickens according to Tkačová and Angelovičová (2013), Klimentová (2019).

#### Peroxide value

Fat peroxide value was determined in fat samples performed 1 day after slaughter of broiler chickens, 6, 9, and 12 months after the slaughter of broiler chickens. The samples were stored for 1 day at room temperature, 6, 9, and 12 months in freezing conditions at -18 °C. The peroxide number is an indicator to examine the oxidation of fats. It expresses the amount of active oxygen in 1.0 g of fat ( $\mu$ mol O<sub>2</sub>.g<sup>-1</sup>). The peroxide value is defined as the amount of peroxide oxygen per 1 kg of fat or oil. Traditionally this was expressed in units of milliequivalents, although if we are using SI units then the appropriate option would be in mmol per kg (N.B. 1 milliequivalents = 0.5 mmol; because 1 mEq of  $O_2 = 1$  $mmol/2 = 0.5 mmol of O_2$ , where 2 is valence). The unit of milliequivalent has been commonly abbreviated as mequiv or even as meq.

The peroxide value is determined by measuring the amount of iodine that is formed by the reaction of peroxides (formed in fat or oil) with iodide ions.

$$2 I^{-} + H_2O + HOOH -> HOH + 2OH^{-} + I_2$$
 (5)

The base produced in this reaction is taken up by the excess of acetic acid present. The iodine liberated is titrated with sodium thiosulphate. (6)

$$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2 I^-$$

The acidic conditions (excess acetic acid) prevent the formation of hypoiodite (analogous to hypochlorite), which would interfere with the reaction.

The indicator used in this reaction is a starch solution where amylose forms a blue to the black solution with iodine and is colorless where iodine is titrated.

A precaution that should be observed is to add the starch indicator solution only near the endpoint (the endpoint is near when fading of the yellowish iodine color occurs) because at high iodine concentration starch is decomposed to products whose indicator properties are not entirely reversible.

#### Principle of determination of peroxide value

The principle of determining the peroxide value is the determination of iodine by titration during its release from iodide by hydroperoxide-unsaturated lipids in an acidic medium.

#### Procedure for the determination of the peroxide value

Erlenmeyer flasks with ground glass joint and glass stoppers and a starch indicator were prepared. 5.0 g of starch was weighed, to which 30.0 mL of water was added. The mixture was mixed thoroughly. 2.0 g of fat was weighed and transferred to each Erlenmeyer flask. Chloroform (10.0 mL) was added to the fat. The flask was then closed and the contents were shaken well by hand until the fat was completely dissolved.

Saturated 1.0 mL aqueous potassium iodide solution and 15.0 mL concentrated acetic acid were added to the dissolved sample. The flask was immediately closed and the contents were manually shaken again for 1 minute. Following this procedure, the sample was stored in a closed flask in a dark place at room temperature with the windows covered with blinds for exactly 5 minutes. Then 75.0 mL of water was added to the sample in the flask and again the contents were shaken thoroughly by hand in a closed flask. After mixing, a prepared starch indicator of 5.0 mL (starch solution) was added to the contents of the flask. The sample thus prepared was titrated with a solution of sodium thiosulfate 0.01 mol.L<sup>-1</sup> c (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O). Its concentration was determined by discoloration. In parallel with the determination of the peroxide value of the fat sample, a blank experiment of the fat-free sample was performed.

Calculation:

$$PV = \frac{[(V_1 - V_0) \times T \times 1000]}{n}$$
(7)

where:

 $PV = peroxide value, \mu mol O_2.g^{-1}$ ,

 $V_0$  = sodium thiosulphate standard solution used in the blank, mL,

 $V_1$  = sodium thiosulphate standard solution used for sample titration, mL,

T = concentration of sodium thiosulphate standard solution used, mol.L<sup>-1</sup>,

n = weighed sample of fat, g.

The weight of extracted fat was determined based on the results of the presumed peroxide value in the thighs of broiler chickens according to Tkačová and Angelovičová (2013), Klimentová (2019).

#### Statistical analysis of results

The data obtained by the measurements were statistically evaluated according to statistical characteristics such as mean (*M*), average value, and standard deviation (*SD*). The difference in the indicator between the groups with each other, i.e. two dependent selections of control and experimental groups were evaluated by Student's t-test. The value of the t-test was commented as statistically significant  $p \le 0.05$  and  $p \le 0.01$ , and statistically non-significant  $p \ge 0.05$ . The statistical evaluation of the results was by the methods of the SAS program package version 8.2.

