Food Science and Applied Biotechnology, 2022, **5**(2), 240-255

UFT Academic Publishing House, Plovdiv

Food Science and Applied Biotechnology
e-ISSN: 2603-3380

Journal home page: www.ijfsab.com
https://doi.org/10.30721/fsab2022.v5.i2



OPEN ACCESS

Research Article

Quality changes of *Longissimus dorsi* and *Semimembranosus* muscles and perirenal adipose tissue during frozen storage of lambs fed dihydroquercetin or dry distilled rose petals supplemented diet

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Abstract

The objective of the study was to evaluate the changes of frozen stored of *Longissimus dorsi* and *Semimembranosus* muscles and perirenal adipose tissue from Bulgarian Dairy Synthetic population lambs, fed a diet supplemented by 7.5 mg dihydroquercetin (DQ) or 545 mg dry distilled rose petals (DDRP).kg^{-1} life weight.d^{-1}. Three groups of 10 male lambs aged 65 days were fed 50 days ad libitum: a control (ground alfalfa + granular compound feed) and two experimental groups (the same diet + the addition of phytonutrients). Samples were collected 1 d post-mortem. A half of them were analyzed immediately. Another half was vacuum-packed, quickly frozen at -40°C and stored for 365 d at -18°C. Supplementing of the lamb's diet with polyphenol-rich phytonutrients reduces with 6.9 -12.9% the aerobic plate count, 9.3 - 25.3% the α-aminoacidic nitrogen, 31.8 - 45.9% the protein carbonyls and 38.9 - 63.4% TBARS ($p \leq 0.05$) more pronounced in the m. Longissimus dorsi from lambs fed with 545 mg DDRP.kg^{-1} life weight.d^{-1}. Further studies are needed to understand whether the use of higher doses of DDRP or DQ as supplements in the small ruminant's diet will be able to provide a more pronounced inhibitory effect on oxidation processes in frozen lamb.

Keywords: frozen storage, lambs, polyphenol-rich phytonutrients, meat, fat, quality

Abbreviations: APC – aerobic plate count; AV – acid value; DAD – diode array detector; DDRP – dry distilled rose petals; DQ – dihydroquercetin; ΔE – total color difference; FAME – fatty acid methyl esters; HPLC – high-performance liquid chromatography; HADH – β-hydroxyacyl CoA-dehydrogenase; GC – gas chromatography; LoD – limit of detection; MDA –malondialdehyde; MHC – myosin heavy chain; MRA – metmyoglobin reductase activity; MUFA – monounsaturated fatty acids; PBC -psychrotrophic bacterial count; POV – peroxide value; SFA – saturated fatty acids; TBARS – 2 thiobarbituric acid reactive substances; TLC – thin layer chromatography; TYMC – total combined yeasts and molds count

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Article history:
Received 12 July 2022
Reviewed 25 August 2022
Accepted 19 September 2022
Available on-line 13 October 2022

https://doi.org/10.30721/fsab2022.v5.i2.210
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Introduction

Lamb meat freezing and storing for a long period of time at -18°C is a practice that has arisen from the new structure of markets in a globalizing world (Coombs 2017). Hypermarket chains require that suppliers of frozen lamb should guarantee an even longer shelf life without adversely affecting its quality and safety (Muela et al. 2016). During longer storage of frozen lamb lipids are oxidized (Pinheiro et al. 2019). For these reasons, a variety of approaches have been proposed to mitigate the effects of freezing on the quality of lamb meat. A new opportunity to stabilize the quality of long-stored frozen lamb is the addition of natural biologically active phytonutrients to the feed (Oh et al. 2016). According to Surai (2014) polyphenols (mainly flavonoids) may be even more effective than vitamins E and C. Among the compounds having antimicrobial and antioxidant properties discuss the bioflavonoid dihydroquercetin (DQ). Its effectiveness in cattle's, pig's, broiler's, chicken's or bee's (Fomichev et al. 2016) feeding has been reported. Another natural source of polyphenols is dry distilled rose petals (DDRP): a by-product of the rose oil industry. They contain more than 30 polyphenolic compounds (Dragoev et al. 2021) and are characterized by antioxidant and antibacterial properties (Baydar & Baydar 2013). Therefore, the aim of this study was to identify the quality changes during frozen storage of m. *Longissimus dorsi*, m. *Semimembranosus* and perirenal adipose tissue from lambs fed a diet supplemented by dihydroquercetin or dry distilled rose petals.

Materials and Methods

Animals and experimental design. The experiments were performed with 30 clinically healthy male lambs aged 65 days, equal in live weight. The lambs were divided into three groups with 10 lambs fed ad libitum for 50 days. The control group (C) was fed with ground alfalfa + pelleted compound feed. The experimental groups (DQ) and (DDRP) were fed with the same diet supplemented with 7.5 mg DQ. kg^{-1} life weight. d^{-1} or 545 mg DDRP. kg^{-1} life weight. d^{-1} respectively. Similar doses have been used in pig diet supplementation (Ivanova et al. 2021), which have complex stomach and physiological peculiarities in digesting food. The composition of the feed, information on the weights of the lambs before and after the trial together with growth rates and intakes and the slaughter procedure are described in detail in previous our work (Stancheva et al. 2021). The experiments were carried out in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Regulation (EC) No. 1099/2009 of the Council, Recommendation 2007/526/EC of the Commission and the Law on Veterinary Medicine in Bulgaria. The experiment was coordinated and approved by the Bulgarian Commission on Scientific Ethics, in compliance with Council Directive 2010/63/EU. Lamb carcasses were chilled for 24 h at 0 - 4°C (day 1 of the experiment). The chilled carcasses were divided into two halves. From the left halves of each group were removed m. *Longissimus thoracis et lumborum* (between 9 and 15 thoracic vertebrae, hereinafter referred to as m. *Longissimus dorsi*), m. *Semimembranosus* and perirenal adipose tissue (fat). The chilled muscles of the left halves of the carcasses were divided into two equal parts. The first - to determine the microbiological status and color characteristics, the second - minced in a mincer with a mesh diameter of 3 mm, collected together to prepare mean sample. Homogenization was done using homogenizer (Mechanika Precyzyjna, Model type ST-2). The final homogenate was sent for further analysis (1 d *post mortem*).

