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The Effect of Dietary Dry Distilled Rose Petals or Dihydroquercetin Supplementation on the Oxidative Stability and Quality of Lamb Muscles and Fat

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Abstract: The study's objective was to determine the changes in oxidative stability of m. *Longissimus dorsi,* m. *Semimembranosus* and perirenal adipose tissue from lambs fed a diet supplemented by 7.5 mg dihydroquercetin/kg life wight/d or 545 mg dry distilled rose petals (DDRP)/kg life wight/d.The experiments were performed with 30 male lambs aged 65 days. They were divided into three groups of 10 animals fed 50 days ad libitum: the control group (with ground alfalfa + granulated compound feed) and two experimental ones (with the same diet + phytonutrients). Samples stored 7 days at 0-4°C were examined. After 7 d of storage in both muscles, the addition of 545 mg DDRP/kg life wight/d contributed to the reduction of α-aminoacidic nitrogen with approximately 0.5 mg Leu/g and the FFA with 0.5-2%. The addition of 7.5 mg dihydroquercetin contributed to the reduction of TBARS with 0.14-0.21 mg MDA/kg, the total color difference, and a slight increase in the share of the MUFA more pronounced in m. *Longissimus dorsi.* The reduction of aerobic plate count and total yeasts and molds count were determined too. Further studies with higher doses of phytonutrient's supplementation are needed to determine if it will provoke a more pronounced oxidative stability.

Keywords: lambs; phytonutrients; meat; fat; quality.

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1. Introduction

The last two decades have seen an increasing demand for organic meat. It is associated with the consumers' concerns about the presence in the meat of residues of pesticides, hormones, and veterinary drugs, including antibiotics [1]. Oxidative stress is another cause of disease-causing pathological changes in ruminants [2]. For these reasons, researchers are increasingly turning their attention to the use of safer alternatives such as plant biologically active feed supplements [3].

Several phytonutrients have been discussed as supplements to ruminant feed, which can be distinguished by their antimicrobial and antioxidant properties and stimulation of the immune effectiveness by modeling the intestinal microflora [4, 5]. One such phytonutrient is

flavonol dihydroquercetin [6]. As an electron donor, it can inhibit free hydroxyl radicals and effectively treat microbial infections and inflammatory processes, tumors, cardiovascular and liver diseases, and overcome conditions of oxidative stress. In addition to its confirmed anti-inflammatory, cardioprotective, lipid-reducing, capillary-protective, and hepatoprotective effects, it also has membrane-protective, immunomodulatory, antiallergic, detoxifying, angioprotective, and gastroprotective properties [6, 7].

Another phytonutrient containing a wide range of polyphenolic components is the dry distilled rose (*Rosa damascena* Mill.) petals (DDRP). They are a by-product of the distillation of rose oil. They are a source of glycosides of quercetin, kaempferol, and gallic acid, flavonol aglycones, a number of anthocyanins, and quercetin with proven cytotoxic, antioxidant, and antimicrobial activity [8, 9].

Oxidation and microbial spoilage are the main reasons for meat deterioration [10, 11]. They reduce its nutritional value, sensory and functional properties [11,12]. Using the animal's metabolism, supplements of dihydroquercetin and DDRP can be included in the diet to inhibit oxidative processes and microbial spoilage of meat while extending its shelf life [9, 13-16]. The effect of phytonutrients with antioxidant properties is specific to lambs and depends on their dose, metabolism, and the digestive system's specific structure [17].

A limited number of publications discuss the effect of dihydroquercetin or DDRP as antioxidant phytonutrient feed supplements on oxidative stability and meat quality in lambs. Therefore, the objective of this study was to monitor the effect of the supplementation to lamb's diet of low doses of dihydroquercetin or dry distilled rose petals on the oxidative stability and the quality of the lamb muscles and perirenal adipose tissue.

2. Materials and Methods

2.1. Lambs and experimental design.

The experiments were performed with 30 clinically healthy male lambs aged 65 days and equal in live weight following European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Council Regulation (EC) No 1099/2009, Commission Recommendation 2007/526/EC and Bulgarian Veterinary Medical Activity Act. The experiment was approved by the Bulgarian Scientific Ethics Committee, and requirements of the Council Directive 2010/63/EC were met. The lambs were housed indoors in the Experimental Farm of the Agricultural Institute, Shumen, Bulgaria, and were divided into one control and two experimental groups, each containing 10 animals being fed for 50 days. The control group (C) was fed with ground alfalfa + granulated compound feed, and the experimental groups (D) and (R) - with the same diet with the addition of 7.5 mg dihydroquercetin/kg/d, or 545 mg of DDRP/kg/d. Feeding the lambs was ad libitum in group boxes, with access to water and salt. Individual daily doses of the supplements were calculated according to the previous weighing of the animals, mixed with supplementary feed, and given with the morning feeding. Justification for the applied doses of phytonutrients, the feed composition, and the slaughter procedure was described in detail earlier [18].

After chilling for 24 h the lamb carcasses were stored at 0 - 4°C. On day 1 of chilled storage, the carcasses were divided into two halves. From the left halves in each group, there were separated and collected parts of m. *Longissimus thoracis et lumborum* (between 9 and 15 thoracic vertebrae, called further as m. *Longissimus dorsi*), m. *Semimembranosus* and perirenal adipose tissue (fat). Chilled muscle samples were ground in a mincer with a holes' diameter of

3 mm after that, were blended on a homogenizer. The same procedure was repeated with the right carcass halves on 7 d of the chilled storage. The values of the studied indicators were determined as the average of nine repetitions.

2.2. Phytonutrients and diet.

The experimental groups (D) and (R) were fed the same diet supplemented with 7.5 mg dihydroquercetin/kg/day or 545 mg DDRP/kg/day. The reasons why the above doses were used are presented in our previous publication [18].

Daily control of the amount of combined feed consumption during the experiment was exercised. Residual feed was weighed and subtracted from the daily amount of feed consumed. Lambs were weighed every two weeks.