# **RESULTS AND DISCUSSION**

# Dry matter in thigh muscle with skin

The average dry matter content in the thigh muscle with skin 1 day, 6, 9, and 12 months after the slaughter of broiler chickens is given in Table 2.

Table 2 Average dry matter content in the chicken thigh muscle with ski	n, g.100 g <sup>-1</sup> .

8 1	8 ,8			
Storage time thigh muscl	e Group	n	$M \pm SD$	t-test
1 day at room temperatur	e control	12	26.91 ±2.03	0.241-
	experimental	12	27.21 ±1.85	0.341-
6 months at -18 °C	control	12	$27.17 \pm 1.44$	0,701-
	experimental	12	28.31 ±1.53	0,701
9 months at -18 °C	control	12	$27.89 \pm 1.41$	0,401-
	experimental	12	$28.26 \pm 2.01$	0,401
12 months at -18 °C	control	12	$28.41 \pm 1.52$	0, 302-
	experimental	12	28.72 ±1.52	0, 302

Note: n = multiciplity, M = mean, SD = standard deviation,

numerical values in the column at t-test marked -: statistically non-significant difference between the control and experimental group (p > 0.05).

In recent years, it has become common use prophylactic drugs and antibiotic growth stimulants in the feeding of broiler chickens and other farm animals. However, the continued use of these compounds has led to results such as the development of resistant bacteria and antibiotic residues in meat and other animal products that pose a risk to public health and the environment. This concern has led many countries, including European Union countries, to limit the use of antibiotics in feed. Therefore,

it is necessary to identify safe alternatives to feed antibiotics (Jazi et al., 2020).

In our study, we focused on the verification of oregano essential oil by application to chicken thighs in relation to oxidative stability depending on the storage time under freezing conditions. Positive results in the application of essential oils whether in feed or on the product itself, are achieved by the effects of bioactive substances contained in the essential oils. These bioactive compounds include hydrocarbons, phenols, esters, alcohols, acids, and steroids, which have a positive effect on the health of broiler chickens and their performance or meat quality (Shirani et al., 2019). **Sharma et al. (2020)** state that essential oils can influence the performance and physiological processes of broiler chickens, both quantitatively and qualitatively.

The aim of the study by Sabikun et al. (2019) was to investigate the *post-mortem* effects on physico-chemical properties and oxidative stability of chicken thigh and breast muscles depending on the storage time in freezing conditions. Chicken breast and thigh muscles were obtained from 24 broiler chickens and measured from slaughter for 30 minutes or 1.5 hours. They were immediately frozen at -75 °C and then stored at -20 °C for 90 days to measure meat quality characteristics. The results showed that longer freezing led to the deterioration of meat quality and greater deterioration of post-rigid frozen muscles. Frozen muscles before stiffening had a higher pH, water holding capacity (about 90%) and sarcomere length with lower melting point and boiling loss than muscles after solidification. The thigh muscles had better physico-chemical properties than the breast muscles, except for loss during cooking. Therefore, immediate freezing could be an effective way to minimize the deterioration of the quality of frozen chicken.

The condition of *rigor-mortis* or *post-mortem* causes various physical and biochemical changes in the muscles through the activity of the proteolytic system after the slaughter of broiler chickens. This proteolytic system is associated with qualitative features such as sensitivity, juiciness, water retention, taste, protein, and structural muscle degradation (Carvalho et al., 2017).

When frozen chicken meat is thawed, the muscles lose weight and lose a lot of water, leading to a reduction in the juiciness of the meat. Their research aimed to evaluate preand post-rigor frozen muscles concerning the quality of chicken meat. The research focused on changes in muscle pH, color, water retention capacity, heating losses, cooking losses, shear strength, sarcomere length, and oxidative stability before and after solidification of frozen thigh and breast muscles during different storage times for 90 days in freezing conditions (-20 °C). Based on the results, a conclusion was formulated. Prolonged post-mortem aging before freezing could have adverse effects on the quality characteristics of frozen chicken muscles. The results showed positive effects of freezing before the onset of rigor-mortis (within 30 minutes after the slaughter of broiler chickens) on the quality of chicken muscles. Frozen muscles before solidification had a higher pH, lower dripping, shorter thawing, and less cooking losses than frozen muscles after solidification (Sabikun et al. (2019).