The muscles from the right halves of the carcasses were placed in plastic bags and vacuum sealed and were frozen at -40°C. The frozen samples were stored for 365 days (1 year) at -18°C. On the 365th day of the frozen storage, the samples were thawed without unpacking. After thawing, the above procedure is repeated.

Phytonutrients. The powdered DQ was supplied by Flavitlife Bio JSCo (Sofia, Bulgaria). It contained 96% dihydroquercetin, 3% dihydro-kaempferol and 1% naringenin. The distilled rose petals were delivered by Bulattars Production Company Ltd (Pavel Bania, Bulgaria). After pressing, the petals were dried for 24 h at 65°C and ground to a particle size < 0.4 mm. Details of the basic polyphenol indices, identification and quantification of polyphenol components and antioxidant activity of the DDRP can be found in previous our work (Dragoev et al. 2021).

Proteolysis and oxidative status of total proteins. The α-aminoacidic nitrogen was determined by the ninhydrin method modified by Garrido et al. (2012).

The protein oxidation was measured spectrophotometrically at 370 nm as protein dinitrophenylhydrazones included in the bases upon absorption of 21.0 $m.M^{-1}.cm^{-1}$ (Mercier et al. 2004). Due to the relatively limited protein content of fats and their very low amount in lamb carcasses similar analyses for perirenal adipose tissue were not performed.

Oxidative status of total lipids. Extraction of total lipids was performed by the method of Bligh and Dyer (1959). The oxidative status of total muscle lipids and those of perirenal adipose tissue are characterized by: the degree of lipolysis expressed by acid value (AV) according to ISO 660 (2009); the levels of lipid hydroperoxides (primary products of lipid peroxidation) expressed by peroxide value (POV) and malondialdehyde (MDA) (secondary products of lipid peroxidation) expressed TBARS (Botsoglou et al. 1994).

The peroxide value 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 obtained Fe^{3+} and SCN (Schmedes & Hølmer 1989) with some modification. The extracted lipids (0.1 g) with 50 µl $FeCl_2$, 50 µl NH_4SCN and $CHCl_3$:CH_3OH (3:5, v/v) were mixed to a volume of 10 ml. The sample was left for 10 min and the absorption was measured at 507 nm against a control prepared from 0.1 g of total lipids, 50 µl of NH_4SCN, $CHCl_3$:CH_3OH (3:5, v/v) to a volume of 10 ml. POV was calculated by equation (1):

$$POV = (A \times 10): (a \times 55.84 \times 22), meqO_2.kg^{-1} lipids \quad (1)$$

where

A is the concentration reported on the standard line, $\mu eqFe^{3+}. ml^{-1}$;

a is the amount of total lipid sample taken for analysis, g;

22 is the conversion factor of $\mu eqFe^{3+}$ to $meqO_2$.

TBARS were determined by measuring the absorption of samples at 532 nm on a Camspec M 550 double beam UV-Vis spectrophotometer (Spectronic CamSpec Ltd, Garforth, UK).

Fatty acid profiles. The fatty acid composition of the total lipids was determined by gas chromatography (GC) after transmethylation of the samples with 2% H_2SO_4 in CH_3OH at 50°C (ISO 12966-2 2011). 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) with mobile phase n-hexane: diethyl ether (97:3, v/c). Gas chromatographic analysis was performed on a GC gas chromatograph Agilent 6890 Plus (Agilent Technologies, Santa Clara, USA) equipped with 5793 mass-selective detector (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 $6°C.min^{-1}$ to 190°C (0 min) at $10°C.min^{-1}$ to 250°C (0 min); the injector and detector temperatures are maintained at 250°C. Hydrogen is a carrier gas at a flow rate of $0.8 ml.min^{-1}$ 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 (ISO 12966-1 2014).

Color properties. The meat color properties were measured in three places on both sides of the two-centimeter incision from each muscle (n = 10) made perpendicular to the direction of the muscle fibers (24 h and after thawing the samples to 1°C on 365 d post mortem) by a Konica Minolta model CR-410 chromometer using the CIE L*, a*, b* system. The calibration step was determined against white reference standard no.18833116 (Y = 94.3, x = 0.3134 and y = 0.3197). The color components were measured nine times. The brightness of the color L* (ranging from 0 black to 100 white): the red component of the color a* (varying from - green to + red) and the yellow component of the color b* (varying from - b blue to + b yellow) were measured at the following settings: aperture = 8 mm, standard observer 2° and light source D65 (Karp et al. 2020).

The total color difference (ΔE) (Tkacz et al. 2020).

was also calculated by equation 2:

$$\Delta E = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \quad (2)$$

where ΔE is the total color difference and ΔL^*, Δa^* and Δb^* are the differences in the values of the color brightness (L*): the red component of the color (a*)

and the yellow component of the color (b*) between the compared samples e.g., between the control and the corresponding experimental sample.

Biogenic amines. From a ground sample 10 g were weighed and transferred to a 50 cm^3 conical flask with 10 cm^3 of 0.4 M perchloric acid. The mixture was stirred on a shaker for 30 min. The homogenized sample was centrifuged for 10 min at 1,000×g and the separated supernatant was filtered through filter paper (Watman 1). To 1 cm^3 of the obtained extract were added 50 µl of the internal standard solution (1,000 ppm 1,7-diaminoheptane solution): 200 µl of 2N sodium hydroxide solution, 300 µl of saturated sodium bicarbonate solution and 2 ml of dansyl chloride solution (10 mg/ml). The sample was incubated in the dark at room temperature for 15 min. A hundred µl of NH_4OH was added to remove residual dansyl chloride. After staying for 5 min at room temperature the volume of the reaction mixture was brought to 5 ml with acetonitrile and centrifuged for 5 min at 2,500×g. This sample was filtered through a syringe filter with a PVDF membrane (pore size 0.45 µl). The filtered sample (20 µl) was injected into the HPLC injector of the Hitachi LaChrom Elite® HPLC system (Hitachi High Technologies America, Inc., Schaumburg, IL, USA): equipped with a diode array detector (DAD): ELITE LaCHrome software and a Purospher STAR column (C18) (4.6 x 150 mm 5 µm particle size). A mixture of acetonitrile and water in a volume ratio of 70:30 was used as the mobile phase. The mobile phase was pre-filtered through a membrane filter with a pore size of 0.45 µm and subjected to degassing in an ultrasonic bath. The flow rate is 0.8 $ml.min^{-1}$. There was a 10 min wait phase before the next analysis so that the equilibrium in the column could be restored. The absorption of the dansylated biogenic amine derivatives was recorded at 254 nm by the UV detector of the HPLC system and the final amounts were calculated using standard curves for five biogenic amines: putrescine, cadaverine, tyramine, histamine and 1,7-diaminoheptane. The results obtained are presented as $mg.kg^{-1}$ of dry matter.