The dihydroquercetin powder isolate was supplied by Flavitlife Bio JSCo (Sofia, Bulgaria). It had a purity of 96%. In addition to dihydroquercetin, the preparation contains 3% dihydrokaempferol and 1% naringenin.

The dry distilled rose petals (DDRP) were delivered by Bulattars Production Company Ltd (Pavel banya, Stara Zagora region, Bulgaria). After pressing, the petals were dried (24 hours, 65°C) and ground to a particle size < 0.4 mm. The 13 glycosides of kaempferol, 10 glycosides of quercetin, 6 glycosides of gallic acid, and 2 flavonol aglycones were identified in dry rose petals. The daily dose of the supplements was calculated according to the previous one and mixed with feed mixture [18] and given to the lambs with the morning feeding.

2.3. Determination of α-aminoacidic nitrogen.

The content of α-aminoacidic nitrogen in lamb was determined by the ninhydrin method with small modifications [19, 20].

2.4. Extraction of the total lipids.

Extraction of the total lipids was performed by the method of Bligh and Dyer [21].

2.5. Free fatty acids determination.

The acid value (AV) of the extracted total lipids was determined according to the procedure of EVS-EN ISO 660:2009 [22]. The extracted lipids were dissolved in ethyl alcohol (99%), heated for 2 min before titration while still hot against 0.1 M NaOH using phenolphthalein as an indicator. Free fatty acids FFA (%) were calculated as 0.503 × AV.

2.6. Peroxide value.

The peroxide value (POV) was measured based on the oxidation of Fe^{2+} to Fe^{3+} in the presence of hydroperoxides and the formation of a color complex between the obtained Fe^{3+} and SCN with some modification [23]. The extracted lipids (0.1 g) were mixed with 50 µl $FeCl_2$, 50 µl NH_4SCN and $CHCl_3$: CH_3OH (3:5, v/ v) to a volume of 10 ml. The sample was left for 10 min. The absorbance was measured at 507 nm using double beam UV-Vis spectrophotometer Camspec M 550 (Spectronic CamSpec Ltd, Garforth, Leeds, United Kingdom) against a control sample prepared from 1 g of total lipids, 50 µl NH_4SCN, $CHCl_3$: CH_3OH (3:5, v/ v) to a volume of 10 ml.

2.7. TBARS.

TBARS were measured spectrophotometrically at 532 nm using Botsoglou et al. [24] using a double beam UV-Vis spectrophotometer Camspec M 550 (Spectronic CamSpec Ltd, Garforth, Leeds, United Kingdom).

2.8. Fatty acids analysis.

The fatty acid composition of the extracted total lipids was determined by gas chromatography (GC) after transmethylation of the samples with 2% H_2SO_4 in absolute CH3OH at 50°C [25]. Fatty acid methyl esters (FAME) were purified by thin-layer chromatography (TLC) on 20x20 cm plates coated with 0.2 mm silica gel 60 G (Merck) layer with a mobile phase n-hexane: diethyl ether (97: 3, v/ v). Gas chromatographic analysis of the FAME was performed on a GC gas chromatograph Agilent 6890 Plus (Agilent Technologies, Santa Clara, USA) equipped with 5793 mass-selective detectors (Agilent Technologies, Santa Clara CA, USA) and with capillary column SP 2380 (30 mx 0.25 mm x 0.25 µm, Supelco, Bellefonte PA, USA). The column temperature was programmed from 70°C (1 min), at 6°C/min to 190°C (0 min), at 10°C/min to 250°C (0 min); the injector and detector temperatures are maintained at 250°C. Hydrogen was a carrier gas at a 0.8 ml/min flow rate, and the separation was 1: 50. The identification of fatty acids was performed by comparing the retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions [26].

2.9. Determination of the meat color characteristics.

The color of the meat was determined in three places on both sides of the two-centimeter cross-section made perpendicular to the muscle fibers (24 h and after chilling the samples to 0 - 4°C on 7 d *post mortem*), after which the values were averaged. The color characteristics of the samples were examined with a Konica Minolta chromameter model CR-410 using the CIE L*, a*, b* system [27].

The total color difference (ΔE) was also calculated by equation (4):

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \qquad (4),$$

where:
ΔE is the total color difference and ΔL*, Δa* and Δb* are the differences in the values of the brightness (L*), the red component (a*), and the yellow component (b*) between the control sample and the corresponding experimental one [28].

2.10. Determination of the microbiological status of lamb meat.

The microbiological status of the samples was established through bacteriological tests carried out following the microbiological criteria of Regulation (EC) № 1441 of 05.12.2007 using ISO 4833:2001 [29].

2.11. Statistical analyses.

A two-way analysis of variance (ANOVA) was used to evaluate the effect of the addition of the two phytonutrients and the chilled storage time on the studied parameters: α-aminoacidic nitrogen, FFA, POV, TBARS, color characteristics (L*, a*, b*, ΔE) and microbiological status of the lamb. The data analysis was performed with the statistical package

Microsoft Excel Office Professional Plus 2010. The differentiation of the mean values was performed using Fisher's test, and significant differences ($P \leq 0.05$) between the treatments were found. The results obtained are presented as mean ± standard deviation (mean ± SD).

3. Results and Discussion

3.1. α-Aminoacidic nitrogen.

The data from Fig. 1 show that on 1 d *post mortem* the levels of α-aminoacidic nitrogen in the studied samples fluctuated within narrow limits (between 2.12 - 2.51 mg/g) and did not significantly differ ($p > 0.05$). After 7 d of storage of the lamb muscles, proteolytic changes were observed, and the content of α-aminoacidic nitrogen increased 2.68 - 4.08 times. This increase was more pronounced in control C and samples D in both muscles. The increased levels of α-aminoacidic nitrogen in the controls are probably due to the reduced activity of Ca^{2+}-ATPase and the increased activity of Mg^{2+}-EGTA-ATPase, enzymes responsible for the denaturation of myosin and troponin-tropomyosin complex in muscle fibers [30]. It was found that after 7 d of chilled storage, the lamb's feed supplementation of 545 mg DDRP/kg/d reduced the accumulation of α-aminoacidic nitrogen by 14.5% in m. *Longissimus dorsi* and by 7.3% in m. *Semimembranosus* respectively.

