IMPROVİNG THE OXİDATİVE STABİLİTY OF PORK BY ANTİOXİDANT TYPE PHYTONUTRİENTS

Dessislava B. Vlahova-Vangelova[1], Dessislav K. Balev[1], Sonya G. Ivanova[2], Jivko L. Nakev[2], Tanya I. Nikolova[2], Stefan G. Dragoev [1,3]♦, David E. Gerrard[4]

[1]Department of Meat and Fish Technology, University of Food Technologies, Maritsa 26, 4020 Plovdiv, Bulgaria
[2]Department of Animal Science, Agricultural Institute, Agricultural Academy, Simeon Veliki 3, 9700 Shumen, Bulgaria
[3]Assembly of Academicians and Corresponding Members, Bulgarian Academy of Sciences, Fifteenth of November 1, 1040 Sofia, Bulgaria
[4]Department of Animal and Poultry Sciences, College of Agriculture and Life Sciences, Virginia Tech, West Campus Drive 175, 3470 Litton-Reaves Hall, Blacksburg, 24061 Virginia, USA

♦Corresponding author: logos2000lt@gmail.com

Abbreviated title: Dihydroquercetin/ dry distilled rose petals as pig's supplements

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Abstract

The objective of this study was to determine the influence of supplementations with 3.5 or 7.5 mg dihydroquercetin (experimental groups D1 and D2) or with 0.255 or 0.545 g dry distilled rose petals (experimental groups R1 and R2)/kg/d added as to pig's combined feed on the parameters of lipolysis expressed by acid value; lipid hydroperoxides expressed by peroxide value, lipid oxidation secondary products expressed by 2-tiobarbituric acid reactive substances (TBARS), pH and L*, a*, b* colour characteristics in m. Longissimus lumborum et thoracis, m. Semimembranosus, backfat and leaf fat stored 24 h and 7d at 2±1°C, or 315 d at -18±1°C. A total of 120 pigs were randomly divided to five groups – a control (C) and four experimental (D1, D2, R1 and R2) each fed 45 d prior to harvest with shown above levels of phytonutrients enriched diets. More pronounced effects were determined (P≤0.05) at frozen storage compared to chilled storage. The oxidative and colour stabilities of chilled (2±1°C) and frozen (-18°C) pork are comparatively higher when pig's diet was supplemented with 3.5 mg dihydroquercetin or 0.255 g dry distilled rose petals/kg/d. The conclusion was made can the supplementation of pig's combined feed (finisher) with 3.5 mg dihydroquercetin or 0.255 g dry distilled rose petals/kg/d is a promising strategy to increase the oxidative stability of lean pork or fat and stabilized pork meat colour without deleterious changes of meat acidity.

Key words: pork, colour, lipid oxidation, dihydroquercetin, dry distilled rose petals

Introduction

Numerous strategies exist to modify the composition of pork by altering protein content, vitamins, fats and fatty acid composition (Loetscher et al., 2013; Pogorzelska et al., 2018). Further, additional technologies in breeding practices increase the productivity of pigs and impact pork quality, specifically, indoor versus outdoor rearing (Pugliese et al., 2005), use of various muscle glycogen-reducing diets (Tikk et al., 2006) and dietary enrichment with vitamin E (Frank., 2005), conjugated linoleic acid (Wiegand et al., 2001), tuna oil (Jaturasitha et al., 2008), grape pomace (Bertol et al., 2017) and astaxanthin (Pogorzelska et al., 2018).

Falowo et al. (2014) specifically describe the positive effects of feed supplementation with natural and synthetic antioxidants on the inhibition of the oxidative degradation of meat. In addition, newly discovered plant-based substances such as Siberian larch (*Larix sibirica* Ledeb) dihydroquercetin (Fomichev et al., 2016), extract of distilled rose (*Rosa damascena* Mill.) petals (Shikov et al., 2012), the goji berry (*Lycium barbarum*) dried fruits and pumpkin powder (Bulambaeva et al., 2014), and others have been studied for benefiting food stability.

The powdered dihydroquercetin isolate mentioned below as dihydroquercetin (DHQ) is capable of donating an electron and as such, inhibits hydroxyl radicals (Kumar and Pandey 2013). DHQ is a powerful antioxidant that deactivates alkylperoxyl and superoxide radicals, reduces haemolysis induced by phospholipase C and inhibits superoxide produced by xanthine oxidase (Chen and Deuster, 2009). The DHQ is a proven capillary protector with hepatoprotective, gastroprotective, anti-inflammatory, antisclerotic, radioprotective, anti-coagulant, anti-inflammatory properties and inhibits the LDL-cholesterol oxidation in blood serum (Artem'eva et al., 2015). It is often used for prevention of oxidative stress and is well accepted as a treatment for select carcinomas, and cardiovascular and liver diseases (Artem'eva et al., 2015). DHQ's anti-radical activity occurs at a concentration of about 0.0001-0.00001% in the absence of mutagenic activity (Fomichev et al., 2016).

A by-products of rose oil processing are distilled rose (*Rosa damascena* Mill) petals. Those by-products contain a wide range of flavonol glycosides and polyphenols with strong antioxidant capacity (Shikov et al., 2012). It was reported the addition of distilled rose (*Rosa Damascena* Mill) petal extracts improves colour stability of the canned strawberries' beverage (Mollov et al., 2007). The dietary supplementation of dry rose (*Rosa damascena* Mill) petals or dihydroquercetin in chicken meat cuts improves their quality, too (Balev et al., 2015).

Therefore, the objective of this study was to determine the influence of two concentrations of dihydroquercetin or dry distilled rose petals respectively on the lipolysis, lipid oxidation, pH and colour stability of chilled (2±1°C) and frozen (-18°C) pork.

Materials and methods

This experiment was designed using ARRIVE guidelines and performed in accordance to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes European Convention for the Protection of Vertebrate Animals, Council Directive 2010/63/EC, Council Directive 2008/120/EC, Commission Recommendation 2007/526/EC, Regulation EC No 1099/2009 and such as Bulgarian Veterinary Activity Act and Bulgarian Ordinance No 20 of 1 November 2012. The experiment was approved by the Bulgarian Scientific Ethics Committee.

Animals were fed an *ad libitum* grower diet to 60 kg live weight and a finisher to 110 kg. Feed was prepared at the Agricultural Institute, Shumen, Bulgaria. Ingredient composition and the energy values of the diets are presented in Table 1, and their chemical composition in Table 2. DHQ preparation from Siberian larch (*Larix sibirica* Ledeb) with purity ≥ 96% was secured from Flavitlife Bio JSCo (Sofia, Bulgaria).