We recorded the average dry matter content of the thigh muscle with skin 1 day after the slaughter of broiler chickens and kept at room temperature in the experimental group of 27.21 g.100 g<sup>-1</sup> compared with the average dry matter content of the thigh muscle with skin in the control group of 26.91 g.100 g<sup>-1</sup>. The difference in dry matter content with skin 1 day after the slaughter of broiler chickens and kept at room temperature was not statistically significant (p > 0.05) between the control and experimental groups. Based on the statistical evaluation of the results expressed by the standard deviation, we can state that the measured values of dry matter content in thigh muscle with skin kept at room temperature 1 day after the slaughter of broiler chickens fluctuated more in the control group compared to the experimental group (SD = 2.03 versus SD = 1.85).

Freezing is the preferred way to preserve food on the world meat export market at more than \$ 13 billion a year. Despite their ability to maintain the quality and safety of meat, problems with freezing and thawing cycles remain a major problem for processors and consumers. Temperature fluctuations or abuse caused by the freeze-thaw cycle stimulate lipid oxidation and accelerated discoloration of the meat surface. Temperature fluctuations, which are a major problem in the cold-chain meat industry, especially in developing countries, are related to physiological and biochemical changes in animal muscles. The storability of meat is usually determined by its appearance, structure, color, aroma, microbial activity, and nutritional value and is affected by frozen storage and subsequent thawing. The main damage to frozen meat during storage is caused by the breakdown of fats and proteins. The meat industry has a strong interest in verifying a meat product that is actually fresh or that has been previously frozen, due to the large price differences between fresh, frozen, and thawed meat. It is difficult for consumers to detect changes in the quality of meat that occur in some products when they have been frozen. Most research on freezing and thawing red meat focuses on reducing moisture loss. Water loss is directly proportional to water retention capacity by muscle proteins, and reduced water content alters key quality parameters such as color and texture. The freeze-thaw cycle is a major contributor to reduced water retention capacity and unacceptably low water retention capacity causes great deterioration of products. However, limited progress has been made in understanding the real mechanisms for changing meat quality in freeze-thaw cycles. The reduction in water retention capacity is directly related to the denaturation of proteins in the muscle fiber structure. Protein oxidation due to freeze-thaw cycles has been largely ignored, especially in the commercial broiler chicken production chain. Therefore, studies are needed to understand the effect of freeze-thaw cycles on protein stability and its relation to lipid and protein oxidation. The result of increasing freezing and thawing cycles is a higher degree of oxidation of lipids and proteins, as evidenced by a higher content of malondialdehyde and carbonyl compounds and a lower content of sulfhydryl groups. Repeated freeze-thaw cycles increase lipid and protein oxidation and reduce water retention and color fastness of chicken meat (Ali et al., 2015).

We recorded an average dry matter content in the thigh muscle with skin stored for 6 months in freezing conditions at -18 °C after the slaughter of broiler chickens in the experimental group of 28.31 g.100 g<sup>-1</sup>. This value is slightly higher than compared to the control group of 27.17 g.100 g<sup>-1</sup>. The difference in dry matter content of the thigh muscle with skin stored for 6 months in freezing conditions at -18 °C after the slaughter of broiler chickens was not statistically significant (p > 0.05) between the control and experimental groups. By statistical evaluation of the results expressed by the standard deviation, we found that the measured values of the dry matter content of thigh muscle with skin stored for 6 months in freezing conditions at -18 °C after the slaughter of broiler chickens fluctuated more in the experimental group compared to control group (SD = 1.53 vs. SD = 1.44).

The average value of dry matter content in the thigh muscle with skin was measured in the experimental group of 28.26 g.100 g<sup>-1</sup> for 9 months of our storage in freezing conditions at -18 °C after the slaughter of broiler chickens and in the control sample 27.89 g.100 g<sup>-1</sup>. The value of the experimental group was slightly higher. The difference in dry matter content of the thigh muscle with skin stored for 9 months in freezing conditions at -18 °C after the slaughter of broiler chickens was not statistically significant (p > 0.05) between the control and experimental groups. According to the statistical evaluation expressed by the standard deviation, we found that the measured values of dry matter content in the thigh muscle with skin, which were stored for 9 months in freezing conditions at -18 °C after the slaughter of broiler chickens fluctuated more in the experimental group than in the control group (SD = 2.01 vs. SD = 1.41).

After 12 months from the slaughter of broiler chickens kept in freezing conditions at -18 °C, the average dry matter content in the thigh muscle with the skin of the experimental group was measured 28.72 g.100 g<sup>-1</sup> and in the control sample 28.41 g.100 g<sup>-1</sup>. The difference in the dry matter content of the thigh muscle with skin stored for 12 months in freezing conditions at -18 °C after the slaughter of broiler chickens was not statistically significant (p > 0.05) between the control and experimental groups.