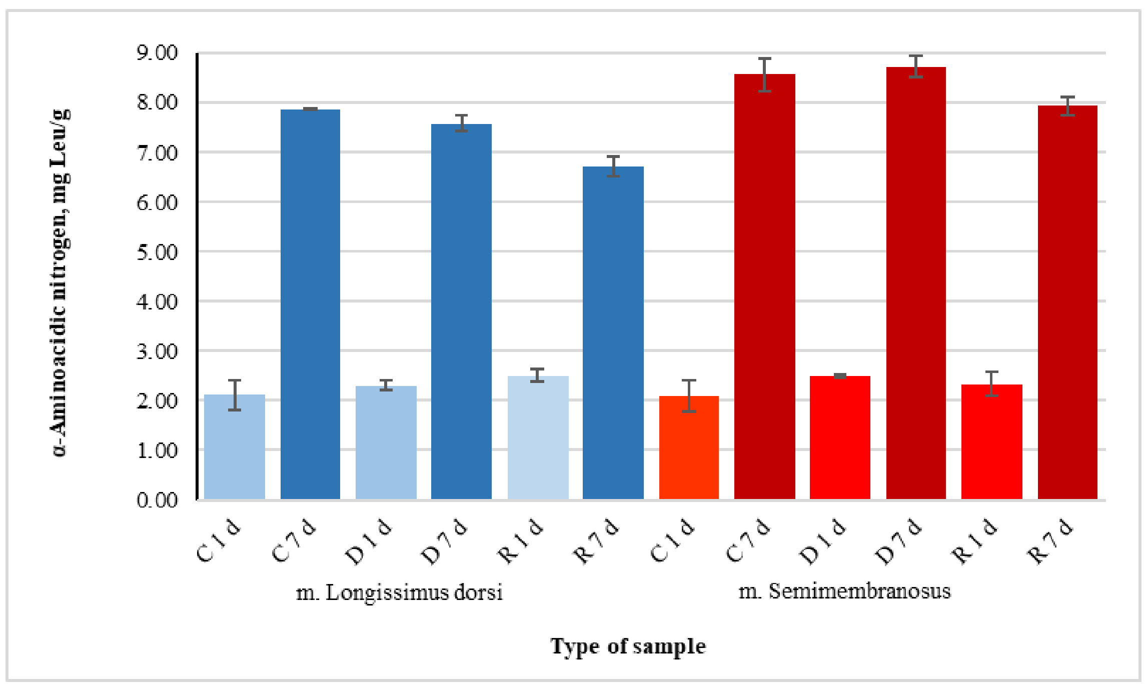

Figure 1. Changes of the α-aminoacidic nitrogen of lamb's m. *Longissimus dorsi* and m. *Semimembranosus* after 7 d storage at 0-4°C.

This phenomenon is more pronounced in samples R from m. *Longissimus dorsi*. Presumably, the heavy myosin chain and troponin T undergo significant hydrolytic degradation while actin remains relatively stable during meat refrigeration [31]. Polyphenols from DDRP may inhibit muscle proteolytic enzyme systems *in vivo* [16]. Biologically active substances such as polyphenols can affect the molecular mechanisms of control of genes, affecting cellular metabolism [31]. The mechanism of this interaction is still unclear. It depends on various factors such as the efficiency of their assimilation, their active concentrations in muscle tissue, metabolic transformation after absorption, which would rather reduce their antioxidant properties [16].

3.2. Free fatty acids (FFA).

The data from Fig. 2 demonstrate that 1 d *post mortem* FFA found in samples D and R in m. *Longissimus dorsi* was 13.8 - 19.5% less ($p \leq 0.05$) compared to control one C. In m. *Semimembranosus* and perirenal adipose tissue FFA are less by approx. 41.2% and resp. 14.7% only in samples R. A similar trend was found after 7 d storage of lamb muscles. The lowest levels of FFA were found in samples D in m. *Semimembranosus* - by 17.5%, in perirenal adipose tissue - by 26.3% and in m. *Longissimus dorsi* by more than 52% compared to control samples C.

These results give us reason to hypothesize that the weaker increase in free fatty acid levels in the samples obtained from lambs fed with feed supplemented with 545 mg DDRP/kg/d is probably also due to an inhibition of the activity of endogenous lipolytic enzyme systems in ruminants [2].

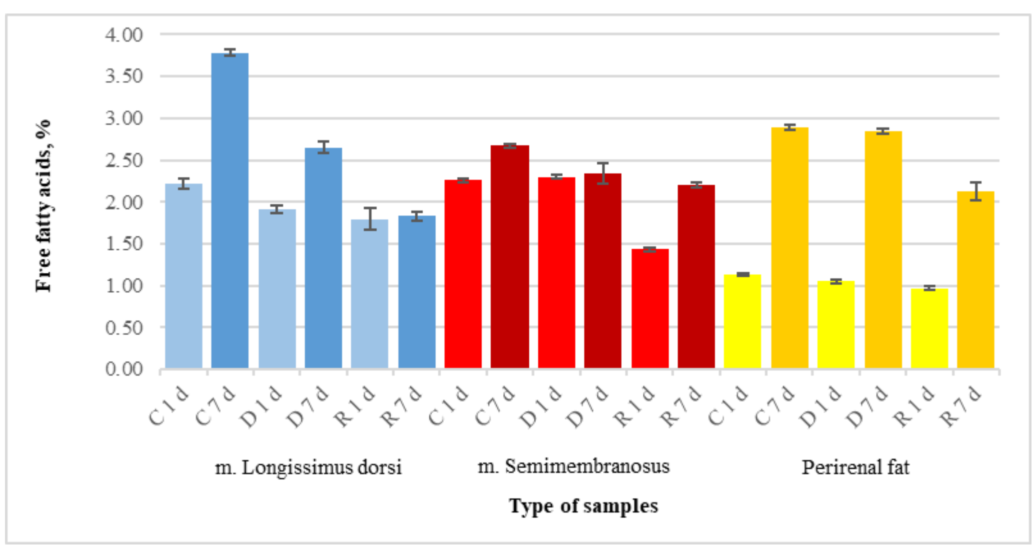

Figure 2. Changes of the percentage of free fatty acids in total lipids extracted from lamb's m. *Longissimus dorsi*, m. *Semimembranosus* and perirenal fatty tissue after 7 d storage at 0-4°C.