Rose distillation (*Rosa damascena* Mill.) was supplied by Bulatarts Productions (Skobelevo village, Bulgaria). Raw material was pressed and air dried (60°C, 6 h). Dry pressed distilled rose petals (DDRP) were finely powdered (<0.4mm) prior to use as a dietary additive.

The study was performed at the Animal Production Experimental Farm at the Agricultural Institute, Shumen - a division of the Agricultural Academy in Sofia, Bulgaria. One hundred and twenty Danube White breed fattening pigs (male: female = 60:60) of equal weight (72.500±1.937kg) and age (155 days old) were used in this experiment. The pigs were randomized by origin, age, sex, weight and distributed into five groups (one control and four experimental), each containing of 24 animals fed for 45 d with different supplementation. The control group (C) was fed basal diet which ingredient composition is shown in Table 1. Bio-concentrates BK14 and BK16 included in the formulations of grower and finisher basal diets were supplied by Vasil Kostov Feed Factory, village Lyuben Karavelovo, Varna District, Bulgaria.

Pigs were housed in barn equipped with individual pens with feeders and drinkers. The temperature was between 19-27°C. Feed was available *ad libitum*. Water was provided by nipples drinkers *ad libitum* too. During the finishing period, they were reared according to the requirements of Bulgarian Ordinances No 21 of 14 December 2005. The DHQ and DDRP supplementation began at an average live weight of 72 kg. Pigs were fed individually twice daily and diets were weighed separately for each pig. Phytonutrients were weighed and dosed individually for each pig according to live weight and expected increase from previous weighing. Supplements were mixed with diets and fed each morning. Supplementations occurred over 40 d until harvest.

One hundred ninety-five days old animals were transported and harvested at a commercial abattoir (Unitemp Ltd, Village of Voyvodinovo, Municipality Maritsa, District Plovdiv, Bulgaria) according to Regulations EC No 1/2005, approved procedures from the Bulgarian

133 Food Safety Authority (Bulgarian Ordinance No 16 of 3 February 2006). After 18 h of lariage
134 pigs were harvested in compliance with the requirement of Art. 9, par. 3 of Bulgarian Ordinance
135 No 15 of 8 May 2009. The pigs were electrically stunned with following exsanguination. The
136 carcasses were scalded, dehaired and eviscerated. Carcasses were cooled for 24 h to 4-7°C.
137 After chilling, meat samples from m. *Longissimus thoracis et lumborum* (hereafter referred
138 to as m. *Longissimus lumborum*, LL) were collected between 12-13 ribs. The lumbar portion of
139 and m. *Semimembranosus* (SM) muscle two adipose tissue depots - backfat (spinal
140 subcutaneous fatty tissue, BF) and leaf fat (soft adipose tissue from chest cavity, LF) were
141 dissected. Separated muscles and fat from each forequarter were vacuum packed and stored at
142 2±1°C. Samples for analysis were taken initially at 24 h postmortem and after 7 d of storage.
143 Additional muscles and fat samples from the right forequarter were vacuum packed and quickly
144 frozen at an air temperature of -35±1°C, and an air flow rate 2±1 m/s. The frozen samples were
145 stored at -18±1°C. Samples were analysis after 315 d (9 months) of frozen storage.
146 Analyses were performed in the laboratory of the Department of Meat and Fish Technology
147 from Technological Faculty of University of Food Technologies, Plovdiv, Bulgaria.
148 Total lipids were extracted from muscle and adipose tissue samples in the dark using the
149 polar and non-polar solvent methods as outlined by Bligh and Dyer (1959). Extracted lipids
150 were stored at 2±1°C in the dark.
151 Approximately one gram of extracted total fat was used for determination of free fatty acids
152 expressed as acid value (AV). Lipolytic changes in the fraction of the total lipids extracted from
153 the samples were determined using procedure described by Zhang et al. (2015). The principal
154 of this titration method is based on the measurement of the number of carboxylic acid groups
155 in lipids, such as a liberated during lipolysis free fatty acids. The AV was presented as the mass
156 of potassium hydroxide (KOH) in mg needed for neutralization of one g of fatty tissue. The
157 analysis were conducted at five repetitions.

158 Primary lipid oxidation products were determined by the peroxide value (POV) using a
159 Camspec M 550 double-ray UV-VIS spectrophotometer (Spectronic Camspec Ltd, Leeds,
160 United Kingdom) by the method described from Yi et al. (2013). Specifically, the improved
161 ferrous oxidation-xylenol orange (mFOX) method was used where the absorbance
162 measurements of colored complex of ferric ions formed by the oxidation of ferrous ions with
163 the reaction of peroxides in the presence of xylenol orange (3, 3′-bis [N, N′-di{carboxymethyl}-
164 aminomethyl]-o-cresolsulfonephthalein) are determined at 650 nm. POV was estimated using
165 a standard curve. The analysis were conducted at five repetitions.
166 Secondary lipid oxidation products, expressed as free malondialdehyde (MDA) were
167 determined using TBARS as an indicator (Botsoglou et al., 1994). Absorbance measurement
168 was performed at 532 nm on a double-lane scanning UV-VIS spectrophotometer Camspec M
169 550 (Spectronic Camspec Ltd, Leeds, United Kingdom). TBARS were calculated as mg of
170 malonaldehyde/kg of meat. MDA results per kg of meat. Samples were run five times.
171 The pH of the samples was measured electropotentiometrically (Young et al., 2004) using a
172 calibrated digital pH meter (Microsyst 2004; Microsyst Ltd, Plovdiv, Bulgaria), equipped with
173 a temperature and combined pH electrode using a 10 g sample diluted with 90 ml distilled water.
174 Measurements were conducted five times.
175 Colour of the muscles and fats was determined according to lightness (L*), redness (a*), and
176 yellowness (b*) values using a Konica Minolta colorimeter CR-410 (CR-400, Konica Minolta,
177 Inc., Tokyo, Japan) with a 2° standard observer and D65 illuminant daily of simulated retail
178 display, the opening of the measuring head – 8 mm, calibration on the white standard: L* -
179 97.83, a* - 0.45, b* - 1.88). The colorimeter was calibrated using a standard white tile covered
180 in the polyvinylchloride film (AMSA, 2012). The samples were measured after unpacking and
181 subsequence blooming for 30 min at 4°C. Measurements were collected on all samples.
182 Samples were averaged from measurements collected at five locations.

A two factor analysis with replications was used to evaluate the effect of type and concentration of phytonutrients and storage time on different traits (pH, L*, a*, b*, AV, POV, TBARS) using statistical software (SPSS version 12.0, SPSS, Thailand) package with an ANOVA. The overall analysis was conducted including all traits in the model (type and concentration of phytonutrients (fixed), time of storage (fixed) and pH, L*, a*, b*, AV, POV, TBARS (covariates). The analyses was conducted for two types of muscles - m. *Longissimus lumborum et thoracis*, m. *Semimembranosus*, as well as backfat and lean fat.