Statistical evaluation of the results, which are expressed by the standard deviation, we found that the measured values of the dry matter content of the thigh muscle with skin stored for 12 months in freezing conditions at -18 °C after the slaughter of broiler chickens was lower in the experimental group compared to control group (SD = 1.36vs. SD = 1.52).

To compare the dry matter content in the thigh muscle of our results, we selected the results of **Haščík et al. (2012)** for a hybrid combination of Ross 308 broiler chickens. Their results are higher compared to ours. They recorded dry matter content  $31.51 \text{ g}.100 \text{ g}^{-1}$  in the control group in which the broiler chickens were fed a feed mixture with coccidiostats and  $30.21 \text{ g}.100 \text{ g}^{-1}$  or  $29.88 \text{ g}.100 \text{ g}^{-1}$  in the experimental group with pollen extract with different doses.

In an experiment with broiler chickens, **Semjon et al.** (2020) investigated by chemical analysis the dry matter content in the thigh muscle of the Cobb 500 hybrid combination, which measured slightly lower values compared to our results. These authors report 26.21 g.100  $g^{-1}$  and 26.04 g.100  $g^{-1}$  of dry matter content in the thigh muscle. Their object of research with broiler chickens was different doses of humic substances in feed mixtures.

## Fat in thigh muscle with skin

The average fat content in the thigh muscle with skin 1 day, 6, 9, and 12 months after the slaughter of broiler chickens is given in Table 3.

The fat content of chicken meat is closely related to the nutrition and feeding of broiler chickens. The lipid profile in these tissues can be assumed to reflect the lipid profile of the feed (type and dose) (Sirri et al., 2003).

The interactions that take place between the nutrients that make up the feed and the synthesis and activity of lipogenic enzymes are responsible for a wide range of lipid storage options in adipose tissue. In addition, the biological activity of some fatty acids stimulates or inhibits specific lipogenic genes encoding enzymes (Jump, 2002).

Sierżant et al. (2018) state in their study that meat obtained from broiler chickens is a source of the high biological value of animal proteins and is therefore valued for the consumer. On the other hand, the consumer prefers chicken meat due to its low-fat content and low energy value compared to meat from large livestock.

Fats are vital substances for proper human nutrition. In addition to providing energy for the body's biological processes, fats contain large amounts of substances such as essential fatty acids or fat-soluble vitamins that only a diet can provide. On the other hand, when obtaining fats from food, it is necessary to know the conditions for their qualitative attributes (**Purriños et al., 2011**), in our case chicken thigh muscle, such as the focus of this study in the methodology.

Fats are involved in processes that affect the taste of meat and contribute to improving its tenderness and juiciness (Amaral et al., 2018).

Therefore, the fat content and its composition are crucial for consumers due to their importance for meat quality and nutritional value (Wood et al., 2004).

However, fats are prone to degradation. Fat oxidation is a major non-microbial cause of deterioration in the quality of meat and meat products (Lorenzo et al., 2012).

Degradation of the chicken begins with the killing of the broiler chicken and continues gradually until the final product is consumed (Chaijan et al., 2017).

Table 3 Average fat content in the chicken thigh muscle with skin, g.100 g<sup>-1</sup>.

Storage time thigh muscle	Group	n	M ±SD	t-test
1 day at room temperature	control	12	2,56 ±0.16	0.222-
	experimental	12	$2.77 \pm 0.09$	0.332-
6 months at -18 °C	control	12	$2.68 \pm 0.15$	0.000-
	experimental	12	$2.57 \pm 0.09$	0.208-
9 months at -18 °C	control	12	$2.72 \pm 0.18$	0.621-
	experimental	12	$2.66 \pm 0.23$	
12 months at -18 °C	control	12	2.76 ±0.13	0.271-
	experimental	12	2.52 ±0.19	0.371-

Note: n = multiciplity, M = mean, SD = standard deviation; numerical values in the column at t-test marked -: statistically non-significant difference between the control and experimental group (p > 0.05).

Therefore, all stages of the processes of handling broiler chickens and chicken meat, processing and storage of chicken meat must be carefully controlled to avoid possible spoilage (**Richards**, 2006) and to minimize economic losses in the meat industry (**Králová**, 2015).