3.3. Peroxide value (POV).

Both on 1 d *post mortem* and after 7 d of the storage (Table 1), relatively low levels of primary lipid oxidation products (POV) were found.

It was observed that both in the samples of fresh muscle and perirenal adipose tissue (1 d *post mortem*) and those after 7 d of storage, the POV varied between 0.103 - 0.119 μeqO_2/g lipids but the differences were not significant ($p > 0.05$). Significantly higher levels of POV (0.126 μeqO_2/g lipids) were found in controls C in m. *Semimembranosus* only on 1 d *post mortem*. Our results correspond to the available data that the total peroxides in lamb were less than in beef or pork [33]. Hydroperoxides are unstable structures. They undergo transformations and turn into lower molecular secondary lipid oxidation products, and at 4°C such reactions need 24 - 36 h [33]. This explains the lower POV we found on 7 d stored chilled meat. The formation of approximately 70% of the non-polar peroxides was due to the change in fatty acid composition and heme iron content in meat [33]. The results showed that the feed supplements of 7.5 mg dihydroquercetin/kg/d and 545 mg DDRP/kg/d when feeding lambs were not sufficient to support the lamb's own integrated antioxidant system and to prevent the negative effects of free radicals and the reactive oxygen species of their metabolism [2, 34].

Table 1. Primary and secondary products of lipid oxidation determined in lamb's m. *Longissimus dorsi,* m. *Semimembranosus* and perirenal adipose tissue stored 7 d at 0-4°C.

Samples	m. *Longissimus dorsi*			m. *Semimembranosus*			Perirenal adipose tissue		
	C	D	R	C	D	R	C	D	R
Peroxide value, μeqO_2/g fat 1 d	0.111 ± 0.010 *ay*	0.119 ± 0.009 *ay*	0.112 ± 0.001 *ay*	0.126 ± 0.005 *bz*	0.116 ± 0.008 *az*	0.113 ± 0.003 *az*	0.108 ± 0.010 *az*	0.103 ± 0.005 *ay*	0.106 ± 0.005 *ay*
Peroxide value, μeqO_2/g fat 7 d	0.113 ± 0.004 *ay*	0.112 ± 0.003 *ay*	0.111 ± 0.003 *ay*	0.109 ± 0.004 *ay*	0.108 ± 0.003 *ay*	0.112 ± 0.007 *ay*	0.103 ± 0.002 *ay*	0.107 ± 0.003 *az*	0.106 ± 0.005 *ay*
TBARS, mg MDA/kg 1 d	0.43 ± 0.04 *by*	0.39 ± 0.06 *by*	0.64 ± 0.10 *dy*	0.70 ± 0.02 *ey*	0.59 ± 0.01 *dy*	0.53 ± 0.01 *cy*	0.24 ± 0.03*ay*	0.20 ± 0.03 *ay*	0.79 ± 0.01 *ez*
TBARS, mg MDA/kg 7 d	0.57 ± 0.10 *dz*	0.43 ± 0.01 *cz*	0.64 ± 0.05 *ey*	0.93 ± 0.05 *iz*	0.72 ± 0.03 *fz*	0.77 ± 0.04 *gz*	0.26 ± 0.03 *az*	0.28 ± 0.03 *bz*	0.43 ± 0.03 *cy*

* Different letters (*a, b, c, d, e, f, g, i*) within the same row indicate significant differences ($p \leq 0.05$); Different letters (*y, z*) within the same column indicate significant differences ($p \leq 0.05$); n = 9; SD - standard deviation;

3.4. TBARS.

Relatively low TBARS values were found on 1 d post mortem and after 7 d of the storage (Table 1). They were ten times as low as the values of TBARS (≤ 5 mg MDA/kg) indicated as the threshold for detecting unpleasant odors and tastes in meat [11, 34]. Similar to our results, there were reported significant differences in malondialdehyde content after 3 d of chilled storage of rabbit meat [30]. On 1 d post mortem in lamb muscles, TBARS were statistically ($p \leq 0.05$) distinguishable but varied within a very small range 0.39-0.70 mg MDA/kg without a one-way trend. For example, in m. *Longissimus dorsi* and in the perirenal adipose tissue, the lowest levels of TBARS were found in samples D. Their values did not differ significantly ($p > 0.05$) from those of control C. In m. *Semimembranosus* the lowest levels of TBARS were found in samples R. In samples D in m. Longissimus dorsi and m. Semimembranosus the lowest levels of TBARS were recorded even after 7 d of lamb storage by 24.5% and 22.5%, respectively. The results obtained can be explained by the fact that the oxidation of lipids and proteins in meat is closely related [32]. The major substance that reacts with TBA to form an adduct absorbing at 532 nm is MDA. To form dimers or trimers, it can react with a wide range of compounds such as amines, amino acids, amino sugars, proteins, and nucleosides. Such chemical transformations practically remove MDA from the reaction system. Its amount decreases, which lowers the concentration of the MDA-TBA complex, and the reported values for TBARS are lower [35]. This explains the relatively lower TBARS values that were reported after 7 d of chilled storage (Table 1). On the other hand, the endogenous antioxidant defense system of the muscles remains active for several days after the animal death. This answers why the statistical differences in TBARS values are more significant only after 7 d refrigeration [33]. The obtained results allow us to conclude that the supplementation of 7.5 mg dihydroquercetin/ kg/d to the lamb's diet can be used as a good tool for inhibiting lipid oxidation processes in m. *Longissimus dorsi* and m. *Semimembranosus* and of reducing the content of secondary products of lipid oxidation by more than 20%. Such a conclusion cannot be drawn for perirenal adipose tissue. The concentration of the 11 studied supplements of two phytonutrients is not sufficient to have an inhibitory effect on lipid oxidation. Lower TBARS levels with supplementation of 7.5 mg dihydroquercetin/kg/d to the lamb's diet demonstrate a positive connection between the intake of this 96% flavonol isolate and the lamb's antioxidant protection in vivo [36].