Comparison of the values of the various indicators was done by the Student and ANOVA t-test of all the experimental samples with the controls on the one hand and inside the factor levels (low and high concentration of the supplemented antioxidant type phytonutrient) for significant differences at the probability $P \leq 0.05$ or $P \leq 0.01$ respectively (Table 3). When the significant effect was found ($P \leq 0.05$), the Duncan New's Multiple Rank test was used to compare the mean values.

Results

Irrespective of muscle or adipose tissue, feed supplementation (DHQ or DDRP) did not affect significantly ($P > 0.05$) on the AV after 315 d of frozen storage (Table 4). Similar results were found in both muscles indicating that DHQ or DDRP supplementation slightly impact on lipolytic changes in muscle tissue at early postmortem stages (Table 4).

Significant ($P \leq 0.05$) differences, though small, were detected in the AV of adipose tissues (Table. 4) after 24 h storage at 2±1°C. The difference between lowest and highest AV value varies from 0.20 mg KOH/g in the leaf fat to 0.26 mg KOH/g in the backfat. Compared to controls, the AV of backfat was lower ($P \leq 0.05$) in group (D1), followed by the group (R2). Feed supplementation with 0.252 g of DDRP/kg/d and 7.5 mg of DHQ/kg/d did not contribute ($P > 0.05$) to the AV reduction (24 h postmortem) in backfat and the leaf fat after storage.

After 7 d storage at 2±1°C (Table 4), AV in LL samples from C, D2 and R1 groups did not differ significantly (P>0.05). For the same studied period AV in D1 and R2 were 8 and 20% higher (P≤0.05) than controls, respectively. AV in SM lipids, group C and D1 were not differ significantly (P≤0.05), whereas R1 and R2 were lower than controls by 3-4% (P≤0.05). In comparison, the AV of SM lipids in D2 pigs was approximately 10% less than controls. In contrast, after 7 d of storage at 2±1°C, both types of adipose tissue had lower (P≤0.05) AV for all experimental groups compared to controls (Table. 4). This reduction was most pronounced in R1 backfat and D1 leaf fat, with 0.12 and 0.18 mg KOH/g, respectively.

Reduction of AV in backfat were most pronounced in pigs supplemented with 3.5 mg DHQ/kg/d as well as in leaf fat from pigs fed 0.255 g DDRP/kg/d supplementation.

Irrespective of muscle type or adipose tissue depot, dietary supplementation of DHQ or DDRP to finishing pigs did not affect the AV of fast-frozen pork after 315 d of storage at -18±1°C.

The DHQ or DDRP supplementation had a little effect on the POV in LL and SM samples after 24 h and after 7 d of storage at 2±1°C (Table 4). The POV of refrigerated stored samples was quite low and varied between 0.68 and 0.86 meqv O_2/kg.

The DHQ or DDRP antioxidant phytonutrients feeding reduced (P≤0.05) POV by 17-27% in D2 and R1 leaf fat after 24 h and after 7 d of storage at 2±1°C compared to controls (Table 4). In backfat, the POV reduction was greatest (P≤0.05) in R1, followed by R2.

The use of DHQ or DDRP as feed supplements inhibited (P≤0.05) the formation of primary lipid oxidation products during extended (9 months) storage at -18±1°C in quick-frozen SM and backfat but was not so effective (P>0.05) in frozen LL (Table 4).

The most pronounced reducing effect on the POV levels (P≤0.05) in long-term frozen storage (9 months) was found in R1 and R2, though positive effects were noted in D1. These

231 results demonstrate greater efficacy of phytonutrients added in lower doses against formation
232 of primary lipid oxidation products in both muscle and fat tissue.

233 The both used concentrations of DDRP showed positive effect ($P \leq 0.05$) in muscle and
234 adipose tissue oxidative stability after 315 d of storage at -18±1°C.

235 After 7 d of storage at 2±1°C in chilled muscles and fats positive impact against oxidation
236 was found after DDRP and 3.5 mg DHQ/kg/d supplementation.

237 Despite the higher used concentration of DHQ (7.5 mg/kg/d) the effect of supplementation
238 on oxidative stability in muscle and adipose tissue for short term chilled storage was insufficient
239 ($P > 0.05$).

240 Variable TBARS results were observed in samples after 24 h of storage at 2±1°C. Samples
241 from the LL did not differ ($P > 0.05$) with respect to TBARS values (Table 4). Conversely, values
242 from the SM and backfat samples were lower ($P \leq 0.05$) in all treated samples compared to
243 controls. In backfat the only exception is D2 which does not change significantly ($P > 0.05$) to
244 C. The lowest ($P \leq 0.05$) TBARS values were found in D1 samples, followed by R2 and R1.
245 After 7 d of storage at 2±1°C, the MDA content of all samples were lower ($P \leq 0.05$) or
246 unchanged compared to controls (Table 4), except was D2 in LL with 17% increasing ($P \leq 0.05$).
247 The lowest secondary products of lipid oxidation were observed in D1 backfat. In SM samples,
248 the lowest ($P \leq 0.05$) TBARS values were found in D1 and R1. Compared to controls, lower
249 TBARS ($P \leq 0.05$) were found in the four treated groups of frozen samples under long-term
250 storage (Table 4). The only exception was D2 in SM which does not change significantly
251 ($P > 0.05$) to C. Formation of secondary lipid oxidation products was minimal in LL of D1 pigs,
252 followed by SM of D1 and R1 pigs, backfat of R2 and R1 pigs and leaf fat samples from D1
253 and D2 pigs. Our results confirm this suggestion by lower TBARS obtained at 7 d postmortem
254 at 2±1°C and after 315 d freezing in enriched with DDRP and DHQ backfat, leaf fat and m.
255 *Semimembranosus* compared to m. *Longissimus lumborum et thoracis*.

In conclusion, this study showed that dietary supplementation of growing pigs with DHQ or DDRP results in reduction of primary and secondary lipid oxidation products during storage LL and SM muscles and adipose samples from backfat and leaf fat depots. This most pronounced effect for LL and SM was found after 3.5 mg DHQ/kg/d and 0.255 g or 0.545 DDRP/kg/d supplementation. For short term chilled storage both used supplements decreased lipid oxidation in adipose tissue. In long term frozen storage greatest oxidation stability was observed after feeding low levels of DHQ (3.5 mg/kg/d) for muscle tissue and high levels of DHQ (7.5 mg DHQ/kg/d) for adipose tissue.

In conclusion, supplementation with a phytonutrient such as DHQ or DDRP leads to a reduced accumulation of primary and secondary lipid oxidation products during storage in both muscle and adipose tissue. This effect is more pronounced in long term versus short term storage.