Microbiological analysis. The microbiological status of the samples was established by bacteriological tests carried out in accordance with the microbiological criteria of Regulation (EC) № 1441 of 05.12.2007 according to ISO 4833 (2003). Upon opening each vacuum pack (n = 10 for each muscle): 15 grams of sample are taken under sterile conditions. This is followed by fine chopping and ten-fold dilution with saline in a Stomacher bag at 230 RPM for 2 min. For this purpose, a Stomacher 400 circulator was used.

Statistical analysis. A two-way ANOVA was used to determine whether the variability of the outcomes is due to chance or to the two factors in the analysis – the type of diet (Control, DQ or DDRP) and time of frozen storage (1 or 365 days) (for each type of tissue, separately): and to determine whether there is a two-way relationship among variables on an outcome. The symbol * indicates the significance ($p \leq 0.05$) of both factors (type of diet and time of storage) and their interaction; and the symbol ** indicates significance ($p \leq 0.05$) only for the factor time of storage. Results were presented as means ± standard error of the means (SEM). The measurements were made in ten repetitions (n = 10). The statistical package Microsoft Excel Office Professional Plus 2010 was used.

Results and Discussion

Changes in the muscle's proteins. The levels of α-aminoacidic nitrogen in the studied samples on 1 d post mortem fluctuated within narrow limits (between 2.10 - 2.50 mg $Leu.g^{-1}$) although they are significantly ($p \leq 0.05$) different (Table 1). After 365 d of storage of frozen lamb muscles at -18°C, the proteolytic processes deepen and the content of α-aminoacidic nitrogen increases 3.6 - 4.2 times. This increase was more pronounced in control samples in both muscles. As a result, mitochondrial and lysosomal enzymes, heme iron and other prooxidants are released from the damaged microstructure of the muscle cells which lead accelerated protein oxidation (Leygonie et al. 2012). Elevated levels of α-aminoacidic nitrogen may also be due to the decreased activity of Ca^{2+} ATPase and the increased activity of Mg^{2+}-EGTA-ATPase - enzymes involved in the denaturation of myosin and troponin-tropomyosin complex in frozen muscles (Benjakul et al. 2003). The addition of phytonutrients to the feed to some extent limits the accumulation of α-aminoacidic nitrogen during the one-year storage of frozen lamb. This phenomenon is more pronounced in samples DDRP in m. *Longissimus dorsi*. Probably in biological systems in vivo the polyphenolic compounds of DDRP inhibit muscle proteolytic enzyme systems by a still unclear

Table 1. Changes in muscle protein fraction affected by feed supplementation with phytonutrients and one-year (365d) of storage at -18°C

Lamb tissue	**m. Longissimus dorsi**			**m. Semimembranosus**		
Parameters	**C**	**DQ**	**DDRP**	**C**	**DQ**	**DDRP**
α-aminoacidic nitrogen, mg Leu.g^{-1} - 1 d	2.10 ± 0.08*	2.31 ± 0.02*	2.43 ± 0.03*	2.10 ± 0.08*	2.50 ± 0.01*	2.34 ± 0.03*
α-aminoacidic nitrogen, mg Leu.g^{-1} - 365 d	9.94 ± 0.04*	8.46 ± 0.05*	7.43 ± 0.07*	10.36 ± 0.01*	9.24 ± 0.18*	9.45 ± 0.11*
Protein carbonyls, nmol DNPH.mg^{-1} protein - 1 d	0.0469 ± 0.0008*	0.0470 ± 0.0012*	0.0319 ± 0.0005*	0.0469 ± 0.0011*	0.0469 ± 0.0008*	0.0488 ± 0.0006*
Protein carbonyls, nmol DNPH.mg^{-1} protein – 365 d	0.0952 ± 0.0004*	0.0954 ± 0.0007*	0.0649 ±0.0007*	0.1083 ± 0.0009*	0.1168 ± 0.0008*	0.2004 ± 0.0028*

C - control group lambs fed with ground alfalfa + pelleted compound feed
DQ - experimental groups lambs fed with the same diet supplemented with the addition of 7.5 mg DQ.kg^{-1} life weight.d^{-1}
DDRP - experimental groups lambs fed with the same diet supplemented with the addition of 545 mg dry distilled rose petals (DDRP).kg^{-1} life weight.d^{-1}
Results are presented as Means ± Standard error of the means (SEM); n = 10

* - indicates significance ($p \leq 0.05$) of both factors (type of diet and time of frozen storage) and their interaction;

mechanism (Surai 2014). Similar trends were found for protein carbonyls whose values compared to control samples were significantly lower in frozen samples DDRP in m. *Longissimus dorsi* as well as in m. *Semimembranosus* (Table 1). The oxidative degradation of frozen meat is provoked by the formation and growth of ice crystals. They cause mechanical damage to membranes and other cellular structures. This increases the concentration of prooxidants in the unfrozen phase of meat. Compared to samples DDRP, levels of protein carbonyls in samples DQ of m. *Semimembranosus* were slightly smaller. These results confirm the hypothesis that the metabolic profile of muscles affects the oxidative stability of muscle proteins. They are evidence that the addition of 545 mg DDRP.kg^{-1} life weight.d^{-1} to lambs' diet can successfully inhibit proteolysis and protein oxidation in m. *Longissimus dorsi*. According to Leygonie et al. (2012) there is a strong interdependence between protein denaturation and protein oxidation (carbonyl formation). More significant protein oxidation enhances myosin heavy chain degradation (MHC) but has little effect on actin degradation (Xue et al. 2013).The elevated levels of protein carbonyls in frozen lamb muscles stored for 365 d at -18°C are probably due to the autooxidation of myoglobin. It is accompanied by the formation of carbonyl derivatives of myofibrillar proteins as a result of their interaction with by-products of lipid oxidation such as malondialdehyde . Our results are in unison with the hypothesis of Traore et al. (2003).that during cold storage protein oxidation levels increase insignificantly in meat.