Even though flavonoids are not well absorbed in the intestines of ruminants, even with the indicated intake of 7.5 mg dihydroquercetin/kg/d, there is clear antioxidant protection [34]. It could also be due to the antioxidant activity of the zinc dihydroquercetin complex, which can

reduce the MDA content and enhance the catalase activity [37]. Obviously, oxidative processes in the muscle lipids are provoked during the lamb refrigeration. As a result of the dynamic transformation of some of the primary lipid peroxidation products, secondary products accumulate relatively lower levels of TBARS in m. *Longissimus dorsi* compared to m. *Semimembranosus* testifies to its stronger oxidative stability due to the more active antioxidant enzyme superoxide dismutase (SOD) and catalase. These enzymes slow down lipid oxidation. They can reduce the superoxide anion and remove hydrogen peroxide from the system [38].

3.5. Fatty acid composition.

No one-way trends in the influence of the supplementation of feed phytonutrients (7.5 mg dihydroquercetin/kg/d or 545 mg DDRP/kg/d) or 7 d chilled storage on the fatty acid compositions of the samples (Table 2). With aging, when goats are fed dry diets containing natural phytonutrients, the content of stearic acid (C18:0) in fat depots decreases, but with increasing the live weight, the levels of saturated fatty acids increase at the expense of monounsaturated ones [39]. The data for lamb's perirenal adipose tissue confirmed the claims of previous researches with the exception of the stearic acid levels (C18:0) in samples D found on 1 d post mortem [39]. Compared to controls C, after 7 d of storage of the samples, the levels of SFA decrease by approx. 5% at the expense of a similar increase in MUFA was found only in samples D of m. *Longissimus dorsi* (Table 2).

Table 2. Changes of fatty acid profiles of total lipids extracted from lamb's m. *Longissimus dorsi*, m. *Semimembranosus* and perirenal fatty tissue after 7 d storage at 0 - 4°C

