MALONDIALDEHYDE LEVELS IN FRESH AND FROZEN TURKEY MEAT

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Abstract. The aim of this study was to determine the levels of malondialdehyde (MDA) variation in healthy and dysfunctional limbs of turkey meat. MDA content in turkey breast and thigh muscle was determined by a high-performance liquid chromatography. For the study, the turkeys selected were of the same breed and age, consisting of male turkeys (breed BIG-6, 147 days of age), grown under the same conditions. Turkeys were divided into four treatment groups: I – healthy turkeys, II – turkeys with pododermatitis, III – turkeys with pododermatitis-arthritis-tendovaginitis, IV – turkeys with pododermatitis-*varus-valgus* deformities. MDA content in fresh and frozen turkey meat was tested at three time intervals: 24 hours and 3 and 6 months following the turkey slaughter.

The results on MDA content showed that the fat of breast and thigh muscles of the control (healthy) turkeys oxidized most intensively. It was found that 24 hours following the turkey slaughter, the MDA content in the breast muscles of control turkeys was by an average of 0.63 µmol/kg higher than in group II, 0.03 µmol/kg higher than in group III, and 0.59 µmol/kg higher than in group IV. The content of MDA in the thigh muscles of control group of turkeys was by 0.18 µmol/kg higher than in the muscles of group II and by 0.29 µmol/kg higher than in the muscles of group IV. The MDA content measured in group III was by 0.41 µmol/kg higher than in the control group.

After a total of 3 months following the turkey slaughter, MDA content in the control (I) group of breast samples was 1.47 µmol/kg higher than in group II, 0.7 µmol/kg higher than in group III, and 1.35 µmol/kg higher than in group IV. The MDA content in the thigh muscles of control turkeys was by 1.43 µmol/kg higher than in group II, 1.93 µmol/kg higher than in group III, and 2.81 µmol/kg higher than in group IV.

After a total of 6 months following the turkey slaughter, the MDA content in the breast samples of control group(I) was by 2.5 µmol/kg higher than in group II, 1.01 µmol/kg higher than in group III, and 3.34 µmol/kg higher than in group IV. The MDA content in the thigh muscles of control group of turkey was by 1.38 µmol/kg higher than in group II, 1.27 µmol/kg higher than in group III, and 2.66 µmol/kg higher than in group IV.

Keywords: turkey meat, limb pathology, lipid oxidation, malondialdehyde.

MALONDIALDEHIDO KIEKIS ŠVIEŽIOJE IR ŠALDYTOJE KALAKUTIENOJE

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Santrauka. Šio tyrimo metu nustatyta malondialdehido (MDA) kiekio kaita sveikų ir galūnių patologijų turinčių kalakutų mėsoje. MDA kiekis kalakutų krūtinėlių ir šlaunelių raumenyse nustatytas efektyviosios skysčių chromatografijos metodu. Tyrimui atrinkti tos pačios veislės ir amžiaus kalakutai patinai (veislė BIG-6, amžius – 147 d.), auginti vienodomis sąlygomis. Paukščiai suskirstyti į keturias tiriamąsias grupes: I – sveiki kalakutai; II – kalakutai su pododermatitais; III – kalakutai su pododermatitais-artritais-tendovaginitais; IV – kalakutai su pododermatitais-*varus-valgus* deformacijomis. MDA kiekis šviežioje ir šaldytoje kalakutienoje tirtas tris kartus: praėjus 24 val., 3 ir 6 mėn. po kalakutų skerdimo.

MDA kiekio tyrimų rezultatai rodo, kad intensyviausiai oksidavosi sveikų kalakutų krūtinėlių ir šlaunelių raumenyse esantys riebalai. Nustatyta, kad, praėjus 24 val. po kalakutų skerdimo MDA I grupės kalakutų krūtinėlių raumenyse buvo vidutiniškai 0,63 µmol/kg daugiau nei II grupės, 0,03 µmol/kg daugiau nei III grupės ir 0,59 µmol/kg daugiau nei IV grupės. MDA I grupės kalakutų šlaunelių raumenyse buvo 0,18 µmol/kg daugiau nei II grupės ir 0,29 µmol/kg nei IV grupės, III grupės – 0,41 µmol/kg buvo daugiau nei I grupės.