No differences ($P>0.05$) were detected between the pH values of LL across all experimental groups studied either at 45 min postmortem or after 7 d of storage at 2±1°C (Table 5). Similar results were also determined for SM. No differences in fat pH at 45 min postmortem were detected, though the pH of backfat and leaf fat was closer to 7.00 than muscle tissue.

After long-term frozen storage, differences in pH value of backfat or leaf fat did not differ from controls (Table 5). However, compared to 45 min postmortem pH values both backfat and leaf fat was lower ($P\leq0.05$). This could be explained by the more rapid lipolysis in the triglycerides of adipose tissue during frozen storage.

After 24 h small but statistically distinct ($P\leq0.05$) pH values were found in muscles stored at 2±1°C. For example, in LL the lowest pH values ($P\leq0.05$) were found in muscle of D2 pigs, whereas muscle from D1 and especially R1, the pH was higher than controls.

The DHQ or DDRP feeding slightly influenced the pH of pork but did not have major effects ($P>0.05$) on colour characteristics of lean or fat, regardless of type of storage used.

281 Similar results were found in the SM. The greatest decrease in SM pH at 24 h postmortem
282 was detected in D1 and R2 pigs.
283 All samples can be characterized as normal (RFN - reddish pink, firm and normal exudative
284 meat). This conclusion is in good agreement with data characterizing the meat colour.
285 After 315 d frozen storage pH in five studied LL and SM samples were found significantly
286 different ($P \leq 0.05$) (Table 5). Compared to controls C pH in LL of D1 pigs after 315 d frozen
287 storage were significantly ($P \leq 0.05$) lower. The pH in LL samples of D2 group did not differ
288 ($P > 0.05$) to controls, whereas pH of group R1 was slightly higher ($P \leq 0.05$). After 315 d frozen
289 storage the pH values in SM of groups D1, D2 and R2 were found lower ($P \leq 0.05$), compared
290 to the controls while pH of group R1 was slightly higher ($P \leq 0.05$). The pH values of both
291 adipose tissue samples for five studied groups stored 24 h and 7 d of pigs were found
292 significantly different ($P \leq 0.05$). The pH in fat decreased with 1.2 - 1.3 units during 7 d of cold
293 storage. After 7 days cold storage of backfat lowest pH values ($P \leq 0.05$) were found in D1, R1
294 and R2 groups. In the same time, pH of D2 backfat was statistically indistinguishable ($P > 0.05$)
295 compared to the controls. The difference between lowest and highest pH level of was 2.65%
296 (Table 5). Similar results were found in pH of leaf fat (Table 5). After 7 d of cold storage pH in
297 D1 and R1 groups was lower ($P \leq 0.05$), whereas in D2 and R2 samples pH was statistically
298 indistinguishable ($P \leq 0.05$) compared to controls. The difference between lowest and highest
299 pH in cold stored leaf fat was 0.08 units or 1.43% (Table 5). Our results confirmed this study
300 by different trend established in pH during refrigeration or frozen storage. The studied
301 concentrations of DHQ or DDRP as pigs' feed supplements show a little impact on pH changes
302 of pork. It is most pronounced in the rigor mortis at 24 h postmortem. Compared to controls,
303 the supplements and their concentrations differ by the muscle and adipose tissue type.

Feeding with DHQ or DDRP increased (P≤0.05) the brightness (L*) and yellowness (b*) and reduced the redness (a*) in both refrigerated and frozen storage pork (Table 6). The colour brightness (L*) was influenced by the storage period but not on the used feed supplement.

At 24 h postmortem the highest (P≤0.05) L* and b* values were recorded in the LL of R1 pigs and in the SM of D2 pigs (Table 6). At the same time, a* values were the highest (P≤ 0.05) in the LL and SM of D2 pigs, respectively. After 7 days refrigerated storage, brightness and yellowness increased (P≤0.05) in the LL and SM of D1 (Table 6).

After long-term frozen storage, the LL and SM had the highest (P≤0.05) L* values in R2 pigs, while b* were the greatest in the LL of D1 pigs and the SM of D2 pigs (Table 6). At the same time, a* values were the lowest (P≤0.05) the LM of R1 pigs and SM of D2 pigs. Differences were noted (P≤0.05) in the color of adipose tissue compared to the muscle tissue (Table 6). Both the backfat and leaf fat were (P≤0.05) brighter and less (P≤0.05) red. The greatest (P < 0.05) decrease in brightness of the backfat and leaf fat was in R2 pig carcasses after 24 h storage at refrigerated temperatures. Storage, frozen or refrigerated increased L* values of fat but were not affected by antioxidant pig feeding (Table 6). A decrease (P≤0.05) in redness (a*) and an increase in yellowness (b*) were recorded in chilled backfat and leaf fat in all experimental groups compared to controls (Table 6). Redness and yellowness were not different across treatments for backfat and leaf fat. These results indicate that the pigs feed supplementation with antioxidant type phytonutrients did not effect on colour characteristics in quick-frozen backfat and leaf fat (Table 6).

In summary, the addition of DHQ or DDRP to feed of fattening pigs does not have a major effect on colour characteristics of muscle and adipose tissue during their frozen and chilled storage. Changes in the colour characteristics of muscle tissue were more pronounced than fats. In backfat and leaf fat the influence of freezing and cooling has a profound effect on quality parameters than the addition of the phytonutrients.

Discussion

One possible reason the obtained AV results is reduction of enzyme-catalyzed lipolysis (Wood et al., 2008) as well as water activity of the sarcoplasm (Dave and Ghaly, 2011) after formation of microscopic ice crystals in the meat fibers due to rapid freezing (Huan et al., 2003). On the other hand, the relatively low levels of AV (James and James, 2012) after storage may could be a consequence of free fatty acids in initiation reactions and further development of lipid oxidation (Zhu et al., 2004). This would be further reduced by rapid freezing immediately after harvest. One possible explanation is comparatively high initial pH of studied muscles (>6.10) (Table 5) which does not allow higher free fatty acids contentment during storage (Buscailhon et al., 1994).

According to Gandemer (2002) the factors storage time and temperature greatly effect on the lipases activity and lipolysis of adipose tissue. It seems that lipolytic changes are not significantly affected by the type of phytonutrients used as supplemented in pig`s diet (Cava et al., 1999).

These data argue that 7 days storage period is too short and lipid oxidation processes is probably in its initial phase. In addition, there is relatively low amount of intramuscular fats and triglycerides in the two muscles studied **(**Fernandez et al., 1999). It is known that oxidation generally begins with phospholipids and if the meat was stored intact, the oxidation occurred in short term refrigerated storage is weak **(**Mercier et al., 2004**)**.