We recorded the average fat content of the thigh muscle with skin 1 day kept at room temperature in the experimental group of 2.77 g.100 g<sup>-1</sup> after the slaughter of broiler chickens compared to the average fat content in the thigh muscle with the skin of the control group of 2.56 g.100 g<sup>-1</sup>. The difference in the fat content of the thigh muscle with the skin was not statistically significant (p > 0.05) between the control and experimental groups. Based on the statistical evaluation of the results expressed by the standard deviation, we can state that the measured values of fat content in the thigh muscle with skin kept at room temperature 1 day after the slaughter of broiler chickens fluctuated more in the control group compared to the experimental group (SD = 0.16 vs. SD = 0.09).

In thigh muscle with skin stored for 6 months in freezing conditions at -18 °C after the slaughter of broiler chickens, the average fat content in the experimental group was recorded at 2.57 g.100 g<sup>-1</sup>. This value is lower compared to a control sample of 2.68 g.100 g<sup>-1</sup>.

The difference in fat content in the thigh muscle with skin stored for 6 months in freezing conditions at -18 °C after the slaughter of broiler chickens was not statistically significant (p > 0.05) between the control and experimental groups. Statistical evaluation of the results expressed by the standard deviation, we found that the measured values of thigh muscle fat content with skin stored for 6 months in freezing conditions at -18 °C after the slaughter of broiler chickens fluctuated more in the control group compared to the experimental group (SD = 0.15 and SD = 0.09). We found the average value of fat content with skin stored for 9 months in freezing conditions at -18 °C after the slaughter of broiler chickens in the experimental group 2.66 g.100 g<sup>-1</sup> and the control group slightly higher 2.72 g. 100 g<sup>-1</sup>. Statistical evaluation of the results expressed by the standard deviation, we found that the measured values of thigh muscle fat content with skin stored for 9 months in freezing conditions at -18 °C after the slaughter of broiler chickens fluctuated more in the experimental group compared to the control group (SD = 0.23 against SD = 0.18).

After 12 months from the slaughter of the broiler chickens when the thigh muscles were stored in freezing conditions at -18 °C, the average fat content in the thigh muscle with the skin of the experimental group was found 2.52 g.100 g<sup>-1</sup> versus control group 2.76 g.100 g<sup>-1</sup>. The difference in the fat content of the thigh muscle with skin stored for 12 months under freezing conditions at -18 °C was not statistically significant (p > 0.05) between the control and experimental groups. By statistical evaluation of the measured results, which are expressed by the standard deviation, we found that the values of fat content in the thigh muscle with the skin of the experimental group stored for 12 months in freezing conditions at -18 °C fluctuated more compared to control samples (SD = 0.19vs. SD = 0.13).

Freeze storage cannot prevent oxidative degradation and microbial or enzymatic degradation (Jay et al., 2005).

It has been known in the past, as is the case today, that chemical preservation methods for meat are quite beneficial in combination with refrigeration to optimize stability, product quality while maintaining freshness and nutritional value (Cassens, 1994).

As reported by Ali et al. (2015), the main degradation of frozen meat during storage is caused by the processes of fat and protein degradation. It is through these changes that it is possible to find out whether a given product is fresh or has been frozen before. Especially in the meat industry, this is important from a price point of view, as fresh products have a higher price than frozen or thawed products.

All foods that contain lipids, even in very small amounts (<1%), can undergo oxidation, leading to yellowing **(Wąsowicz et al., 2004)**.

Common livestock species, such as beef and lamb (ruminants), contain higher levels of saturated fatty acids in muscle mass and adipose tissue compared to chicken meat, which contains higher levels of polyunsaturated fatty acids (Wood et al., 2008).

#### Acid value in the thigh muscle with skin

The average acid value in the thigh muscle with skin 1 day after the slaughter of broiler chickens, 6, 9, and 12 months from the slaughter of broiler chickens is given in Table 4.

Storage time thigh muscle	Group	n	M ±SD	t-test
1 day at room temperature	control	12	4.68 ±1.16	0.5(2-
	experimental	12	4.31 ±1.13	0.563-
6 months at -18 °C	control	12	$6.46 \pm 0.88$	0.196-
	experimental	12	6.08 ±1.19	
9 months at -18 °C	control	12	$7.68 \pm 1.07$	0.273-
	experimental	12	$7.21 \pm 1.18$	
12 months at -18 °C	control	12	9.78 ±1.23	0.401-
	experimental	12	9.26 ±1.16	$0.481^{-}$

Table 4 Average acid value in the chicken thigh muscle with skin, g.100 g<sup>-1</sup>.

