

Use of Lithium Ascorbate to reduce stress for improvement in pork quality

K.S. Ostrenko*, A.N. Ovcharova, O.V. Sofronova

All-Russia Research Institute of Animal Physiology, Biochemistry, and Nutrition, Branch of Ernst Russian Institute of Animal Breeding Federal Science Center for Animal Husbandry, Federal State Budgetary Scientific Institution
248010 Borovsk, p. Institute, Russian Federation

*Corresponding author E-mail: Ostrenkoks@gmail.com, +79109166658

Journal of Livestock Science (ISSN online 2277-6214) 11: 95-100

Received on 03/04/20; Accepted on 4/6/2020

doi. 10.33259/JLivestSci.2020.95-100

Abstract

The specificity of post-mortem redox processes in pork with the development of Porcine Stress Syndrome (PSS), which is the syndrome of pig stress, is considered. Pre-slaughter stress leads to the increased breakdown of glycogen, a slight decrease in the pH of muscle tissue during autolysis and a significant pH shift to the acidic side. To study the physicochemical parameters of muscle tissue after deboning, samples of the longest muscle were taken at the level of 9–12 thoracic vertebrae, 400 g of each half-carcass. Changes in the pH of meat in the experimental groups correlate with the indicators of volatile fatty acids (VFA). So, in the experimental groups, the level of VFA is lower: in the group with a dosage of 10 mg / kg of lithium ascorbate, their level was 3.07 % lower than in the control, which is an indicator of meat quality. During the studies, higher pH values in the first experimental group by 3 % relative to the control group were recorded. When the pH balance is shifted to the acidic side, acidosis occurs, which negatively affects muscle contraction in living organisms. In the post-slaughter period, its occurrence prevents the contraction of muscle fibers, which increases to a certain limit the organoleptic quality of meat products. With increasing the stress resistance, lithium ascorbate prevents the activation of metabolic processes under the action of catecholamines and reduces the level of organic acids in muscle fibers. The use of lithium ascorbate as a stress protector makes it possible to reduce these negative effects and leads to the increase the values of organoleptic analysis. According to the effect on meat quality, it is recommended to include lithium ascorbate in a standard diet in a dosage of 10 mg/kg of the body weight during the entire feeding period. The given scheme of the lithium ascorbate use will allow achieving the best organoleptic and physicochemical qualities of meat products.

Key words: stress; lithium ascorbate; Porcine Stress Syndrome (PSS), physicochemical properties; lactic acid; pork

Introduction

Over the past decade, priority in pig production has been given to increasing muscle growth with a consistent decrease in fat depots (Cook et al., 1996, 2012). Fat thickness greatly affects the quality of meat since the corresponding fat layer prevents the hams from excessive water loss and deterioration of organoleptic characteristics (Detmer et al., 2011). In addition to the fat thickness, the loss of water in the dry hardened hams also depends on its amount stored in the muscles since the presence of intermuscular and intramuscular fat improves the organoleptic properties of meat (Ostrenko et al., 2019). The amount and composition of intramuscular fat are important characteristics that determine the quality of meat and, therefore, consumer recognition. The resulting contrasting requirements increase the need to elucidate the factors underlying the qualitative properties in order to find a balance between what is important for the consumer and promising for the industry. The efforts have been made so far to improve the quality of pork, and scientific research has focused on the aspects that affect the quality characteristics and composition of final meat products (Gajana et al., 2013; Rey-Salgueiro et al., 2018; Magomedaliev et al., 2019; Niyazov et al., 2019). The lack of unity in the ongoing research may depend on statistical methods, such as information, calculation of genetic parameters regardless of the fact that the authors have genomic data or information only of the breeding basis or phenotypic and genetic characteristics of the studied population. We considered this problem from the view of possible violations of physiological and biochemical mechanisms leading to a decreasing in the quality of meat under the influence of attributive industrial stresses that occur during slaughter.

High huddling, loading and unloading, adverse weather conditions, lack of food and water, the length of transportation, mixing with animals from other groups, restraint and fatigue are the known stress factors for pigs (Vermeulen et al., 2016). High stress levels increase susceptibility to diseases, reduce life expectancy, impair growth and reproduction, cause body damage and behavioral abnormalities, and also reduce the quality of meat (Bazhovet al., 2013).

Post-mortem redox processes in pork are characterized by the development of Porcine Stress Syndrome (PSS) – the syndrome of pork stress. Pre-slaughter stress leads to the increased decomposition of glycogen, a slight decrease in the pH of muscle tissue during autolysis, and a significant shift in pH to the acidic side (Bazhovet al., 2013). So, 45 minutes after slaughter, the pH is 5.4, which leads to the formation of meat with PSE (Pale, soft, and exudative) signs. Accelerated glycolysis is associated with the damage to the sarcoplasmic reticulum in the PSE-muscle tissue and the release of Ca^{2+} (Moore, et al., (2012)). Besides, in the PSE-muscle tissue during autolysis, calcium ions are not released from the sarcoplasmic reticulum to the same extent as in Normal (NOR) meat, which prevents the development of deep post-mortem rigidity in exudative muscle tissue (Vermeulen, et al., (2015)). Researchers have noted corrective actions to the problem of meat with PSE and Dry, firm, and dark (DFD) properties, although preventing the formation of raw materials with deviations in the process of autolysis can lead to minimum production of low-quality meat (Ustyantseva et al., 2009). It is possible to reduce the risk of obtaining meat with PSE signs with the *in vivo* use of adaptogenic preparations that normalize neurohumoral regulation and reduce the risk of meat with signs of PSE (Rey-Salgueiro et al., 2015).

It is known that at present in some regions of Russia the amount of pork with PSE signs obtained from slaughtering animals from industrial complexes is 35–40 %, and it is 25–30 % from farms.