Praėjus 3 mėn. po kalakutų skerdimo, MDA I grupės kalakutų krūtinėlių raumenyse buvo 1,47 µmol/kg daugiau nei II grupės, 0,7 µmol/kg daugiau nei III grupės ir 1,35 µmol/kg daugiau nei IV grupės. MDA I grupės kalakutų šlaunelių raumenyse buvo 1,43 µmol/kg daugiau nei II grupės, 1,93 µmol/kg daugiau nei III grupės ir 2,81 µmol/kg daugiau nei IV grupės.

Praėjus 6 mėn. po kalakutų skerdimo, MDA I grupės kalakutų krūtinėlių raumenyse buvo 2,5 μmol/kg daugiau nei II grupės, 1,01 μmol/kg daugiau nei III grupės ir 3,34 μmol/kg daugiau nei IV grupės krūtinėlių raumenyse. MDA I grupės kalakutų šlaunelių raumenyse buvo 1,38 μmol/kg daugiau nei II grupės, 1,27 μmol/kg daugiau nei III grupės ir 2,66 μmol/kg daugiau nei IV grupės šlaunelių raumenyse.

Raktažodžiai: kalakutiena, galūnių patologijos, riebalų oksidacija, malondialdehidas.

Introduction. In recent years, the public has been interested in nutritional aspects of meat consumption and the demand has increased dramatically. Meat is one of the most valuable food products and provides a number of nutritional factors: moisture, protein, fat, carbohydrates, vitamins, enzymes and minerals. The nutritional contents found in the carcass of the animal is influenced by factors such as the species, breed, sex, age, fat content, feeding content, anatomical parts of the carcass, technological aspects of processing, storage and warehousing (Blaha et al., 2001).

Turkey meat compared to other types of meat is rich in proteins, vitamins, and minerals and low in fat. Turkey growing conditions are key factors influencing the quantity and quality of their meat.

Intensive breeding of turkeys can result in pathological conditions such as: pododermatitis, arthritis-tendovaginitis and *varus-valgus* deformities in the limbs. These diseases often lead to pain, suffering and, as a result, to increase feather plucking, cannibalism, growth retardation, increased mortality, and reduced carcass quality. The pathological conditions that affect the health of the birds can also affect the turkey meat quality and ultimately result in an economic loss.

The intensive growth of turkeys results in rapid weight gain, which leads to morphological changes in tissue composition such as the increase of breast muscle yield, hypertrophy of muscle fibres and formation of giant fibres, while the growth of the connective tissue and blood vessels still maintain an average rate. Such a growth difference in the bird can lead to hypoxia, acidosis, or myopathy (Taubert, 2001; Julian, Gazdzinsky, 2000). The thin network of blood vessels within the muscle is not able to supply the oxygen and nutrient levels needed. The elimination of metabolic products, especially of the CO_2 and lactic acid can also be affected by this thin network and thus may lead to muscle degeneration (Elminowska-Wenda et al., 2005; Branscheid et al., 2004 a,b).

Lipids are present in muscles as structural components of the muscle membranes, as droplets of triacylglycerol between muscle fibres and as adipose tissue (marbling fat). These lipids, or more precisely their fatty acids, contribute to a wide range of quality attributes. The fatty acids composition of meat will affect the profile of compounds produced in lipid oxidation. The abundance of unsaturated fatty acids will favour the abstraction of a hydrogen atom and the start of the oxidation process (Wilfred Ruban, 2009).