Note: n = multiciplity, M = mean, SD = standard deviation; numerical values in the column at t-test marked -: statistically non-significant difference between the control and experimental group (p > 0.05).

**Leonel et al. (2007)** analyzed breast and thigh muscle. They considered that higher levels of fat found in the thigh muscle compared to the breast muscle could indicate a higher probability of oxidative processes in the thigh muscle. The different fat content in the thigh muscle and the breast muscle is not related to the oxidative processes of fat.

These conclusions were reported in the study by **Gardini** (2000).

However, there is a more recent study which states that muscles with a higher fat content show a greater tendency to oxidize through a continuous chain reaction of free radicals (**Ruban**, 2009).

The main goal of the meat industry and food safety researchers is to understand the mechanisms of fat oxidation and to identify the most effective methods of managing this process (Domínguez et al., 2018).

Lipid oxidation leads to a deterioration of some quality characteristics of the meat, such as taste, texture, and color, and also reduces the shelf life along with the formation of some toxic compounds (Mohamed et al., 2008).

A similar view is reported by Ali et al. (2015).

We recorded the average acid value in the thigh muscle with skin for 1 day stored at room temperature after the slaughter of broiler chickens in the experimental group of 4.31 mg KOH.g<sup>-1</sup> compared to a control sample of 4.68 mg KOH.g<sup>-1</sup>. The difference in the average value of the acid value of thigh muscle fat with skin for 1 day kept at room temperature after the slaughter of broiler chickens was not statistically significant (p > 0.05) between the control and experimental group. Based on the statistical evaluation of the results expressed by the standard deviation, we can state that the measured acid value of thigh muscle fat with skin kept at room temperature 1 day after slaughter of broiler chickens fluctuated slightly more in the control group compared to the experimental group (SD = 1.16 vs. SD = 1.13).

In thigh muscle with skin stored for 6 months in freezing conditions at -18 °C after the slaughter of broiler chickens, we recorded the average acid value in the experimental group of 6.08 mg KOH.g<sup>-1</sup>. This value is lower compared to the control group of 6.46 mg KOH.g<sup>-1</sup>. The difference in the acid value in thigh muscle with skin stored for 6 months in freezing conditions at -18 °C after the slaughter of broiler chickens was not statistically significant (p > 0.05) between the control and experimental group. Statistical evaluation of the results expressed by the standard deviation, we found that the measured values of the acid value of thigh muscle with skin stored for 6 months in freezing conditions at -18 °C after the slaughter of broiler chickens fluctuated more in the experimental group compared to the control group (SD =1.19 vs. SD = 0.88).

In the thigh muscle stored under freezing conditions at -18 °C 9 months after the slaughter of broiler chickens, the average acid value was measured in the experimental group of 7.21 mg KOH.g<sup>-1</sup> and the control sample 7.68 mg KOH.g<sup>-1</sup>. No statistically significant difference (p > 0.05) was found between the control and experimental group in the in acid value of thigh muscle with skin stored for 9 months in freezing conditions at -18 °C after the slaughter of broiler chickens. By statistical evaluation of the results expressed by the standard deviation, we found that the measured acid values of thigh muscle fat with skin stored for 9 months in freezing conditions at -18 °C after the slaughter of broiler chickens fluctuated more in the experimental group compared to the control group (SD = 1.18 vs. SD = 1.07).

In thigh muscles stored for 12 months in freezing conditions at -18 °C after the slaughter of broiler chickens, we recorded an average acid value in the experimental thigh muscle group with the skin of 9.26 mg KOH.g<sup>-1</sup> and the control group 9.78 mg KOH.g<sup>-1</sup>. The difference in measured acid values of thigh muscle with skin stored for 12 months in freezing conditions at -18 °C after the slaughter of broiler chickens was not found statistically significant (p > 0.05) between the control and experimental group. Statistical evaluation of the results, which are expressed by the standard deviation, we found that the measured acid values of thigh muscle with skin stored for 12 months in freezing conditions at -18 °C after the slaughter of broiler chickens fluctuated more in the control group compared to the experimental group (SD = 1.23 and vs. *SD* = 1.16).

It is well known that unsaturated fatty acids and oxygen are components that react during the fat oxidation process. In addition, other components may promote or prevent oxidative reactions. Fats can be oxidized in three main ways, which involve complex reactions: autoxidation, enzymatically catalyzed oxidation, and photooxidation. Of the three mechanisms, the most important process of lipid oxidation in meat is autoxidation, which is a continuous chain reaction of free radicals (Cheng, 2016).