Samples	**m. *Longissimus dorsi***			**m. *Semimembranosus***			**Perirenal fatty tissue**		
Fatty acid, % of FAME	**C**	**D**	**R**	**C**	**D**	**R**	**C**	**D**	**R**
C 12:0 - 1 d	0.2 ± 0.02	0.1 ± 0.01	0.2 ± 0.03	0.1 ± 0.05	0.5 ± 0.02	0.6 ± 0.03	0.3 ± 0.01	0.5 ± 0.03	0.9 ± 0.03
C 12:0 - 7 d	0.2 ± 0.03	0.2 ± 0.03	0.3 ± 0.02	0.4 ± 0.01	0.5 ± 0.04	0.5 ± 0.05	0.3 ± 0.03	0.4 ± 0.02	0.2 ± 0.03
C 14:0 - 1 d	2.5 ± 0.06	5.8 ± 0.03	3.4 ± 0.02	2.8 ± 0.07	5.1 ± 0.08	4.8 ± 0.08	2.9 ± 0.06	4.3 ± 0.03	6.2 ± 0.05
C 14:0 - 7 d	2.8 ± 0.05	2.6 ± 0.04	3.2 ± 0.05	3.0 ± 0.06	3.8 ± 0.05	4.2 ± 0.05	2.5 ± 0.01	4.3 ± 0.04	2.9 ± 0.05
C 15:0 - 1 d	0.1 ± 0.04	0.7 ± 0.01	0.4 ± 0.03	0.4 ± 0.03	0.5 ± 0.03	0.4 ± 0.02	0.0	0.1 ± 0.04	0.1 ± 0.01
C 15:0 - 7 d	0.4 ± 0.04	0.5 ± 0.03	0.4 ± 0.02	0.4 ± 0.01	0.6 ± 0.02	0.5 ± 0.06	0.4 ± 0.03	0.5 ± 0.01	0.5 ± 0.03
C 16:0 - 1 d	23.2± 0.08	34.4± 0.02	25.3± 0.05	22.5± 0.10	29.3± 0.11	28.6± 0.10	19.0± 0.08	23. ± 0.07	29.8± 0.02
C 16:0 - 7 d	22.9± 0.05	25.1± 0.10	30.5± 0.11	23.2± 0.14	28.2± 0.10	29.2± 0.11	21.4± 0.05	23.1± 0.10	29.9± 0.05
C 16:1 - 1 d	1.4 ± 0.01	1.9 ± 0.06	2.0 ± 0.08	1.4 ± 0.02	2.1 ± 0.04	1.5 ± 0.02	0.9 ± 0.01	1.6 ± 0.05	1.6 ± 0.08
C 16:1 - 7 d	1.2 ± 0.03	2.2 ± 0.04	1.4 ± 0.09	1.3 ± 0.06	2.0 ± 0.10	1.5 ± 0.04	1.3 ± 0.03	1.7 ± 0.07	2.1 ± 0.04
C 17:0 - 1 d	1.4 ± 0.03	1.7 ± 0.02	1.4 ± 0.05	1.3 ± 0.03	1.4 ± 0.06	1.0 ± 0.01	1.6 ± 0.02	2.4 ± 0.05	1.3 ± 0.05
C 17:0 - 7 d	1.5 ± 0.09	1.9 ± 0.07	1.3 ± 0.04	1.3 ± 0.03	2.0 ± 0.10	1.5 ± 0.07	1.7 ± 0.10	1.7 ± 0.01	1.6 ± 0.06
C 17:1 - 1 d	0.5 ± 0.03	0.5 ± 0.06	0.8 ± 0.04	0.5 ± 0.01	0.6 ± 0.02	0.7 ± 0.05	0.3 ± 0.04	0.5 ± 0.02	0.5 ± 0.04
C 17:1 - 7 d	0.4 ± 0.02	1.1 ± 0.02	0.5 ± 0.03	0.5 ± 0.03	0.6 ± 0.02	0.7 ± 0.06	0.5 ± 0.08	0.6 ± 0.03	0.8 ± 0.02
C 18:0 - 1 d	13.5± 0.10	15.5± 0.07	12.7± 0.08	15.1± 0.08	13.2± 0.13	14.5± 0.09	21.7± 0.10	23.5± 0.17	16.8± 0.11
C 18:0 - 7 d	18.3± 0.05	10.5± 0.10	17.2± 0.15	16. ± 0.10	11.8± 0.16	17.4± 0.06	18.3± 0.05	15.1± 0.16	11. ± 0.14
C 18:1 - 1 d	48.8± 0.13	33.4± 0.11	47.9± 0.08	49.1± 0.17	40.1± 0.08	42.5± 0.19	45.2± 0.10	36.7± 0.15	40.6± 0.11
C 18:1 - 7 d	47.4± 0.11	51.0± 0.06	41.5± 0.10	47.0± 0.05	46.5± 0.12	39.9± 0.19	49.5± 0.06	48.0± 0.21	47.9± 0.13
C 18:2 - 1 d	7.1 ± 0.08	3.9 ± 0.05	4.9 ± 0.02	5.9 ± 0.07	5.4 ± 0.05	4.6 ± 0.03	7.1 ± 0.07	5.9 ± 0.08	0.8 ± 0.04
C 18:2 - 7 d	4.7 ± 0.07	4.6 ± 0.04	3.2 ± 0.05	6.1 ± 0.05	4.0 ± 0.06	4.5 ± 0.08	3.6 ± 0.05	4.3 ± 0.05	2.2 ± 0.03
C 18:3 - 1 d	0.4 ± 0.03	1.6 ± 0.02	0.3 ± 0.03	0.6 ± 0.04	0.9 ± 0.05	0.1 ± 0.01	0.6 ± 0.02	0.8 ± 0.03	0.6 ± 0.01
C 18:3 - 7 d	0.0	0.0	0.2 ± 0.03	0.2 ± 0.02	0.0	0.0	0.1 ± 0.02	0.0	0.0
SFA – 1 d	**41.7 ± 0.01**^{ay}	**58.6 ± 0.01**^{iz}	**44.0 ± 0.01**^{cy}	**42.4 ± 0.02**^{by}	**50.7 ± 0.08**^{ez}	**50.5 ± 0.05**^{ey}	**45.7 ± 0.03**^{dz}	**54.3 ± 0.02**^{fz}	**55.7 ± 0.04**^{gz}
SFA – 7 d	**46.3 ± 0.08**^{dz}	**41.0 ± 0.08**^{ay}	**53.2 ± 0.04**^{ez}	**44.8 ± 0.08**^{bz}	**46.8 ± 0.04**^{dy}	**53.3 ± 0.08**^{ez}	**44.9 ± 0.04**^{by}	**45.3 ± 0.08**^{cy}	**46.9 ± 0.01**^{dy}
UFA – 1 d	**58.3 ± 0.02**^{iz}	**41.4 ± 0.08**^{ay}	**56.0 ± 0.04**^{fz}	**57.6 ± 0.03**^{gz}	**49.3 ± 0.02**^{dy}	**49.5 ± 0.06**^{dz}	**54.3 ± 0.08**^{ey}	**45.7 ± 0.02**^{cy}	**44.3 ± 0.04**^{by}
UFA – 7 d	**53.7 ± 0.08**^{by}	**59.0 ± 0.04**^{ez}	**46.9 ± 0.03**^{ay}	**55.2 ± 0.04**^{dy}	**53.2 ± 0.05**^{bz}	**46.7 ± 0.04**^{ay}	**55.1 ± 0.08**^{dz}	**54.7 ± 0.03**^{cz}	**53.1 ± 0.04**^{bz}
MUFA – 1 d	**50.8 ± 0.03**^{ey}	**35.9 ± 0.01**^{ay}	**50.8 ± 0.08**^{ez}	**51.1 ± 0.02**^{fz}	**43.0 ± 0.04**^{cy}	**44.8 ± 0.02**^{dz}	**46.6 ± 0.02**^{ey}	**39.0 ± 0.08**^{by}	**42.9 ± 0.04**^{cy}
MUFA – 7 d	**49.0 ± 0.06**^{by}	**54.4 ± 0.03**^{ez}	**43.4 ± 0.09**^{ay}	**48.9 ± 0.02**^{by}	**49.2 ± 0.03**^{by}	**42.2 ± 0.08**^{ay}	**51.4 ± 0.03**^{dz}	**50.4 ± 0.06**^{cz}	**50.9 ± 0.02**^{cz}
PUFA – 1 d	**7.5 ± 0.03**^{iz}	**5.5 ± 0.01**^{dz}	**5.2 ± 0.08**^{cz}	**6.5 ± 0.02**^{fz}	**6.3 ± 0.04**^{ez}	**4.7 ± 0.02**^{bz}	**7.7 ± 0.02**^{jz}	**6.7 ± 0.08**^{gz}	**1.4 ± 0.04**^{ay}
PUFA – 1 d	**4.7 ± 0.06**^{ey}	**4.6 ± 0.03**^{ey}	**3.4 ± 0.09**^{by}	**6.3 ± 0.02**^{fy}	**4.0 ± 0.03**^{cy}	**4.5 ± 0.08**^{ey}	**3.7 ± 0.03**^{cy}	**4.3 ± 0.06**^{dy}	**2.2 ± 0.02**^{az}

* Different letters (*a, b, c, d, e, f, g, i*) within the same row indicate significant differences ($p \leq 0.05$);
Different letters (*y, z*) within the same column indicate significant differences ($p \leq 0.05$); n = 9; SD - standard deviation;

The established divergent differences in the fatty acid profiles of the three studied samples from muscle and perirenal adipose tissues, on the one hand, are probably due to the relatively small proportion of phospholipids (polar lipid fraction) compared to one of the non-polar fats (triacylglycerols). On the other hand, the different content of MUFA in the two studied muscles and perirenal fat depends on the metabolic muscle type and the topographic-anatomical specificity of the adipose tissue [40]. The complex influence of breed, age, and dietary conditions on the fatty acid composition of lipids in goat meat was also reported [39]. Additional research is needed to elucidate the nature of the interactions between these factors and the doses of phytonutrient supplements with antioxidant properties applied to growing-up lambs. It is important to consider the fact that adding polyphenolic compounds for a longer period or at higher doses can cause a prooxidant effect in meat and accelerate oxidative processes, including adversely affecting the fatty acid metabolism and composition [41]. It should also be borne in mind that ruminants' digestions associated with food fermentation and polyphenols are poorly absorbed in the ruminants [2, 42]. This may require the administration of significantly higher doses than those used in monogastric animals.