Changes in the total lipids of lamb's muscles and perirenal fatty tissue. The AV of the lipids extracted from m. *Longissimus dorsi* in samples DQ and DDRP on 1 d post mortem is less ($p \leq 0.05$) with approx. 15% and AV from perirenal adipose tissue is less with about 15% and resp. 7%, compared to controls (Table 2). A similar trend was found after 365 d of frozen storage where the AV in samples DQ was more than 17% and in samples DDRP it was approx. 5% lower than the one of the controls. Both on 1 d post mortem and after 365 d of frozen storage this indicator has the lowest values in samples DQ of m. Semimembranosus. After 365 d of frozen storage, the AV of perirenal adipose tissue was found to be the lowest in samples DQ.

Relatively low levels of both primary (POV) and secondary (TBARS) products of lipid oxidation were found, both on 1 d post-mortem and after 365 d frozen storage (Table 2). This was the idea of the experiment: to provide a set of antioxidants through feed supplements to help the lambs' organism to build an integrated antioxidant system. It was established that in all samples on 1 d post mortem the POV varies between 0.105-0.126 $\mu eqO_2.g^{-1}$ lipids. After 365 d of frozen storage some reduction in POV levels was found compared to those found on 1 d post-mortem. They vary between 0.064-0.075 $\mu eqO_2.g^{-1}$ lipids. An exception was observed only in control samples of m. *Semimembranosus* which are significantly higher, both on 1 d post-mortem (0.126 $\mu eqO_2.g^{-1}$ lipids) and after 365 d of frozen storage (0.123 $\mu eqO_2.g^{-1}$ lipids) resp. (Table 2). Tissue hydroperoxides (derivatives of free radicals) are unstable structures with proven toxicity (Yi et al. 2013). They undergo chemical transformations and turn into secondary products of lipid oxidation. Such reactions need 2- 4 h at 37°C to carry out (Yi et al. 2013). This explains the relatively low POV established on 1 d post-mortem. The formation of approx. 70% of the non-polar peroxides due to the change in fatty acid composition and heme iron content (Yi et al. 2013). This can explain the lower POV values found in the samples after 365 d of frozen storage.

It was found that TBARS in two lamb muscles were statistically ($p \leq 0.05$) distinguishable but varied within an extremely small range: 0.21-0.70 mg MDA.kg^{-1} (1 d post mortem) and 0.10-0.41 mg MDA.kg^{-1} (after 365 d of frozen storage) (Table 2). In samples of perirenal adipose tissue TBARS ranged between 0.10-0.79 mg MDA.kg^{-1} (Table 2) and in two muscle frozen samples DDRP and DQ it was lower than the one in control samples. As mentioned above, MDA was one of the secondary derivatives of lipid-oxidation reactions. It can react with the breakdown products of proteins and form carbonyl derivatives. Therefore, the oxidation of proteins and lipids is interrelated (Benjakul et al. 2003). The MDA can react with a wide range of compounds or form dimers or trimers. Consequently, the amount of MDA that can react with TBA decreases, resulting in reduced TBARS. This explains the relatively lower levels reported by us for TBARS after 365 days of frozen storage

Table 2. Changes in the lipid fraction of muscle and perirenal adipose tissues affected by feed supplementation with phytonutrients and one-year (365 d) of frozen storage at -18°C

Lamb tissue	**m. *Longissimus dorsi***			**m. *Semimembranosus***			**Perirenal adipose tissue**		
Parameters	**C**	**DQ**	**DDRP**	**C**	**DQ**	**DDRP**	**C**	**DQ**	**DDRP**
Acid value, mg KOH.g^{-1} - 1 d	4.41±0.05*	3.80±0.02*	3.64±0.03*	4.85±0.04*	4.65±0.05*	2.85±0.02*	2.25±0.02*	2.09±0.01*	1.92±0.01*
Acid value, mg KOH.g^{-1} - 365 d	5.53±0.04*	4.59±0.03*	5.04±0.03*	5.86±0.03*	5.44±0.02*	5.53±0.02*	3.84±0.03*	3.30±0.03*	3.98±0.02*
Peroxide value, $\mu eqO_2.g^{-1}$ lipids - 1 d	0.111±0.002*	0.119±0.001*	0.112±0.002*	0.126±0.001*	0.116±0.001*	0.113±0.001*	0.108±0.001*	0.105±0.001*	0.105±0.001*
Peroxide value, $\mu eqO_2.g^{-1}$ lipids-365d	0.064±0.001*	0.074±0.001*	0.071±0.001*	0.123±0.001*	0.075±0.001*	0.074±0.001*	0.074±0.001**	0.072±0.002**	0.073±0.001**
TBARS, mg MDA.kg^{-1} - 1 d	0.43±0.011*	0.34±0.022*	0.64±0.025*	0.70±0.042*	0.59±0.003*	0.53±0.042*	0.24±0.013*	0.21±0.010*	0.31±0.012*
TBARS, mg MDA.kg^{-1} -365 d	0.20±0.012*	0.20±0.004*	0.10±0.008*	0.41±0.037*	0.15±0.007*	0.19±0.007*	0.54±0.009*	0.33±0.012*	0.79±0.007*

C - control group lambs fed with ground alfalfa + pelleted compound feed
DQ - experimental groups lambs fed with the same diet supplemented with the addition of 7.5 mg DQ.kg^{-1} life weight.d^{-1}
DDRP - experimental groups lambs fed with the same diet supplemented with the addition of 545 mg dry distilled rose petals (DDRP).kg^{-1} life weight.d^{-1}
Results are presented as Means ± Standard error of the means (SEM); n = 10
* - indicates significance ($p \leq 0.05$) of both factors (type of diet and time of frozen storage) and their interaction;
** - indicates significance ($p \leq 0.05$) for the factor time of storage only;

Due to sufficient unfrozen water in the samples at -18°C biochemical reactions may occur, although with less speed (Leygonie et al. 2011).