Lipid oxidation in food industry is one of the main problems, which reduces the quality of the meat and has an impact on food rancidity and accumulation of toxic compounds (Gorelik et al., 2008; Paniangvait et al., 1995; Ahn et al., 1992; Ladikos and Lougovois, 1990). Fat oxidation is an interaction of active oxygen and free radicals present in tissues together with interaction of polyunsaturated fatty acids (PUFA) present in lipids. As a result of this interaction, the generated lipid hydroperoxides are unstable and break down further into aldehydes, ketones, alcohols, acids and hydrocarbons (St. Angelo, 1996). These secondary oxidation products change food quality in its colour, texture, smell, taste, and they are responsible for rancidity of food (Grau et al., 2000; Fernandez et al., 1997; Botsoglou et al., 1994). In addition, the oxidation of adipose tissue during the ongoing interactions with free radicals initiate cell damage and can lead to pathological processes (Chopra, Griffin, 1985).

One of the most toxic products of unsaturated fatty acid oxidation is malondialdehyde (MDA), which has carcinogenic, mutagenic and cytotoxic properties (Mendes et al., 2009; Del-Rio et al., 2005). MDA is highly reactive in biological medium and binds to proteins and nucleic acids in the -SH and $–NH_2$ groups, thus creating a tertiary lipids oxidation products (Pilz et al., 2000; Esterbauer, Zollner, 1989). MDA causes intracellular oxidative stress, which stimulates irregularities of red blood cell membrane, reacts with DNA, and it is mutagenic to human (Del-Rio et al., 2005; Cline et al., 2004; Tesoriere et al., 2002; Riggins, Marnett, 2001).

One of the methods of food preservation in the food industry is cooling, which has very little inhibitory effect on lipids oxidation. Studies have shown that cold storage of meat, depending on the time, increases the accumulation of lipids oxidation products in it (Rey et al., 2001; Abdel-Kader, 1996).

Since a long-term freezing of meat does not protect it from oxidative processes and the accumulation of MDA, it is important to determine the MDA content in fresh and frozen turkey. This study was conducted to determine the MDA levels in healthy and pathological turkey leg meat.

The aim of this study was to determine the effect of fat oxidation in turkey meat over time within the limb pathologies. This was conducted by assessing the levels of MDA content in fresh and frozen meats.

Materials and methods. For the study, the turkeys selected were of the same breed, age and weight (20 kg), consisting of 20 male turkeys (breed BIG-6, 147 days of age), grown under the same conditions.

Post-mortem inspection turkeys were divided into four treatment groups: group I - healthy turkeys, group II - turkeys with pododermatitis, group III - turkeys with pododermatitis-arthritis-tendovaginitis and group IV - turkeys with pododermatitis-*varus-valgus* deformities. For the determination of MDA content, 100 g of breast

and thigh muscle samples were taken from each of the four groups consisting of 5 male turkeys. Malondialdehyde content of the samples was tested at three time intervals: 24 hours, and 3 and 6 months following the turkey slaughter. The collected samples were frozen at -18°C temperature prior to analysis (3 and 6 months).

The malondialdehyde content in turkey breasts and thighs was determined by high performance liquid chromatography method described by R. Mendes (Mendes et al., 2009). For this purpose, a high pressure gradient HPLC system Varian ProStar (Varian Corp., USA) was used, consisting of two ProStar 210 pumps, automatic sampling module Prostar 410 and Prostar 363 fluorescence detector. The separation of the Malondialdehyde – 2 – thiobarbituric acid (MDA – TBA) compound was performed by HPLC using a 5 µm particle size, 250 mm long and 4.6 mm internal diameter Gemini C18 (Phenomenex, USA) chromatographic column. The mobile phase consisting of 50 mM KH_2PO_4, methanol and acetonitrile with a mixing ratio of 72/17/11 was supplied with 1.0 ml per 1 min increments. MDA-TBA compound was identified and quantified by measuring the fluorescence at E_{ex} 525, E_{em} 560 nm wavelengths. The sample injection volume was of 10 µl. Data collection and evaluation was performed by using Galaxy Workstation (Varian Corp., USA) operating system.