3.6. Color properties.

Divergent significantly ($p \leq 0.05$) changes in the color properties measured in the samples were found in m. *Longissimus dorsi* and m. *Semimembranosus*, both on 1 d post mortem and after 7 d of storage (Table 3).

Table 3. Changes of the color properties of lambs' m. *Longissimus dorsi* and m. *Semimembranosus* after 7 d storage at 0-4°C.

Samples	m. *Longissimus dorsi*			m. *Semimembranosus*		
	C	D	R	C	D	R
L* - 1 d	41.12 ± 0.12^{ay}	44.91 ± 0.53^{dz}	44.15 ± 0.17^{cy}	45.28 ± 0.08^{ez}	41.67 ± 0.19^{by}	44.97 ± 0.19^{dy}
L* - 7 d	43.27 ± 0.58^{bz}	42.60 ± 0.37^{by}	46.60 ± 1.06^{cz}	40.75 ± 0.86^{ay}	42.48 ± 0.45^{bz}	46.12 ± 0.44^{cz}
a* - 1 d	18.27 ± 0.19^{cy}	17.66 ± 0.30^{by}	18.03 ± 0.11^{bz}	16.77 ± 0.21^{ay}	19.75 ± 0.15^{ez}	18.88 ± 0.20^{dy}
a* - 7 d	19.33 ± 0.40^{cz}	19.10 ± 0.27^{cz}	17.75 ± 1.13^{ay}	19.70 ± 0.64^{dz}	18.98 ± 0.13^{cy}	18.73 ± 0.21^{by}
b* - 1 d	2.03 ± 0.04^{az}	3.20 ± 0.03^{cz}	3.46 ± 0.02^{dy}	3.06 ± 0.24^{cy}	2.50 ± 0.07^{bz}	4.04 ± 0.06^{ez}
b* - 7 d	1.95 ± 0.51^{ay}	2.33 ± 0.19^{ay}	3.93 ± 0.13^{bz}	3.87 ± 0.19^{bz}	2.37 ± 0.05^{ay}	3.70 ± 0.21^{by}
ΔE - 1 d		4.01	3.36		4.71	2.34
ΔE - 7 d		0.80	4.20		2.40	5.46

* Different letters (*a, b, c, d, e*) within the same row indicate significant differences ($p \leq 0.05$);
Different letters (*y, z*) within the same column indicate significant differences ($p \leq 0.05$); n = 9; SD - standard deviation;

Exceptions are the values for the brightness (L*), the red (a*), and the yellow (b*) color component established in the controls C and samples D from m. *Longissimus dorsi* after 7 d of chilled storage and the red (a*) color component found on 1 d and 7 d of chilled storage in samples R of m. *Semimembranosus*. No significant ($p > 0.05$) differences were found between the levels of the indicated samples (Table 3). Neither of the two phytonutrients studied had a one-way effect on the color properties of the chilled lamb muscles. Compared to the controls C on 1 d post mortem, the total color difference (ΔE) in samples D was greater than the one found for samples R (4.01 and 4.71, respectively, compared to 3.36 and 2.34 for samples from m. *Longissimus dorsi* and m. *Semimembranosus*, respectively). After 7 d of storage, the opposite trend was established. The ΔE reported for samples D was significantly smaller (0.80 and 2.40) compared to the one found for samples R (4.20 and 5.46) respectively in m. *Longissimus dorsi* and m. *Semimembranosus*. The pigment responsible for meat color stability is the water-soluble sarcoplasmic protein myoglobin [27].

It undergoes auto-oxidative transformations. During refrigeration connected with exposure to oxygen from the air and light, iron and heme myoglobin are oxidized to metmyoglobin - a pigment with an unattractive grey-brown color [27]. An enzyme system was also found in the muscle tissue that exhibited metmyoglobin reductase activity (MRA). It is able to reduce metmyoglobin back to myoglobin [43]. In fresh meat (1 d post mortem), the enzyme retains high activity, and the formed metmyoglobin is rapidly reduced to deoxymyoglobin. During the 7- day chilled storage of meat at 0-4°C the activity of MRA does not decrease significantly. Under the action of oxygen and light on the surface of the meat, oxidative processes take place [31]. Initially, they are associated with the formation of oxymyoglobin, but as the shelf life progresses, it can self-oxidize iron from heme from Fe^{2+} to Fe^{3+} and thus be transformed into metmyoglobin [43]. Because all forms of myoglobin oxidation are interconnected, once the lipid peroxidation process is initiated, prooxidants are formed that react with oxymyoglobin and contribute to its conversion to metmyoglobin [17]. Since meat oxidation is an autocatalytic chain reaction initiated by lipid hydroperoxides that are transferred to the myoglobin fraction, we can relate the observed color changes to those of lipid oxidation found (Table 1).

3.7. Microbiological status.

On 1 d post mortem, no significant ($p > 0.05$) differences were found in APC and in TYMC in any of the samples in m. *Longissimus dorsi*, m. *Semimembranosus* and perirenal adipose tissue (Table 4).

After 7 d of storage APC and TYMC of samples D and R in lamb's m. *Longissimus dorsi*, m. *Semimembranosus* and perirenal adipose tissue were significantly ($p \leq 0.05$) smaller compared to controls C. The comparison between APC and resp. TYMC found in samples D, and R showed no significant ($p > 0.05$) differences either in m. *Longissimus dorsi* and m. *Semimembranosus* or in perirenal fatty tissue.