Enzymatic and photooxidation mechanisms differ from autoxidation only by the formation of hydroperoxides during the initiation phase (Chaijan et al., 2017).

The mechanism of free radicals, despite the explanation of many of the changes observed in meat, does not provide a detailed and complete description of the changes induced in the reactants and derived products during the oxidation process. The main challenge, therefore, is to complete a scheme that can fully explain all the agents, intermediates, and reactions in fat oxidation (Ghinimi et al., 2017).

# Peroxide value in thigh muscle with skin

The average peroxide value in the thigh muscle with skin 1 day, 6, 9, and 12 months after the slaughter of broiler chickens is given in Table 5.

We found the average peroxide value in the thigh muscle with the skin after 1 day of storage at room temperature after the slaughter of the broiler chickens in the experimental group of 0.99 µmol O<sub>2</sub>.g<sup>-1</sup> compared with the control group of 1.11 µmol O<sub>2</sub>.g<sup>-1</sup>. The difference in peroxide value in the thigh muscle with skin for 1 day kept at room temperature after the slaughter of broiler chickens was statistically significant ( $p \le 0.01$ ) between the control and experimental groups. Based on the statistical evaluation of the results expressed by the standard deviation, we can state that the measured values of peroxide value in the thigh muscle with skin kept at room temperature 1 day after the slaughter of broiler chickens fluctuated more in the control group compared to the experimental group (SD = 0.52 vs. SD = 0.43).

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An increase in hydroperoxides is observed in the initial stages of fat oxidation because the level of formation is higher than during decomposition. These compounds are unstable. The process of decomposition of hydroperoxides is larger than the process of formation in more advanced stages of oxidation, therefore a decrease in the content of hydroperoxides (peroxide value) is observed. This means that low peroxide levels can result in early as well as advanced fat oxidation (Estévez et al., 2009).

The peroxide value is a used indicator to know the degree of oxidation. Previous research as well as review and arguments rather point to its effectiveness in the initial stages of oxidation processes (Shahidi and Wanasundara, 2002).

From this aspect, it can be concluded that in the advanced stages of fat oxidation, the peroxide value indicator as an oxidation indicator could lead to an underestimation of the degree of oxidation (**Ross and Smith, 2006**).

Table 5 Average peroxide value in the chicken thigh muscle with skin, g.100 g<sup>-1</sup>.

Storage time thigh muscle	Group	n	$M \pm SD$	t-test
1 day at room temperature	control	12	$1.11 \pm 0.52$	0.004++
	experimental	12	$0.99 \pm 0.43$	0.004
6 months at -18 °C	control	12	$1.37 \pm 0.68$	0.076-
	experimental	12	$1.02 \pm 0.56$	$0.076^{-}$
9 months at -18 °C	control	12	$2.47 \pm 0.93$	0.082-
	experimental	12	$2.01 \pm 0.98$	0.083-
12 months at -18 °C	control	12	$3.48 \pm 1.23$	0.021+
	experimental	12	$2.96 \pm 1.09$	0. 031+

Note: n = multiciplity, M = mean, SD = standard deviation; numerical values in the column at t-test marked ++, +: statistically significant difference between the control and experimental group ( $p \le 0.01$ ,  $p \le 0.05$ ); numerical values in the column at t-test marked -: statistically non-significant difference between the control and experimental group (p > 0.05).

In thigh muscle with skin stored for 6 months in freezing conditions at -18 °C after the slaughter of broiler chickens, we recorded the average peroxide value in the experimental group of 1.02 µmol O<sub>2</sub>.g<sup>-1</sup>. This value is lower than the compared control sample 1.37 µmol O<sub>2</sub>.g<sup>-1</sup>. The difference in peroxide value in thigh muscle with skin stored for 6 months in freezing conditions at -18 °C after the slaughter of broiler chickens was not statistically significant (p > 0.05) between the control and experimental group. By statistical evaluation of the results expressed by the standard deviation, we found that the measured peroxide values of the thigh muscle with skin stored for 6 months in freezing conditions at -18 °C after the slaughter broiler chickens fluctuated more in the control group compared to the experimental group (SD = 0.68 vs. SD = 0.56).