The muscle tissue of 5 g was extracted with 10 ml of 7.5% trichloroacetic acid solution by homogenization (IKA T18 basic Ultra-Turrax, IKA Laboratory equipment, Germany) for 1 min. at 5000 rpm (rotations per minute) speed and filtered through filter paper (AlbetFP 589/2, Hahnemühle Fine Art GmbH; Germany). The filtrate was centrifuged for 15 minutes at 4000 rpm (Sigma 2-5, Sigma Laborzentrifugen; Germany). The derivatization of samples was performed with 2-thiobarbituric acid (TBA). This was performed by extracting 0.5 ml of the supernatant layer, which was then transferred into a glass screw tube, and a further 1.5 ml of 40 mM TBA solution was added and mixed thoroughly (MS2, IKA Works, Inc. USA). The mix was placed in the heating furnace at 97°C for 60 min. (UFE 400, Memmert; Germany). The mix was then cooled under running water and then placed in a freezer (-18°C) for a duration of 25 minutes. Following the freezing, 3 ml of methyl alcohol was added to the cooled mixture, mixed thoroughly and filtered through a 0.2 µm PTFE membrane filter (Acrodisc CR 25 mm Syringe Filter, Pall Life Sciences) into the chromatography vials.

The MDA-TBA compound was quantified by comparison between peak area of MDA-TBA compound in sample and peak area of this compound in standard solution.

Intramuscular fat content was determined by the Soxhlet apparatus (ISOLAB, Germany), extracting with chloroform for 8 hours (Januškevičius et al., 2011).

Statistical analysis was performed using Sigma Plot 2000 and Statistica 6.0 Statsoft Inc. 2300 East14thstreet, Tulsa, OK 74104, USA statistical packages. The data of at least five independent cases and the averages of the standard biases were statistically compared by performing the Student's t-test; the differences between the mean values were significant when p≤ 0.05.

Results and discussion

The experimental results on MDA content are presented in Tables 1 and 2 and Figures 1 and 2.

Table 1. **Intramuscular fat content** (%) **and MDA levels** (µmol/kg) **in turkey breast muscle**

Group	Intramuscular fat content, %	MDA µmol/kg 24 hours after slaughter	MDA µmol/kg 3 months after slaughter	MDA µmol/kg 6 months after slaughter
I	1.32±0.03	1.93±0.37	2.86±0.15	5.06±1.55
II	1.21±0.04 *	1.30±0.22	1.39±0.22 ***	2.56±0.49
III	1.74±0.09 ***	1.90±0.62	2.16±0.39	4.05±2.51
IV	1.46±0.08	1.34±0.25	1.51±0.28 **	1.72±0.44

I – control group (healthy turkeys), II – turkeys with pododermatitis, III – turkeys with pododermatitis-arthritis-tendovaginitis, IV – turkeys with pododermatitis-*varus-valgus* deformities; *p<0.05; **p<0.01; ***p<0.001 (depending on the time factor after the turkey slaughter, the control group was compared to treated groups)

Table 2. **Intramuscular fat content** (%) **and MDA level µmol/kg of turkey thigh muscles**

Group	Intramuscular fat content, %	MDA µmol/kg 24 hours after slaughter	MDA µmol/kg 3 months after slaughter	MDA µmol/kg 6 months after slaughter
I	5.12±0.14	2.02±0.34	5.61±0.74	9.04±1.52
II	3.35±0.11 ***	1.84±0.36	4.18±0.76	7.66±0.89
III	3.82±0.17 ***	2.43±0.68	3.68±0.66	7.77±2.21
IV	3.87±0.14 ***	1.73±0.33	2.80±0.45 *	6.38±0.94

I – control group (healthy turkeys), II – turkeys with pododermatitis, III – turkeys with pododermatitis-arthritis-tendovaginitis, IV – turkeys with pododermatitis-*varus-valgus* deformities; *p<0.05; **p<0.01; ***p<0.001 (depending on the time factor after the turkey slaughter, the control group was compared to treated groups)