Table 4. Changes of the aerobic plate count and total combined yeasts and molds count of lamb's m. *Longissimus dorsi*, m. *Semimembranosus* and perirenal fatty tissue after 7 d storage at 0-4°C.

Samples	**m. *Longissimus dorsi***			**m. *Semimembranosus***			**Perirenal fatty tissue**		
Parameter	**C**	**D**	**R**	**C**	**D**	**R**	**C**	**D**	**R**
APC, lg cfu/g - 1d	4.25 ± 0.07 ^{ay}	4.20 ± 0.08 ^{ay}	4.27 ± 0.04 ^{ay}	4.27 ± 0.08 ^{ay}	4.25 ± 0.06 ^{ay}	4.26 ± 0.05 ^{ay}	5.11 ± 0.08 ^{ay}	5.05 ± 0.09 ^{ay}	5.07 ± 0.05 ^{ay}
APC, lg cfu/g - 7d	7.38 ± 0.07 ^{bz}	6.77 ± 0.10 ^{az}	6.74 ± 0.15 ^{az}	7.42 ± 0.08 ^{bz}	6.81 ± 0.09 ^{az}	6.78 ± 0.11 ^{az}	7.18 ± 0.11 ^{bz}	6.94 ± 0.12 ^{az}	6.91 ± 0.13 ^{az}
TYMC, lg cfu/g - 1d	4.31 ± 0.01 ^{ay}	4.30 ± 0.03 ^{ay}	4.25 ± 0.08 ^{ay}	4.33 ± 0.03 ^{ay}	4.35 ± 0.04 ^{ay}	4.28 ± 0.06 ^{ay}	4.67 ± 0.03 ^{ay}	4.66 ± 0.05 ^{ay}	4.61 ± 0.09 ^{ay}
TYMC, lg cfu/g - 7d	4.94 ± 0.06 ^{bz}	4.58 ± 0.02 ^{az}	4.54 ± 0.03 ^{az}	4.99 ± 0.07 ^{bz}	4.59 ± 0.04 ^{az}	4.58 ± 0.02 ^{az}	5.03 ± 0.04 ^{bz}	4.80 ± 0.07 ^{az}	4.81 ± 0.08 ^{az}

* Different letters (*a* or *b*) within the same row indicate significant differences ($p \leq 0.05$);
Different letters (*y* or *z*) within the same column indicate significant differences ($p \leq 0.05$); n = 9; SD - standard deviation;

Regardless of the relatively small doses of administration and the relatively short period of feeding with both phytonutrients, their intake had a certain bactericidal effect after seven days of storage of chilled lamb's m. *Longissimus dorsi*, m. *Semimembranosus* and perirenal fatty tissue. This effect is probably due to the antibacterial action of the flavonoids contained in the studied phytonutrients [44]. Our results agree with previously reported, according to which the total number of bacteria usually increases with the storage time and on 7 d after death does not exceed 108 cfu/g [30].

Dietary supplementation of lambs with 7.5 mg dihydroquercetin/kg/d or 545 mg DDRP/kg/d does not unidirectional affect the quality of chilled lamb after 7 d of storage. The POV levels in both *Longissimus dorsi* and *Semimembranosus* muscles and the perirenal adipose tissue did not depend on the supplementation of 7.5 mg dihydroquercetin/ kg/d to the lamb's diet.

On 1 d post mortem, the content of α-aminoacidic nitrogen and APC and TYMC in the muscle and perirenal adipose tissue are not affected by the supplementation of the lamb's diet with any of the two phytonutrient supplements. On the contrary, TBARS was low and ranged within 0.4 - 0.7 mg MDA/kg without a one-way trend, whereas the FFA of m. *Longissimus dorsi* with both supplements was reduced by about ⅙. In m. *Semimembranosus* and perirenal adipose tissue, only the supplementation of the lamb's diet with 545 mg DDRP/kg/d had a similar reducing action of FFA.

After 7 d of storage, the supplementation of 545 mg DDRP/kg/d to the lamb's diet contributed to a 2 - 6% decrease in pH in both muscle and perirenal fatty tissues; reduced the accumulation of α-aminoacidic nitrogen by 7 - 14% and TBARS by more than 20% but only in m. *Longissimus dorsi* and m. *Semimembranosus*. Both studied phytonutrients supported the reduction of APC and TYMC in m. *Longissimus dorsi* and m. *Semimembranosus* as well as in the perirenal fatty tissue. In contrast, the reduction of FFA, approximately a 5% decrease in SFA at the expense of an increase in MUFA, and a smaller total color difference were found only with the supplementation of 7.5 mg dihydroquercetin/kg/d to the lamb's diet as the established changes were more pronounced in m. *Longissimus dorsi*.

Further studies are needed to answer whether the use of higher doses of DDRP and dihydroquercetin as lamb's dietary supplements may be able to provoke a more pronounced inhibitory effect on lipid oxidation and fatty acid composition of meat and fats.

4. Conclusions

The supplemented by 545 mg DDRP/kg life wight/d lamb's diet contributes for a reduction of α-aminoacidic nitrogen and TBARS but only in m. Longissimus dorsi and m. Semimembranosus after 7 d of storage at 0-4°C. Both studied phytonutrients supported the reduction of APC and TYMC in m. Longissimus dorsi and m. Semimembranosus as well as in the perirenal fatty tissue. In contrast, the 5% reduction of FFA, a decrease of SFA at the expense of an increase in MUFA, and a smaller total color difference were found only with the supplementation of 7.5 mg dihydroquercetin/kg life wight/d to the lamb's diet as the established changes were more pronounced in m. *Longissimus dorsi*. Further studies are needed to answer whether the use of higher doses of DDRP and dihydroquercetin as lamb's dietary supplements may be able to provoke a more pronounced inhibitory effect on lipid oxidation and fatty acid composition of meat and fats.

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Conflicts of Interest

The authors declare no conflict of interest.

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