The addition of DDRP to the lamb's diet can be used as an appropriate tool for inhibiting lipolysis in muscle tissue. Neither this supplement nor the addition of DQ significantly the initiation and development of lipid peroxidation in lamb muscles but they reduce by about 44% the levels of MDA in perirenal adipose tissues. At the intake 7.5 mg DQ. kg^{-1} life weight. d^{-1} and 545 mg DDRP. kg^{-1} life weight. d^{-1} the polyphenolic compounds are insufficient to provide a more pronounced effective antioxidant protection. In conclusion, negative temperature and one-year frozen storage factors are mainly responsible for the reduction of oxidative processes in the fraction of total muscle lipids. POV and TBARS decrease significantly ($p \leq 0.05$) after 365 d of frozen storage. Further studies are needed to determine whether administering higher doses of DQ and DDRP in small ruminants will provide a more pronounced inhibitory effect on lipid oxidation in muscles.

Fatty acid profiles of lamb's muscles and perirenal fatty tissue. Changes in the fatty acid compositions of total lipids extracted from m. *Longissimus dorsi*, m. *Semimembranosus* and perirenal adipose tissue after 365 d of frozen storage at -18°C are too divergent (Table 3). Feeding dry diets enriched with phytonutrients increases the levels of unsaturated fatty acids as well as stearic acid (C18:0) in muscle lipids and fat depots (Banskalieva et al. 2000) but we found a similar tendency only in samples DQ. After 365 d frozen storage the lowest content of SFA was found in samples DDRP of m. *Longissimus dorsi* and in samples DQ of m. *Semimembranosus* and perirenal adipose tissue at the expense of an increase in the proportion of monounsaturated fatty acids (MUFA) (Table 3). Additional experiments are needed to elucidate the nature of the interactions between breed, age and feeding conditions that affect the fatty acid composition of lamb's lipids and fats (Banskalieva et al. 2000). The differences found may be due to the relatively small proportion of the phospholipid fraction against the fraction of neutral lipids and the different content of MUFA in both studied muscles and in perirenal adipose tissue.

Color characteristics. After 365 d of storage at -18°C a significant ($p \leq 0.05$) increase in color brightness (L*) red (a*) and yellow (b*) color components in controls of m. *Longissimus dorsi* and m. *Semimembranosus* was determined (Table. 4). Both phytonutrients did not have a one-way effect on the color characteristics of the frozen lamb muscles. Compared to the controls C on 1 d post mortem, the total color difference (ΔE) in samples DQ is bigger than that for samples DDRP. After 365 d of storage at -18°C, the trend is reversed. The ΔE in samples DQ is significantly smaller compared to samples DDRP in both muscles. The myoglobin is responsible for the meat color stability after freezing and thawing (Leygonie et al. 2012), because it denatures and the iron heme is oxidized which leads to the formation of gray-brown metmyoglobin. One-day post-mortem the enzyme system with metmyoglobin reductase activity (MRA) is very active and the formed metmyoglobin is rapidly reduced to deoxymyoglobin and oxidized back to oxymyoglobin. After 365 d storage at -18°C MRA is rapidly inactivated by the mitochondrial cytoplasm enzyme β-hydroxyacyl CoA-dehydrogenase (HADH). Its activity decreases as a result, metmyoglobin is formed on the surface of the meat. Therefore, meat oxidation is a chain autocatalytic reaction initiated by lipid hydroperoxides that is transferred to the myoglobin fraction (Zhou et al. 2016). It was found the stabilization of the brightness (L*) and the red (a*) component of the color and contributes to ($p \leq 0.05$) an increase in the yellow (b*) component by approx. 1.5 times in samples DDRP of frozen m. *Longissimus dorsi* (365 d). For comparison, in the samples DQ a significant increase of the brightness (L*) by 13% of the red (a*) component by 6% and of the yellow (b*) component of the color - 2.7 times was found. Samples DDRP of frozen m. *Semimembranosus* (365 d) showed ($p \leq 0.05$) a decrease in the brightness (L*) by more than 15%, in the red (a*) component by a little over 3% and in the yellow (b*) component of the color by about 32% (Table 4). In parallel, in samples DQ of frozen m. *Semimembranosus* (365 d) the color brightness (L*) is reduced by 2% only, the red (a*) component of the color by 3.5% and the yellow (b*) component is increased 1.77 times (Table 4)

Table 3. Changes in the fatty acid composition of total lipids from muscle and perirenal adipose tissues affected by feed supplementation with phytonutrients and one-year (365 d) of frozen storage at -18°C