The POV decrease in chilled fat can be explained by the strong antioxidant action of both DDRP (Shikov et al., 2012), as well as DHQ (Fomichev et al., 2016). One possible explanation is higher antioxidant accumulation after feed supplementation in pig fats exerting protective effect against oxidation (Frank, 2005). This is probably due to oxidative stability of the frozen pork during storage at -18°C resides in an initial protracted lag period (Andersen et al., 1991).

354 According to Amaral et al. (2018) the main determinants for stability of raw meats to lipid
355 oxidation are content of free ionic iron and myoglobin, ferric reducing ability, PUFA as well as
356 antioxidant content in meat as mentioned above. As the muscle tissue contained more
357 myoglobin and free ionic iron than adipose tissue is expected oxidation to be faster in the LL
358 and SM muscles. Similar results have been reported by Loetscher et al. (2013) with poultry
359 supplemented with rosemary leaves, rosehip fruits, chokeberry pomace, and entire nettle.
360 All measured TBARS values up to day 7 ranging from 0.202 to 0.664 and indicate that the
361 meat is fresh (Hasty et al., 2002). According to Frank (2005) dietary supplementation with
362 phytonutrients reach in flavonoids such as catechin, epicatechin and quercetins increased in
363 vivo concentrations of tocopherols mainly in pig's fat. These results indicate that the dietary
364 DHQ or DDRP addition had antioxidant effect on meat lipids and confirmed previous reports
365 (Loetscher et al., 2013) with positive effects on oxidative stability of the meat after feed
366 supplementation.
367 Similar results were reported by Bertol et al. (2017) after grape pomace supplementation of
368 pigs. One possible reason for rapid post-slaughter pH decline occurred after animal stress, both
369 prior to and during processing (Channon et al., 2018). However, the largest difference was just
370 0.28 units (Table 5) and was within the pH limits recommended by Warriss (2000). pH values
371 at 45 min and 24 h of the both examined muscles do not perform deviations due to stress and
372 were within the pH limits recommended by Warriss (2000).
373 Usually, during the long time frozen storage the protein buffer systems desaturate releasing
374 hydrogen ions and the content of water-soluble compounds increased. As a result pH decreases
375 (Leygonie et al., 2012). On the other hand, many researchers (Daszkiewicz et al., 2018) did not
376 found significant changes in pH or established increasing and note that pH changes during long
377 time frozen storage were correlated with type of the muscle and the animals' breed. Neethling
378 et al. (2015) attributed the increase of b* values to the increase of metmyoglobin in muscle

tissue. Our results confirmed previous results established that denaturation of globin in the muscle pigment, makes myoglobin more susceptible to auto-oxidation during chilled or frozen storage residues to changes in the meat color (Leygonie et al., 2012). The lower activity of metmyoglobin-reducing enzymes, reduced redox stability of oxymyoglobin, physical processes related water freezing in the surface layer effects on chilled/frozen meat colour (Daszkiewicz et al., 2018) by reducing a* value and increasing colour yellowness (b*), too.

One possible reason for slightly decrease in a* value in SM and LL from R1 and D1 pigs could be an increased antioxidant content in the tissues after dietary feed enrichment which would protect myoglobin from oxidation. Similar results were reported (Bertol et al., 2017) after pigs feed enrichment with grape pomace. The lowest redness ($P \leq 0.05$) in the SM and LL of R2 and D2 pigs (Table 6) showed the importance of feed supplement concentration and confirmed previous results demonstrated that antioxidant effect depend of the type and the quantity of phytonutrient, and even pro-oxidant effect (Loetscher et al., 2013). Similar variations in the instrumental colour of the LL have been reported for chilled pork (Bertol et al., 2017).

In conclusion, this study showed that dietary supplementation of growing pigs with DHQ or DDRP results in reduction of primary and secondary lipid oxidation products during storage LL and SM muscles and adipose samples from backfat and leaf fat depots.

In summary, we can conclude that the supplementation of pigs with DHQ or DDRP is a promising strategy to increase the oxidative stability of lean pork or fat and stabilized pork meat colour without deleterious changes of meat acidity.

This paper has been proof-read by a native speaker of English who is a Full Professor and head of the Department of Animal and Poultry Sciences in the College of Agriculture and Life Sciences, Virginia Tech, Blacksburg, Virginia, USA.

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525 Table 1. Ingredient composition and the energy value of the two fodder mixtures
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Components	Grower[1] Used in the period of adolescence (live weight 20 - 60 kg)	Finisher[1] Used during the fattening period (live weight 60 - 110 kg)
Maize, %	15.00	13.00
Barley, %	25.00	10.00
Wheat, %	27.00	50.00
Wheat bran, %	8.00	7.00
Vitamin/mineral premix Bio-concentrate-14[1], %	25.00	–
Vitamin/mineral premix Bio-concentrate-16[1], %	–	20.00
Total:	**100.00**	**100.00**
Digestible energy, MJ	13.46	13.72
Metabolizable energy, MJ	12.92	13.18

527 [1]Bio-concentrates BK14 and BK16 included in the formulations of grower and finisher basal
528 diets were supplied by Vasil Kostov Feed Factory, village Lyuben Karavelovo, Varna
529 District, Bulgaria.
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544 Table 2. Proximate compositions of two diets used

Item	Grower Used in the period of adolescence (live weight 20 - 60 kg)	Finisher Used during the fattening period (live weight 60 - 110 kg)
Moisture, %	17.10±0.67	15.70±0.57
Dry matter, %	82.90±0.73	84.30±0.68
Organic substances, %	78.22±0.74	79.91±0.69
Crude protein, %	15.75±0.83	15.02±0.54
Crude lipids, %	2.81±0.52	2.42±0.56
Crude fibers, %	4.79±0.91	3.84±0.87
Ash, %	4.68±0.38	4.39±0.32
Nitrogen free extract (NFE), %	54.87±1.01	58.63±1.22
Lysine,%	0.80±0.09	0.72±0.08
Calcium, %	1.31±0.31	1.26±0.28
Phosphorus, %	0.85±0.14	0.31±0.13

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561 Table 3. The percent of variation in pH, L*, a*,b*, AV, POV, TBARS modelled against
562 concentration of phytonutrients and storage time
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Significant predictors (Phytonutrient type and concentration and storage time)	R2 (%)
m. Longissimus lumborum	
pH	5.56
L *	10.63
a *	27.39
b *	6.14
AV	26.67
POV	71.22
TBARS	25.43
m. Semimembranosus	
pH	37.58
L *	30.51
a *	12.10
b *	68.92
AV	30.54
POV	71.58
TBARS	44.33
Backfat	
pH	66.11
L *	38.16
a *	27.25
b *	12.62
AV	38.01
POV	77.34
TBARS	57.95
Leaf fat	
pH	1.70
L *	42.12
a *	35.16
b *	13.58
AV	30.54
POV	75.05
TBARS	56.79