In thigh muscle with skin stored for 9 months in freezing conditions at -18 °C after the slaughter of broiler chickens, we recorded an average peroxide value in the experimental group of 2.01 µmol O<sub>2</sub>.g<sup>-1</sup> and a control sample of 2.47  $\mu$ mol O<sub>2</sub>.g<sup>-1</sup>. The average value of the experimental group is lower. The difference in peroxide value of thigh muscle with skin stored for 9 months in freezing conditions at -18 °C after the slaughter of broiler chickens was not statistically significant (p > 0.05) between the control and experimental groups. According to the statistical evaluation expressed by the standard deviation, we found that peroxide values in the thigh muscle with skin stored for 9 months in freezing conditions at -18 °C after the slaughter of broiler chickens fluctuated more in the experimental group compared to the control sample (SD =0.98 vs. SD = 0.93).

In thigh muscle with skin stored for 12 months in freezing conditions at -18 °C after the slaughter of broiler chickens, we recorded an average peroxide value in the experimental group of 2.96  $\mu$ mol O<sub>2</sub>.g<sup>-1</sup> and the control group of 3.48  $\mu$ mol O<sub>2</sub>.g<sup>-1</sup>. The difference in peroxide

value in thigh muscle with skin stored for 12 months under freezing conditions at -18 °C after the slaughter of broiler chickens was statistically significant ( $p \le 0.05$ ) between the control and experimental groups. By statistical evaluation of the results, which are expressed by the standard deviation, we found that the measured values of peroxide value in thigh muscle with skin stored for 12 months in freezing conditions at -18 °C after the slaughter broiler chickens fluctuated more in the control group compared to the experimental group (SD = 1.23 vs. SD = 1.09).

Based on the comparison of our results of the peroxide value of thigh muscles with the results of the experiment Klimentová and Angelovičová (2019) it can be stated, that the values differ slightly. The authors did not find a statistically significant difference in peroxide value between the experimental group using oregano essential oil and the control group. In our experiment, a statistically significant difference ( $p \leq 0.01$ ) was confirmed for 1 day of sample storage at room temperature between the control and experimental group. The second difference compared to the above study was observed, when the thigh muscle was stored in freezing conditions at -18 °C for 12 months, while our results showed ( $p \leq 0.05$ ) a statistically significant difference between the control and experimental groups. The study of these authors includes the evaluation of the peroxide number of frozen chicken thighs by the application of oregano essential oil as a feed supplement for broiler chickens. The authors recommended the use of oregano essential oil in feed mixtures in a proportion of 0.05%.

Research results of **Marcinčák et al. (2008)** showed that oregano essential oil was effective in slowing down the oxidation of fats compared to the control group. Another fact found by these authors was that the thigh muscle was more prone to fat oxidation compared to the breast muscle  $(p \le 0.05)$ , which is a different opinion than reported **Gardini (2000), Leonel et al. (2007).** According to these authors, the different fat content in the thigh muscle and the breast muscle is not related to the oxidative processes of fat. Their conclusion is identical with the opinion of **Ruban (2009)**, which states that muscles with a higher fat content show a greater tendency to oxidize through a continuous chain reaction of free radicals.

# CONCLUSION

The evaluated results of the application of oregano essential oil to chicken thighs resulted in the following conclusion:

(a) no statistically significant difference was found in dry matter content, fat content of thigh muscle with skin when stored for 1 day at room temperature, 6, 9 and 12 months under freezing conditions at -18 °C compared to a control group whose starter and growth feed mixtures contained coccidiostats,

(b) no statistically significant difference was found in the acid value of thigh muscle with skin when stored for 1 day at room temperature, 6, 9 and 12 months under freezing conditions at -18 °C compared to a control group whose starter and growth feed mixtures contained coccidiostats,

(c) statistically significant difference was found in the peroxide value of thigh muscle with skin when stored for 1 day at room temperature and 12 months under freezing conditions at -18 °C, and no statistically significant difference when stored for 6, 9 months under freezing conditions at -18 °C compared to a control group whose starter and growth feed mixtures contained coccidiostats.

In conclusion, we can state that maintaining the oxidative stability of chicken meat means knowing the factors that affect it and prepare the conditions for its maintenance. The rate and extent of lipid oxidation in muscle tissue depends on many factors, including broiler chicken feed, handling of meat after slaughter. Chicken meat is generally prone to oxidative degradation because it is characterized by a high concentration of polyunsaturated fatty acids. With a sufficient number of effective antioxidants using chicken meat can be a homoeostatic system that remains valid or without of oxidized compounds and reactive components.

These issues are the subject of further research in the field of oxidative stability of chicken meat.

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# **Conflict of Interest:**

The authors declare no conflict of interest.

# **Ethical Statement:**

The use of animals in this research was approved by the Ethics Committee of the Slovak University of Agriculture in Nitra following the legislation of the Slovak Republic.

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