Lamb tissue	**m. Longissimus dorsi**			**m. Semimembranosus**			**Perirenal adipose tissue**		
Fatty acids	**C**	**DQ**	**DDRP**	**C**	**DQ**	**DDRP**	**C**	**DQ**	**DDRP**
C 12:0 - 1 d	0.2 ± 0.01	0.1 ± 0.01	0.2 ± 0.02	0.1 ± 0.01	0.5 ± 0.02	0.6 ± 0.03	0.3 ± 0.01	0.5 ± 0.01	0.9 ± 0.01
C 12:0 - 365 d	0.2 ± 0.02	0.4 ± 0.01	0.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.02	0.3 ± 0.01	0.1 ± 0.02	0.3 ± 0.01	0.5 ± 0.02
C 14:0 - 1 d	2.5 ± 0.01	5.8 ± 0.01	3.4 ± 0.01	2.8 ± 0.02	5.1 ± 0.01	4.8 ± 0.01	2.9 ± 0.01	4.3 ± 0.01	6.2 ± 0.01
C 14:0 - 365 d	4.1 ± 0.02	4.4 ± 0.02	2.3 ± 0.02	5.2 ± 0.01	3.0 ± 0.01	3.6 ± 0.02	3.0 ± 0.03	3.7 ± 0.02	5.2 ± 0.01
C 15:0 - 1 d	0.1 ± 0.03	0.7 ± 0.02	0.4 ± 0.01	0.4 ± 0.01	0.5 ± 0.03	0.4 ± 0.02	traces	0.1 ± 0.01	0.1 ± 0.01
C 15:0 - 365 d	0.4 ± 0.01	0.5 ± 0.01	0.4 ± 0.02	0.6 ± 0.02	0.9 ± 0.01	1.6 ± 0.01	0.9 ± 0.01	0.4 ± 0.02	0.7 ± 0.03
C 16:0 - 1 d	23.2 ± 0.02	34.4 ± 0.01	25.3 ± 0.01	22.5 ± 0.02	29.3 ± 0.01	28.6 ± 0.02	19.0 ± 0.01	23.2 ± 0.01	29.8 ± 0.02
C 16:0 - 365 d	27.4 ± 0.03	29.2 ± 0.02	24.8 ± 0.01	37.2 ± 0.02	27.6 ± 0.03	31.5 ± 0.01	26.3 ± 0.01	24.1 ± 0.02	28.1 ± 0.02
C 16:1 - 1 d	1.4 ± 0.02	1.9 ± 0.01	2.0 ± 0.02	1.4 ± 0.01	2.1 ± 0.03	1.5 ± 0.01	0.9 ± 0.02	1.6 ± 0.02	1.6 ± 0.03
C 16:1 - 365 d	1.6 ± 0.01	1.4 ± 0.01	1.3 ± 0.03	1.8 ± 0.02	2.4 ± 0.01	2.5 ± 0.02	1.4 ± 0.01	1.1 ± 0.02	2.4 ± 0.01
C 17:0 - 1 d	1.4 ± 0.02	1.7 ± 0.01	1.4 ± 0.01	1.3 ± 0.01	1.4 ± 0.01	1.0 ± 0.02	1.6 ± 0.01	2.4 ± 0.02	1.3 ± 0.01
C 17:0 - 365 d	1.8 ± 0.02	1.7 ± 0.01	2.0 ± 0.02	1.9 ± 0.01	2.5 ± 0.02	2.3 ± 0.02	2.2 ± 0.01	1.8 ± 0.02	1.7 ± 0.02
C 17:1 - 1 d	0.5 ± 0.01	0.5 ± 0.02	0.8 ± 0.01	0.5 ± 0.02	0.6 ± 0.01	0.7 ± 0.01	0.3 ± 0.02	0.5 ± 0.01	0.5 ± 0.02
C 17:1 - 365 d	0.8 ± 0.01	0.8 ± 0.02	0.9 ± 0.02	0.9 ± 0.02	1.7 ± 0.01	1.8 ± 0.01	0.8 ± 0.01	0.5 ± 0.01	0.9 ± 0.01
C 18:0 - 1 d	13.5 ± 0.02	15.5 ± 0.01	12.7 ± 0.02	15.1 ± 0.03	13.2 ± 0.03	14.5 ± 0.02	21.7 ± 0.01	23.5 ± 0.01	16.8 ± 0.03
C 18:0 - 365 d	10.8 ± 0.02	16.6 ± 0.02	6.2 ± 0.03	6.0 ± 0.01	6.4 ± 0.01	1.7 ± 0.03	8.2 ± 0.02	3.4 ± 0.03	7.5 ± 0.01
C 18:1 - 1 d	48.8 ± 0.01	33.4 ± 0.03	47.9 ± 0.01	49.1 ± 0.03	40.1 ± 0.03	42.5 ± 0.03	45.2 ± 0.01	36.7 ± 0.03	40.6 ± 0.03
C 18:1 - 365 d	51.7 ± 0.03	42.8 ± 0.03	61.4 ± 0.03	45.5 ± 0.01	53.7 ± 0.02	52.6 ± 0.01	56.4 ± 0.03	63.1 ± 0.01	49.1 ± 0.01
C 18:2 - 1 d	7.1 ± 0.02	3.9 ± 0.01	4.9 ± 0.01	5.9 ± 0.01	5.4 ± 0.02	4.6 ± 0.02	7.1 ± 0.03	5.9 ± 0.01	0.8 ± 0.01
C 18:2 - 365 d	0.7 ± 0.01	1.2 ± 0.01	0.3 ± 0.03	0.2 ± 0.03	0.5 ± 0.01	traces	traces	0.7 ± 0.02	3.2 ± 0.01
C 18:3 - 1 d	0.4 ± 0.02	1.6 ± 0.03	0.3 ± 0.02	0.6 ± 0.01	0.9 ± 0.01	0.1 ± 0.02	0.6 ± 0.01	0.8 ± 0.02	0.6 ± 0.02
C 18:3 - 365 d	0.1 ± 0.02	0.1 ± 0.01	traces	traces	traces	0.1 ± 0.02	traces	traces	0.1 ± 0.01
C 20:0 - 1 d	NF	NF	NF	NF	NF	NF	NF	NF	NF
C 20:0 - 365 d	NF	NF	NF	NF	NF	0.1 ± 0.01	NF	NF	NF
C 20:2 - 1 d	NF	NF	NF	NF	NF	NF	NF	NF	NF
C 20:2 - 365 d	0.1 ± 0.02	traces	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.1 ± 0.02	traces	0.6 ± 0.01	traces

Table 3. Continued

Saturated fatty acids SFA – 1 d	**41.7 ± 0.03***	**58.6 ± 0.01***	**44.0 ± 0.02***	**42.4 ± 0.01***	**50.7 ± 0.03***	**50.5 ± 0.02***	**45.7 ± 0.01***	**54.3 ± 0.01***	**55.7 ± 0.01***
Saturated fatty acids SFA – 365 d	**44.9 ± 0.01***	**53.1 ± 0.03***	**35.9 ± 0.01***	**51.2 ± 0.02***	**40.6 ± 0.03***	**41.5 ± 0.01***	**40.9 ± 0.01***	**33.7 ± 0.01***	**44.0 ± 0.03***
Unsaturated fatty acids UFA – 1 d	**58.3 ± 0.01***	**41.4 ± 0.03***	**56.0 ± 0.01***	**57.6 ± 0.01***	**49.3 ± 0.01***	**49.5 ± 0.02***	**54.3 ± 0.03***	**45.7 ± 0.01***	**44.3 ± 0.01***
Unsaturated fatty acids UFA – 365 d	**55.1 ± 0.01***	**46.8 ± 0.03***	**64.1 ± 0.01***	**48.8 ± 0.02***	**59.4 ± 0.01***	**58.5 ± 0.01***	**59.1 ± 0.01***	**66.3 ± 0.01***	**56.0 ± 0.02***
Monounsaturated fatty acids MUFA – 1 d	*50.8 ± 0.01**	*35.9 ± 0.01**	*50.8 ± 0.03**	*51.1 ± 0.01**	*43.0 ± 0.01**	*44.8 ± 0.01**	*46.6 ± 0.01**	*39.0 ± 0.03**	*42.9 ± 0.01**
Monounsaturated fatty acids MUFA – 365 d	*54.2 ± 0.02**	*45.6 ± 0.01**	*63.7 ± 0.02**	*48.5 ± 0.03**	*58.7 ± 0.01**	*58.3 ± 0.01**	*59.1 ± 0.03**	*65.0 ± 0.02**	*52.7 ± 0.01**
Polyunsaturated fatty acids PUFA – 1 d	*7.5 ± 0.01**	*5.5 ± 0.01**	*5.2 ± 0.03**	*6.5 ± 0.01**	*6.3 ± 0.01**	*4.7 ± 0.01**	*7.7 ± 0.01**	*6.7 ± 0.03**	*1.4 ± 0.01**
Polyunsaturated fatty acids PUFA – 365 d	*0.9 ± 0.01**	*1.3 ± 0.01**	*0.4 ± 0.01**	*0.3 ± 0.01**	*0.7 ± 0.01**	*0.2 ± 0.01**	*NF**	*1.3 ± 0.01**	*3.3 ± 0.01**