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569Table 4. Lipolytic and oxidative changes in pork lipids during different types of storage represented by the AV, POV and TBARS expressed as means (± SEM)

	Storage time	LL (m. Longissimus lumborum)					SM (m. Semimembranosus)					Backfat					Leaf fat				
		(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)
AV, mg KOH/g	24 h 2±1 °C	0.40 ±0.04^{a,y}	0.40 ±0.03^{a,y}	0.42 ±0.05^{a,y}	0.44 ±0.02^{a,y}	0.40 ±0.03^{a,y}	0.50 ±0.05^{a,y}	0.45 ±0.05^{a,y}	0.43 ±0.05^{a,y}	0.44 ±0.03^{a,y}	0.40 ±0.07^{a,y}	0.50 ±0.05^{b,c,y}	0.37 ±0.05^{a,y}	0.63 ±0.04^{d,y}	0.55 ±0.02^{c,y}	0.42 ±0.03^{a,b,y}	0.65 ±0.04^{c,y}	0.45 ±0.05^{a,b,y}	0.55 ±0.05^{b,y}	0.55 ±0.02^{b,y}	0.45 ±0.03^{a,y}
	7 d 2±1 °C	0.65 ±0.05^{a,z}	0.70 ±0.05^{a,b,z}	0.64 ±0.05^{a,z}	0.68 ±0.05^{a,b,z}	0.78 ±0.05^{b,z}	0.73 ±0.03^{a,z}	0.74± 0.04^{a,z}	0.66 ±0.04^{a,z}	0.70 ±0.02^{a,z}	0.71 ±0.03^{a,z}	0.75± 0.01^{c,z}	0.67± 0.03^{a,b,z}	0.74± 0.01^{c,z}	0.63± 0.03^{a,z}	0.72± 0.02^{b,c,z}	0.84 ±0.04^{c,z}	0.64 ±0.04^{a,z}	0.76 ±0.03^{b,z}	0.66 ±0.03^{a,z}	0.76 ±0.06^{b,c,z}
	315 d -18±1 °C	0.10 ±0.02^{a,x}	0.12 ±0.01^{a,x}	0.11 ±0.01^{a,x}	0.12 ±0.01^{a,x}	0.12 ±0.01^{a,x}	0.11 ±0.01^{a,x}	0.12 ±0.01^{a,x}	0.11 ±0.01^{a,x}	0.11 ±0.0^{a,x}	0.11 ±0.01^{a,x}	0.11 ±0.01^{a,x}	0.11 ±0.01^{a,x}	0.11 ±0.01^{a,x}	0.11 ±0.01^{a,x}	0.11 ±0.01^{a,x}	0.11 ±0.01^{a,x}	0.12 ±0.01^{a,x}	0.12 ±0.01^{a,x}	0.11 ±0.01^{a,x}	0.12 ±0.01^{a,x}
POV, meqv O_2/kg	24 h 2±1 °C	0.83 ±0.05^{a,b,x}	0.87 ±0.03^{b,x}	0.79 ±0.02^{a,x}	0.79 ±0.04^{a,x}	0.84 ±0.03^{a,b,x}	0.83 ±0.02^{b,x}	0.83 ±0.03^{b,x}	0.84 ±0.03^{b,x}	0.80 ±0.03^{b,x}	0.68 ±0.03^{a,x}	0.86 ±0.01^{c,x}	0.82 ±0.02^{b,x}	0.83 ±0.01^{b,x}	0.67 ±0.04^{a,x}	0.70 ±0.05^{a,x}	0.88 ±0.03^{c,x}	0.87 ±0.03^{c,x}	0.75 ±0.03^{b,x}	0.69 ±0.02^{a,x}	0.95 ±0.03^{d,x}
	7 d 2±1 °C	0.83 ±0.05^{a,x}	0.78 ±0.02^{a,x}	0.77 ±0.03^{a,x}	0.79 ±0.05^{a,x}	0.75 ±0.04^{a,y}	0.86 ±0.03^{a,x}	0.85 ±0.02^{a,x}	0.86 ±0.03^{a,x}	0.83 ±0.01^{a,x}	0.81 ±0.04^{a,x}	0.87 ±0.01^{b,x}	0.85 ±0.03^{b,x}	0.85 ±0.02^{b,x}	0.74 ±0.05^{a,y}	0.76 ±0.03^{a,x}	0.94 ±0.02^{c,y}	0.93 ±0.02^{c,y}	0.81 ±0.04^{b,x}	0.74 ±0.01^{a,y}	0.92 ±0.04^{c,x}
	315 d -18±1 °C	1.39 ±0.02^{a,y}	1.52 ±0.02^{b,y}	1.48 ±0.03^{b,y}	1.48 ±0.04^{b,y}	1.51 ±0.02^{b,z}	1.51 ±0.02^{b,y}	1.56 ±0.07^{b,y}	1.67 ±0.01^{c,y}	1.47 ±0.02^{b,y}	1.31 ±0.07^{a,y}	1.85 ±0.02^{c,y}	1.79 ±0.03^{b,y}	1.80 ±0.02^{b,y}	1.78 ±0.04^{b,z}	1.71 ±0.01^{a,y}	1.65 ±0.01^{a,z}	1.69 ±0.05^{a,z}	1.80 ±0.04^{b,y}	1.64 ±0.02^{a,z}	1.61 ±0.04^{a,y}
TBARS, mg MDA/kg	24 h 2±1 °C	0.27 ±0.03^{a,x}	0.28 ±0.02^{a,x}	0.31 ±0.02^{a,x}	0.25 ±0.04^{a,x}	0.26 ±0.03^{a,x}	0.60 ±0.05^{c,y}	0.24 ±0.03^{a,x}	0.32 ±0.03^{b,x}	0.26 ±0.02^{a,x}	0.26 ±0.01^{a,x}	0.58 ±0.06^{c,x}	0.20 ±0.02^{a,x}	0.50 ±0.02^{c,x}	0.46 ±0.05^{b,x}	0.42 ±0.01^{b,x}	0.50 ±0.05^{b,x}	0.44 ±0.02^{b,x}	0.47 ±0.02^{b,x}	0.44 ±0.03^{a,b,x}	0.39 ±0.02^{a,x}
	7 d 2±1 °C	0.29 ±0.05^{a,x}	0.34 ±0.02^{a,y}	0.44 ±0.02^{b,y}	0.29 ±0.03^{a,x}	0.32 ±0.03^{a,x}	0.63 ±0.04^{c,y}	0.28 ±0.03^{a,x}	0.38 ±0.03^{b,x}	0.29 ±0.02^{a,x}	0.33 ±0.02^{a,b,y}	0.67 ±0.03^{d,x}	0.23 ±0.04^{a,x}	0.54 ±0.02^{c,y}	0.49 ±0.02^{b,x}	0.45 ±0.03^{b,x}	0.57 ±0.03^{c,x}	0.47 ±0.01^{b,x}	0.49 ±0.02^{b,x}	0.47 ±0.02^{a,x}	0.43 ±0.04^{a,x}
	315 d -18±1 °C	0.49 ±0.03^{c,y}	0.34 ±0.01^{a,y}	0.43 ±0.04^{b,y}	0.40 ±0.01^{b,y}	0.41 ±0.03^{b,y}	0.51 ±0.05^{b,x}	0.37 ±0.06^{a,y}	0.58 ±0.03^{b,y}	0.38 ±0.02^{a,y}	0.43 ±0.03^{c,z}	1.31 ±0.08^{c,y}	1.27 ±0.02^{c,y}	1.06 ±0.03^{b,z}	1.03 0.05^{b,y}	0.90 ±0.04^{a,y}	3.07 ±0.05^{d,y}	1.30 ±0.02^{a,y}	1.28 ±0.15^{a,y}	2.10 ±0.03^{c,y}	1.55 ±0.01^{b,y}