The results of the MDA content show that the breast muscles of the most intensive oxidation of turkey fat were observed in the control group. During the first research phase, the content of MDA levels in the control group of turkey breasts had an average of 0.63 µmol/kg higher than in group II. The control group also had an average of 0.59 µmol/kg higher MDA levels than group IV. However, MDA levels of turkey meat in group III was close to the control group and there was no statistical difference observed. A similar trend was also observed in the later stages of research. After 3 months post-mortem, the control group of turkey breast muscle had an average MDA level of 1.47 µmol/kg (105.76%) higher than in group II ($p<0.001$). Similarly, the MDA levels in the control group were higher than group IV levels by an average of 1.35 µmol/kg (89.40%) ($p<0.01$). Additionally, and consistent with phase one of the studies, the MDA levels in the control group were marginally higher by an average of 0.70 µmol/kg (32.41%) than in group III and were not statistically different. In the third stage of research, these differences became even more pronounced. The control group of turkey breast muscle MDA levels were by an average of 2.50 µmol/kg (97.66%) higher than in group II, 1.01 µmol/kg (24.94%) higher than in group III, and 3.34 µmol/kg (194.19%) higher than in group IV. Fat oxidation was most stable in group IV of turkey breast muscles. During the study of six months, the MDA content of the breast muscles in group IV increased by an average of 0.38 µmol/kg (28.36%). Meanwhile, the same index over the same six months time period, the control group breast muscles had increased by an average of 162.18%, groups II and III had increased by averages of 96.92% and 113.16% respectively.

The most intensive oxidation of fat was observed in breast and thigh muscles of control group. After a total of 24 hours following the turkey slaughter, MDA was mainly found in poultry of group III. During the second and third research phases, oxidation of fat was most advanced in the muscles of control group. After a total of 3 months of the turkey slaughter, the MDA level in the control group was by an average of 34.21% higher than the levels in group II, 52.24% higher than in group III, and 100.36% ($p<0.05$) higher than in group IV. Frozen for 6 months preserved samples of MDA in the control group were by an average of 18.02% higher than in group II, 16.34% higher than in group III and 41.69% higher than in group IV of the turkey thigh muscle samples. Within 6 months of sample storage, the MDA concentration in the thighs of the control group of turkeys increased by 4.46 times ($p<0.01$) on the average, in group II the concentrations increased 4.16-fold ($p<0.001$), group III 3.20-fold ($p<0.05$) and group IV 3.69-fold ($p<0.01$).

The studies conducted within these experiments showed that turkey thighs contained higher MDA concentrations than breast muscles. This was observed in all stages of the study. A greater degree of fat oxidation can be explained by a higher amount of the intramuscular fat in thighs, and thus more characteristic polyunsaturated fatty acid (PUFA) being typical of poultry. According to Marcinčak et al. (2003), the intensity of fat oxidation can influence the levels of triglycerides and phospholipids releasing polyunsaturated fatty acids.