C - control group lambs fed with ground alfalfa + pelleted compound feed

DQ - experimental groups lambs fed with the same diet supplemented with the addition of 7.5 mg DQ.kg^{-1} life weight.d^{-1}

DDRP - experimental groups lambs fed with the same diet supplemented with the addition of 545 mg dry distilled rose petals (DDRP).kg^{-1} life weight.d^{-1}

Results are presented as Means ± Standard error of the means (SEM); n = 10

* - indicates significance ($p \leq 0.05$) of both factors (type of diet and time of frozen storage) and their interaction;

Traces - below the limit of detection (LoD)

NF - not found;

Table 4. Changes in muscle color characteristics affected by feed supplementation with phytonutrients and one-year (365d) of storage at -18°C

Lamb tissue	**m. Longissimus dorsi**			**m. Semimembranosus**		
Samples	**C**	**DQ**	**DDRP**	**C**	**DQ**	**DDRP**
L* -1 d	41.12 ± 0.04*	44.91 ± 0.15*	44.15 ± 0.05*	45.28 ± 0.02*	41.67 ± 0.06*	44.97 ± 0.08*
L* - 365 d	47.45 ± 0.08*	50.94 ± 0.08*	44.87 ± 0.02*	49.46 ± 0.09*	40.66 ± 0.09*	37.95 ± 0.30*
a* - 1 d	18.27 ± 0.06*	17.66 ± 0.09*	18.03 ± 0.03*	16.77 ± 0.06*	19.75 ± 0.05*	18.88 ± 0.06*
a* - 365 d	20.61 ± 0.03*	18.75 ± 0.05*	18.36 ± 0.06*	18.03 ± 0.16**	19.04 ± 0.07**	18.23 ± 0.29**
b* - 1 d	2.03 ± 0.01*	3.20 ± 0.01*	3.46 ± 0.01*	3.06 ± 0.07*	2.50 ± 0.03*	4.04 ± 0.02*
b* - 365 d	8.45 ± 0.09*	8.56 ± 0.04*	5.27 ± 0.01*	6.73 ± 0.15*	4.43 ± 0.04*	2.76 ± 0.05*
ΔE - 1 d		**4.01**	**3.36**		**4.71**	**2.34**
ΔE - 365 d		**3.96**	**4.67**		**9.45**	**12.18**

C - control group lambs fed with ground alfalfa + pelleted compound feed
DQ - experimental groups lambs fed with the same diet supplemented with the addition of 7.5 mg DQ.kg^{-1} life weight.d^{-1}
DDRP - experimental groups lambs fed with the same diet supplemented with the addition of 545 mg dry distilled rose petals (DDRP).kg^{-1} life weight.d^{-1}
Results are presented as Means ± Standard error of the means (SEM); n =10
* - indicates significance ($p \leq 0.05$) of both factors (type of diet and time of frozen storage) and their interaction;
**- indicates significance ($p \leq 0.05$) of factors type of diet and interaction between the factors, excluding the time of storage;

Table 5. Presence of biogenic amines after one year (365 d) of frozen storage at -18°C obtained from animals fed with phytonutrient supplements

Lamb tissue	**m. Longissimus dorsi**			**m. Semimembranosus**			**Perirenal adipose tissue**		
Samples	**C**	**DQ**	**DDRP**	**C**	**DQ**	**DDRP**	**C**	**DQ**	**DDRP**
Biogenic amines									
Putrescine -1 d	NF	NF	NF	NF	NF	NF	NF	NF	NF
Putrescine – 365 d	traces	traces	traces	traces	traces	traces	traces	traces	traces
Cadaverine – 1 d	NF	NF	NF	NF	NF	NF	NF	NF	NF
Cadaverine – 365 d	traces	traces	traces	traces	traces	traces	traces	traces	traces
Histamine – 1 d	NF	NF	NF	NF	NF	NF	NF	NF	NF
Histamine – 365 d	traces	traces	traces	traces	traces	traces	traces	traces	traces
Tyramine -1 d	NF	NF	NF	NF	NF	NF	NF	NF	NF
Tyramine -365 d	traces	traces	traces	traces	traces	traces	traces	traces	traces

C - control group lambs fed with ground alfalfa + pelleted compound feed
DQ - experimental groups lambs fed with the same diet supplemented with the addition of 7.5 mg DQ.kg^{-1} life weight.d^{-1}
DDRP - experimental groups lambs fed with the same diet supplemented with the addition of 545 mg dry distilled rose petals (DDRP).kg^{-1} life weight.d^{-1}
NF - not found;
Traces - below the limit of detection (LoD) 10 mg.kg^{-1};

Table 6. Microbiological status of m. *Longissimus dorsi* after one year (365 d) of frozen storage at -18°C obtained from animals fed with phytonutrient supplements