570 $^{a, b, c}$ Means in the same row for individual muscle or fat with different superscript letters differ significantly (P≤0.05).

571 $^{w, x, y, z}$ Means in the same column for individual muscle or fat with different superscript letters differ significantly (P≤0.05).

572 SEM- standard error of the mean.

Table 5. Changes in the pork pH during different types of storage expressed as means (± SEM)

pH	LL (m. Longissimus lumborum)					SM (m. Semimembranosus)					Backfat					Leaf fat				
	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)
45 min	6.25 ±0.08^{a,z}	6.21 ±0.02^{a,z}	6.19 ±0.04^{a,z}	6.15 ±0.06^{a,y}	6.18 ±0.08^{a,z}	6.24 ±0.03^{a,z}	6.20 ±0.04^{a,z}	6.18 ±0.05^{a,z}	6.16 ±0.05^{a,z}	6.17 ±0.04^{a,z}	6.89 ±0.05^{a,z}	6.91 ±0.04^{a,z}	6.99 ±0.07^{a,z}	6.95 ±0.05^{a,z}	6.98 ±0.06^{a,z}	6.87 ±0.06^{a,z}	6.81 ±0.05^{a,z}	6.89 ±0.05^{a,z}	6.84 ±0.06^{a,z}	6.86 ±0.05^{a,z}
24 h 2±1 °C	5.71 ±0.02^{b,x}	5.79 ±0.03^{c,x}	5.62 ±0.02^{a,w}	5.90 ±0.04^{d,x}	5.72 ±0.05^{b,c,x}	5.70 ±0.03^{b,x}	5.60 ±0.04^{a,w}	5.69 ±0.05^{a,b,w}	5.71 ±0.01^{b,w}	5.61 ±0.02^{a,w}	5.82 ±0.03^{d,x}	5.68 ±0.02^{b,y}	5.55 ±0.01^{a,w}	5.73 ±0.01^{c,y}	5.66 ±0.10^{b,c,x}	6.52 ±0.05^{a,x}	6.59 ±0.04^{a,b,x}	6.51 ±0.06^{a,x}	6.56 ±0.02^{a,x}	6.62 ±0.01^{b,x}
7 d 2±1 °C	5.90 ±0.05^{a,y}	5.89 ±0.04^{a,y}	5.88 ±0.02^{a,y}	5.91 ±0.01^{a,x}	5.90 ±0.01^{a,y}	6.01 ±0.03^{a,b,y}	5.98 ±0.03^{a,b,y}	6.00 ±0.04^{a,b,y}	6.03 ±0.01^{b,x}	5.99 ±0.01^{a,y}	5.65 ±0.02^{c,w}	5.54 ±0.04^{a,b,x}	5.60 ±0.02^{b,x}	5.50 ±0.06^{a,x}	5.58 ±0.02^{a,b,x}	5.61 ±0.03^{b,c,y}	5.57 ±0.01^{a,b,x}	5.60 ±0.01^{b,c,w}	5.55 ±0.03^{a,w}	5.63 ±0.03^{c,w}
315 d -18±1 °C	5.84 ±0.03^{b,y}	5.73 ±0.03^{a,x}	5.78 ±0.03^{a,x}	5.92 ±0.05^{c,x}	5.73 ±0.03^{a,x}	5.96 ±0.04^{b,y}	5.88 ±0.03^{a,x}	5.88 ±0.02^{a,x}	6.06 ±0.01^{c,y}	5.91 ±0.01^{a,b,x}	6.66 ±0.03^{a,y}	6.67 ±0.03^{a,y}	6.70 ±0.03^{a,y}	6.68 ±0.08^{a,y}	6.67 ±0.05^{a,y}	6.64 ±0.05^{a,y}	6.68 ±0.04^{a,y}	6.69 ±0.03^{a,y}	6.65 ±0.04^{a,y}	6.69 ±0.02^{a,y}

$^{a, b, c}$ Means in the same row for individual muscle or fat with different superscript letters differ significantly (P≤0.05).

$^{w, x, y, z}$ Means in the same column for individual muscle or fat with different superscript letters differ significantly (P≤0.05).

SEM- standard error of the mean.