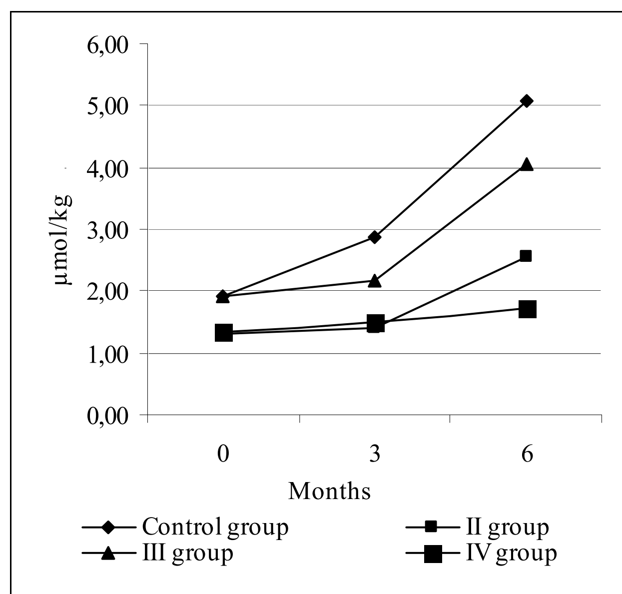

Fig 1. **MDA content variation in turkey breast muscles**

Control group – healthy turkeys, II group – turkeys with pododermatitis, III group- turkeys with pododermatitis-arthritis-tendovaginitis, IV group – turkeys with pododermatitis-*varus-valgus* deformities.

Intramuscular fat content in the breast muscle samples of different groups of turkey ranged from 1.21% to 1.74%. In sample group II, intramuscular fat content was found lower than in the control group by 0.11%. However the results obtained from sample groups III and IV were higher than thse from control group by a difference of 0.42% and 0.14% respectively (Table 1). The rate of variation of intramuscular fat can be several times higher than in other parts of the meat. The most variation of intramuscular fat is in broiler meat and the least is in turkey meat (Jukna et al., 2007).

In this study, there were significant differences ($p<0.001$) in the intramuscular fats indicators between the different turkey thigh muscle groups examined as seen in Table 2. The highest content of intramuscular fat was determined in the samples from the control group at 5.12%, while in sample groups II, III and IV intramuscular fat content were found significantly lower values at 1.77%, 1.30% and 1.25% respectively. It is conceivable that a higher intramuscular fat content of poultry meat leads to increased tissue releasing PUFA concentrations, and in addition the increased MDA levels are observed. This may also be the reason for the observed higher MDA concentration and more intensive oxidation of fat in the thighs of control group of tested turkey. Unfortunately the available studies do not explain the intense oxidation of fat in turkey breast analysed in the control group.

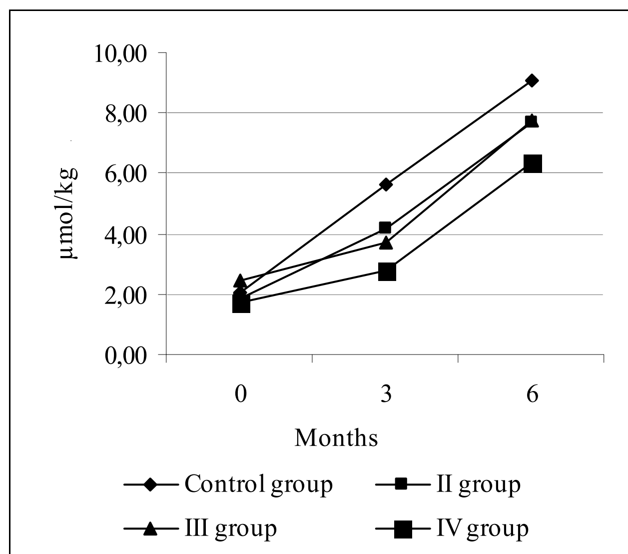

Fig 2. **MDA content variation in turkey thigh muscles**

Control group – healthy turkeys, II group – turkeys with pododermatitis, III group – turkeys with pododermatitis-arthritis-tendovaginitis, IV group – turkeys with pododermatitis-*varus-valgus* deformities.

Our studies have shown that the most intensive lipid oxidation of turkey muscles was in control groups (healthy turkeys). During storage lipid oxidation increased in concentration both in thighs and breasts, but the highest increase was observed in thigh muscles.

Turkeys with pathological conditions of the limbs most frequently show clinical symptoms such as abnormal movements, limping, paralysis, joint and bone deformity, distortion of the toes and joint inflammation, causing pain and suffering. Birds appear tired, passive, tend to sit longer, reduces their utilization of feed, muscle atrophy is likely to occur. Due to illness and stress, glycogen and fat reserves in muscles may be exhausted before they turkey slaughtering, therefore in sick birds muscles containing less fat content results in lower PUFA releasing and at the same time lower MDA levels.