Lamb tissue	**m. Longissimus dorsi**			**m. Semimembranosus**			**Perirenal adipose tissue**		
Parameter	**C**	**DQ**	**DDRP**	**C**	**DQ**	**DDRP**	**C**	**DQ**	**DDRP**
Aerobic plate count (APC): lg cfu.g^{-1} - 1 d	4.25 ± 0.02*	4.20 ± 0.03	4.27 ± 0.01*	4.24 ± 0.01*	4.21 ± 0.02*	4.22 ± 0.01*	4.29 ± 0.02*	4.24 ± 0.01*	4.29 ±0.02*
Aerobic plate count (APC): lg cfu.g^{-1} - 365 d	5.26 ± 0.02*	4.85 ± 0.01*	4.58 ± 0.01*	5.25 ± 0.02*	4.89 ± 0.01*	4.68 ± 0.01*	5.35 ± 0.02*	4.94 ± 0.03*	4.66 ± 0.01*
Total yeasts and molds count (TYMC): lg cfu.g^{-1} - 1 d	4.31 ± 0.01*	4.30 ± 0.01*	4.25 ± 0.03*	4.33 ± 0.03*	4.29 ± 0.01*	4.28 ± 0.01*	4.34 ± 0.02*	4.31 ± 0.01*	4.32 ± 0.02*
Total yeasts and molds count (TYMC): lg cfu.g^{-1} - 365 d	NF**	NF**	NF**	NF**	NF**	NF**	NF**	NF**	NF**
Psychrotrophic bacterial count (PBC): lg cfu.g^{-1} - 1 d	3.73 ± 0.01*	3.92 ± 0.03*	3.91 ± 0.01*	3.88 ± 0.02*	3.73 ± 0.02*	3.71 ± 0.01*	4.00 ± 0.01*	4.12 ± 0.01*	4.11 ± 0.01*
Psychrotrophic bacterial count (PBC): lg cfu.g^{-1} - 365 d	4.28 ± 0.02*	4.60 ± 0.01*	4.59 ± 0.01*	4.26 ± 0.01*	4.58 ± 0.03*	4.57 ± 0.01*	4.44 ± 0.01**	4.48 ± 0.02**	4.47 ± 0.02**

C - control group lambs fed with ground alfalfa + pelleted compound feed
DQ - experimental groups lambs fed with the same diet supplemented with the addition of 7.5 mg DQ.kg^{-1} life weight.d^{-1}
DDRP - experimental groups lambs fed with the same diet supplemented with the addition of 545 mg dry distilled rose petals (DDRP).kg^{-1} life weight.d^{-1}
Results are presented as Means ± Standard error of the means (SEM); n = 10
*- indicates significance ($p \leq 0.05$) of both factors (type of diet and time of frozen storage) and their interaction;
** - indicates significance ($p \leq 0.05$) for the factor time of storage only;
NF - not found;

In summary, adding the DQ (Fomichev et al. 2016) or the rich in polyphenolic components DDRP (Dragoev et al. 2021) to the lamb's feed leads to divergent changes in color characteristics in both studied muscles after their 365 days of frozen storage. The total color difference in samples DQ is smaller than in samples DDRP.

Biogenic amines. The levels of biogenic amines are lower than the limit of detection (LoD - 10 mg.kg^{-1}) of the analytical method in all tested samples (Table 5). These results indicate that a major role in limiting the formation of biogenic amines in lamb muscle and adipose tissues is played by the freezing factor and not by of adding DQ or DDRP to lambs' feed.

Microbiological changes. Compared to control samples, adding DQ or DDRP to lambs' feed did not lead to significant changes in psychrotrophic bacterial count (PBC) and total combined yeasts and molds count (TYMC) in frozen lamb m. *Longissimus dorsi*, m. *Semimembranosus* and perirenal adipose tissue (Table 6). On the other hand, samples DQ and DDRP show a lower APC, more pronounced in samples DDRP. Regardless of the relatively small doses and the relatively short period of application of phytonutrients as feed supplements their intake has a certain bactericidal effect after one year of frozen storage of lamb m. *Longissimus dorsi*. Our results contradict the opinion of Leygonie et al. (2012) who believe that during freezing microorganisms fall into a latent state which effectively stops microbial growth. According to Leygonie et al. (2012) aerobic microflora can regain their activity during the air defrosting of the meat as it is a much slower process than freezing. Our results (Table 6) show the absence of molds and yeast in the frozen lamb samples after 365 d of frozen storage and do not confirm the above hypothesis. On the contrary, the increase in the PBC found in our study after 365 d of frozen lamb storage was in line with the results reported by Vieira et al. (2009) found after 90 d of frozen storage of aged beef. Moreover, compared to the control sample, samples DQ and DDRP showed a slightly higher number of PBC. There is indirect evidence that flavonoids help the animal organism build an integrated antioxidant system by preventing the harmful effects of free radicals. Antioxidant compounds imported with phytonutrients are likely to affect psychrotrophic bacteria in muscle tissue in a similar way.

Conclusions

The supplementation of the lambs' diet with the addition of 7.5 mg DQ. kg^{-1} life weight. d^{-1} and resp. of 545 mg dry distilled rose petals. kg^{-1} life weight. d^{-1} did not uniquely affect the quality of the quick-frozen m. *Longissimus dorsi*, m. *Semimembranosus* and perirenal adipose tissue after 365 d of storage at -18°C. Changes in POV, biogenic amines, PBC and TYMC are not affected by the addition of phytonutrients to lambs' feed but mainly by the factor of one-year frozen storage. The color characteristics change differently in both muscles. Compared to controls, the total color difference (ΔE) in the addition of DDRP was smaller than that of DQ. Supplementing the lambs' diet with phytonutrient-rich polyphenols contributes to some reduction in APC, α-aminoacidic nitrogen, protein carbonyls and TBARS but with small amplitudes. It also reduces the content of SFA at the expense of increasing MUFA. These trends are more pronounced in m. Longissimus dorsi from lambs fed with feed supplemented with 545 mg DDRP. kg^{-1} life weight. d^{-1}. Additional studies are needed to clarify the nature of the interactions between the factors breed, age, temperature and shelf life as well as feeding conditions, including to answer the question of whether the use of higher doses of DDRP or DQ as dietary supplements in small ruminants will be able to provide a more pronounced inhibitory effect on lipid oxidation and fatty acid composition of lamb muscles and fats? When determining the doses of these phytonutrients in these future studies it should be taken into account that the antioxidant activity of polyphenols, respectively of flavonoids in biological systems in vivo, undergoes metabolic transformation and their very high doses can act as prooxidants and inhibit various enzymes and the absorption of certain minerals and vitamins.

Acknowledgements

This work was supported by the Bulgarian National Science Fund (BNSF): Ministry of Education and Science of Republic of Bulgaria of state contract No DN 06/8 of 17 December 2016 "Study of the mechanism of biological active compounds of plant origin accumulation in the organism of Bulgarian

breed agricultural animals and their impact on the meat quality as a natural functional food".

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