593 Table 6. Changes in the instrumental colour characteristics (L *, a *, b *) of m. Longissimus lumborum (LL), m. Semimembranosus (SM),
594 backfat and leaf fat during different types of storage expressed as means (± SEM)
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Colour parameters	Storage time	LL (m. Longissimus lumborum)					SM (m. Semimembranosus)					Backfat					Leaf fat				
		(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)
L*	24 h 2±1 °C	43.57 ±0.70^{a,x}	46.47 ±0.16^{c,x}	46.34 ±0.29^{c,x}	48.89 ±0.27^{d,x}	45.06 ±0.86^{b,x}	41.22 ±0.62^{a,x}	44.21 ±0.24^{c,x}	46.01 ±0.23^{d,x}	45.75 ±0.24^{d,x}	43.15 ±0.06^{b,x}	73.91 ±0.25^{d,x}	73.10 ±0.11^{c,x}	72.59 ±0.23^{b,x}	73.30 ±0.19^{c,x}	70.52 ±0.30^{a,x}	78.97 ±0.29^{d,x}	76.42 ±0.24^{b,x}	76.58 ±0.32^{b,x}	77.20 ±0.13^{c,x}	75.57 ±0.18^{a,x}
	7 d 2±1 °C	43.17 ±0.56^{a,x}	50.01 ±0.29^{d,z}	48.10 ±0.55^{c,y}	48.67 ±0.67^{c,x}	44.92 ±0.43^{b,x}	41.05 ±0.34^{a,x}	47.97 ±0.27^{d,z}	46.27 ±0.64^{c,x}	45.52 ±0.71^{c,x}	43.28 ±0.38^{b,x}	79.34 ±0.37^{a,y}	79.25 ±0.25^{a,y}	79.09 ±0.54^{a,y}	79.24 ±0.53^{a,y}	79.01 ±0.49^{a,y}	79.80 ±0.25^{a,y}	79.71 ±0.30^{a,y}	79.62 ±0.46^{a,y}	79.63 ±0.37^{a,y}	79.59 ±0.44^{a,y}
	315 d -18±1 °C	46.33 ±0.21^{a,y}	47.93 ±0.31^{b,y}	50.09 ±0.57^{c,z}	50.92 ±0.28^{c,y}	54.41 ±0.28^{d,y}	44.54 ±0.68^{a,y}	45.82 ±0.44^{b,y}	47.22 ±0.50^{c,y}	48.85 ±0.23^{d,y}	50.71 ±0.18^{e,y}	79.69 ±0.43^{a,b,}	79.64 ±0.21^{b,y}	79.15 ±0.27^{a,b,y}	79.18 ±0.40^{a,b,y}	79.06 ±0.22^{a,y}	80.02 ±0.42^{a,y}	79.93 ±0.29^{a,y}	79.84 ±0.31^{a,y}	79.73 ±0.30^{a,y}	79.77 ±0.25^{a,y}
a*	24 h 2±1 °C	15.70 ±0.11^{e,z}	13.61 ±0.04^{c,y}	12.87 ±0.16^{a,y}	15.03 ±0.07^{d,z}	13.39 ±0.08^{b,z}	13.15 ±0.07^{d,x}	12.54 ±0.06^{b,x}	11.79 ±0.32^{a,y}	12.32 ±0.11^{b,y}	12.76 ±0.18^{b,x}	3.74 ±0.55^{c,x}	3.16 ±0.49^{a,x}	3.17 ±0.32^{a,x}	3.19 ±0.12^{a,x}	3.49 ±0.38^{b,x}	4.24 ±0.14^{b,x}	4.04 ±0.31^{a,b,x}	4.15 ±0.22^{c,b,x}	3.81 ±0.11^{a,x}	3.62 ±0.35^{a,c,x}
	7 d 2±1 °C	13.71 ±0.17^{b,x}	13.08 ±0.47^{b,x}	12.13 ±0.13^{a,x}	13.60 ±0.25^{b,y}	12.05 ±0.38^{a,x}	13.31 ±0.27^{c,x}	12.81 ±0.48^{b,c,x}	11.05 ±0.28^{a,x}	11.77 ±0.41^{a,x}	12.63 ±0.29^{b,x}	5.49 ±0.22^{b,z}	4.88 ±0.26^{a,y}	4.77 ±0.29^{a,y}	4.86 ±0.31^{a,y}	4.83 ±0.25^{a,y}	5.83 ±0.18^{b,z}	5.41 ±0.19^{a,z}	5.42 ±0.14^{a,z}	5.43 ±0.17^{a,z}	5.34 ±0.21^{a,z}
	315 d -18±1 °C	15.35 ±0.21^{c,y}	12.91 ±0.25^{bx}	12.56 ±0.3^{b,x,y}	11.88 ±0.11^{a,x}	12.77 ±0.13^{b,y}	14.27 ±0.12^{e,y}	13.71 ±0.11^{d,y}	12.03 ±0.44^{a,z}	12.71 ±0.06^{b,z}	13.31 ±0.07^{c,y}	4.54 ±0.20^{a,y}	4.50 ±0.19^{a,y}	4.30 ±0.21^{a,y}	4.41 ±0.43^{a,y}	4.39 ±0.19^{a,y}	4.77 ±0.35^{a,y}	4.82 ±0.28^{a,y}	4.86 ±0.16^{a,y}	4.74 ±0.34^{a,y}	4.91 ±0.20^{a,y}
b*	24 h 2±1 °C	3.72 ±0.05^{a,x}	4.39 ±0.05^{c,x}	4.24 ±0.14^{c,x}	5.07 ±0.09^{d,y}	3.93 ±0.20^{b,x}	3.03 ±0.05^{a,x}	3.05 ±0.01^{a,x}	4.53 ±0.09^{d,y}	4.06 ±0.04^{b,x}	4.22 ±0.04^{c,y}	3.67 ±0.21^{a,x}	4.52 ±0.10^{c,x}	4.31 ±0.48^{b,c,x}	4.23 ±0.17^{b,x}	4.40 ±0.14^{b,c,x}	4.48 ±0.32^{a,x}	4.92 ±0.18^{b,x}	4.95 ±0.25^{b,x}	4.91 ±0.23^{b,x}	4.93 ±0.09^{b,x}
	7 d 2±1 °C	3.54 ±0.12^{a,x}	5.48 ±0.23^{c,y}	5.34 ±0.13^{c,z}	4.55 ±0.22^{b,x}	3.90 ±0.28^{a,x}	3.02 ±0.26^{a,x}	5.11 ±0.38^{c,y}	4.16 ±0.35^{b,x}	4.68 ±0.40^{b,y}	4.00 ±0.32^{b,x}	4.87 ±0.33^{a,y}	5.49 ±0.35^{a,b,y}	5.68 ±0.38^{b,y}	5.50 ±0.21^{b,y}	5.61 ±0.13^{b,y}	6.19 ±0.27^{a,y}	6.70 ±0.10^{b,z}	6.60 ±0.10^{b,z}	6.69 ±0.11^{b,z}	6.63 ±0.11^{b,z}
	315 d -18±1 °C	4.31 ±0.01^{a,y}	5.51 ±0.06^{d,y}	5.17 ±0.02^{c,y}	5.20 ±0.04^{c,y}	4.57 ±0.23^{b,y}	4.13 ±0.08^{a,y}	5.72 ±0.06^{b,z}	6.70 ±0.03^{e,z}	6.39 ±0.26^{d,z}	6.01 ±0.04^{c,z}	5.64 ±0.13^{a,z}	5.66 ±0.25^{a,y}	5.60 ±0.06^{a,y}	5.69 ±0.23^{a,y}	5.72 ±0.10^{a,y}	6.11 ±0.23^{a,y}	6.01 ±0.24^{a,y}	6.07 ±0.17^{a,y}	5.99 ±0.20^{a,y}	6.09 ±0.19^{a,y}

596 $^{a, b, c}$ Means in the same row for individual muscle or fat with different superscript letters differ significantly (P≤0.05).
597 $^{x, y, z}$ Means in the same column for individual muscle or fat with different superscript letters differ significantly (P≤0.05).
598 SEM- standard error of the mean