Polyunsatured fatty acids (PUFA) of muscle membrane cells are particularly susceptible during storage to peroxidation when the degree of unsaturation membrane lipids is increased with reduction of oxidative stability of muscle (Granit et al., 2001). Furthermore, other factors that will affect the lipid oxidation of muscle foods are exposure to light, oxygen availability, temperature conditions and microbial growth (Skibsted et al., 1998).

Oxidative deterioration is the major quality loss in muscle food products. The negative effect of oxidation is involved not only in economic loss, but also is related to human health risk.

Results from animal investigations and biochemical studies indicate that ingestion of food containing lipid peroxidation products increases frequencies of cancer and atherosclerosis also cardiovascular diseases (Esterbauer, 1993).

Studies have shown that exposure of human erythrocytes to a very low concentration (50 µmolar) of MDA brought about early redox impairment, leading to depletion of reduced glutathione, glucose-6-phosphate dehydrogenase and oxygenated hemoglobin (Tesoriere et al., 2002).

Conclusions

1. Turkey hind limb pathologies did not influence the MDA content of their breast muscles, since the most intense oxidation was observed in intramuscular fat of the turkey control group. After a total of 24 hours following turkey slaughter, the MDA content in the control group was measured at 1.93 (µmol/kg), whereas this amount was lower in sample groups II, III and IV and showed an overall difference to the control group of 0.63, 0.03 and 0.59 (µmol/kg) respectively.

After a total of 3 months following the turkey slaughter, MDA content in breast samples of the control group was measured at 2.86 (µmol/kg), whereas this amount was lower in sample groups II, III and IV and showed an overall difference to the control group of 1.47 ($P<0.001$), 0.7 and 1.35 µmol/kg ($P<0.01$) respectively.

After a total of 6 months following turkey slaughter, MDA level in the control group of breast samples was measured at 5.06 µmol/kg, whereas sample groups II, III and IV measurements were lower and showed an overall difference to the control group of 2.50, 1.01 and 3.34 µmol/kg respectively.

2. The highest oxidation of intramuscular fat in thigh muscles of turkeys was determined in the control group. After a total of 24 hours following turkey slaughter, the MDA content in the control group was 2.02 µmol/kg. While the levels in sample groups II and IV showed lower values with an overall difference to the control group of 0.18 and 0.29 µmol/kg respectively. The difference in group III to the control group was higher than other groups with and overall difference of 0.41 µmol.

After a total of 3 months following the turkey slaughter, the MDA content in thigh samples of the control group increased to 5.61µmol/kg, and the respective differences between the control group and the sample groups had also increased. The sample groups were lower with and overall differences observed between the control group and sample groups II, III and IV of 1.43, 1.93 and 2.81 µmol/kg ($P<0.05$) respectively.

After a total of 6 months following turkey slaughter, the MDA level in the thigh samples of control group was the highest – 9.04 µmol/kg. All sample groups still measured lower in value when compared to the control group. The overall differences observed between the control group and sample groups II, III and IV were 1.38, 1.27 and 2.66 µmol/kg respectively.

3. The highest intramuscular fat content was determined in healthy turkey thigh muscle and measured at 5.12 %. The fat content in the sample groups II, III and IV was significantly lower showing overall differences of 1.77 ($P<0.001$), 1.30 ($P<0.001$) and 1.25 % ($P<0.001$) respectively, comparing with the control group. Intramuscular fat content in the control group of turkey

breast muscles was 1.32%. Group II was lower than the control group and showed an overall difference of 0.11% (P<0.05). However, in groups III and IV the fat content measured higher values, and the overall difference between the control group and these sample groups was 0.42 (P<0.001) and 0.14% respectively.

4. At all stages of the study, the MDA content μmol/kg was higher in thigh muscle than in breast muscle. A greater degree of lipid oxidation in thighs can be explained by a higher intramuscular fat content in them.

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