Using new feed additives during an extended period of time, it becomes necessary to control and evaluate their impact on the final product. Due to the fact that lithium ascorbate was used throughout the entire period of fattening, it was necessary to check whether this preparation affects biochemical and organoleptic quality indicators of meat products. In the analysis of the previous studies, the use of lithium ascorbate was proved to increase the productivity of animals and to cause the stimulation of metabolic processes in pigs. There was a need to evaluate the effect of lithium on the process of meat ageing and the organoleptic indicators.

The purpose of the research was to study the effect of the adaptogens and stress-protector of lithium ascorbate on the quality of pig meat of the Irish Landrace breed.

Material and methods

The experiments were carried out in closed joint stock company "Shumyatino" (village Shumyatino, Maloyaroslavets district, Kaluga region, Russian Federation, 54°59' north latitude and 36°20' east longitude). We took five groups of pigs from Irish Landrace breed with 10 animals per each group. The experimental and control groups were formed from the piglets of 2 months of age. The diet and the technological process did not differ from the basic technology of fattening and growing piglets. Lithium ascorbate was administered with food in the following dose (mg per one kg of body weight): group 1 – 10; 2 – 5; 3 – 2; 4 – 0.5 throughout the entire feeding period. The control group of piglets was on the main diet without the addition of the preparation. During the experiment, feeding diets were compiled in accordance with the Russian Institute of Animal Breeding Standards using the Optima Expert Food complex, and the feeding level was calculated with the expectation of receiving from 500 to 700 g of the average daily live weight gain. The rations consisted of combined feed types DF-5 (Dry feed), DF-6, and DF-7. Animals were kept

indoors in stalls. The indoor climate was maintained automatically according to the hygiene requirements. Water was freely available. The total growing cycle was 210 days.

Meat quality was determined in accordance with NSS (National State Standard) 9959-2015 “Meat and Meat Products. General Conditions for Organoleptic Assessment” (with amendment), the categorization of carcasses was determined according to NSS 1213-79 “Pigs for Slaughter- Technical conditions”. The pH level, VFA and other indicators were determined 24 hours after slaughter. To study the physicochemical parameters of muscle tissue after deboning, the samples of the longest muscle were taken at the level of 9–12 thoracic vertebrae, 400 g of each half-carcass. An organoleptic assessment was done to determine the meat appearance, its consistency, flavor, the condition of fat, tendons, and the quality of broth (color, transparency, smell, and taste).

Statistic processing of the results of the organoleptic analysis was carried out in accordance with the development of “Methodological Instructions for the Use of the Point Scale” (10-point system). Each of these indicators was assigned a certain maximum number of points. The differences in meat quality parameters between the control and experimental groups were defined by the single factor method taking into account the Student *t*-criteria. The data in Tables presented as mean \pm standard deviation.

Results

The research was carried out 24 hours after the slaughter and cooling of carcasses. The carcasses of pigs by their appearance were pale pink with a crust of drying up, the muscles were dense and elastic, and the fossa by feeling with a finger quickly leveled off. In the section, the muscles were slightly moist, pale pink, not leaving a wet spot on the filter paper. The flavor was specific to fresh pork. The fat was pale pink. Tendons were dense and resilient, and the surface of the joints was smooth and shiny. The broth during heating to 80–85 °C was transparent and aromatic. Thus, organoleptic methods confirmed the freshness of pork, which was also shown by microscopy of samples: single cocci per visual field and the absence of traces of tissue decay (Table 1).

The content of volatile fatty acids (VFA), positive reaction to peroxidase and a negative reaction with copper sulfate testified to the freshness of pig carcasses and normal post-mortem maturation of meat from animals that had received lithium ascorbate (Table 1). During bacteriological studies of internal organs, the cultures on beef-extract agar (BEA) and the medium of endopathogenic microflora were not detected. When comparing the organoleptic evaluation scores of meat (Table 2), we registered that the dose of 10 mg/kg of lithium ascorbate administered to the fattening pigs did not impair the appearance, flavor, taste, texture, and the juiciness of meat. When analyzing the organoleptic evaluation of the meat broth of the experimental group according to its taste and concentration in the overall assessment, there was a slight tendency to increase the scores. The use of lithium ascorbate as a stress protector can reduce these negative effects, which leads to an increase of the values of organoleptic analysis.

The effect of lithium ascorbate on increasing the categorization was established, which indicated the effectiveness of the use of adaptogens in feeding technology (Table 3). We also observed that the introduction of lithium ascorbate during entire feeding period reduces the load on the corticotropic axis, allows the use of the obtained energy to super-maintain growth, makes it possible to realize the genetic potential of the breed, and affects the categorization of carcasses during slaughter. An increase in the number of carcasses of category II and III was registered.

Discussion

Stress forms the oxygen debt in the muscles and is accompanied by a decrease in the concentration of oxyhemoglobin in the blood. A high concentration of adrenaline causes the breakdown of glycogen in the liver and muscles.

After slaughter, the biochemical processes occurring in the tissues continue; autolysis occurs, but their functional value changes, which leads to a change in properties with more significant changes in muscle tissue. In the muscles, activation of enzymes responsible for the decomposition reactions takes place. There is an accumulation of lactic acid, which causes the process of “acidification” of muscle tissue with the pH shift to the acidic side. This biochemical process enhances the resistance of carcasses to the action of putrefactive microflora. The use of lithium ascorbate relieves the stress from the hypothalamic-pituitary-adrenal axis and makes it possible to maintain a sufficient supply of glycogen in the muscle cells during the period of growing and fattening. In the post-mortem period, the glycogen continues to break down to glucose and pyruvate. Furthermore, due to the enzyme lactate dehydrogenase, pyruvate is reduced to the lactic acid; however, in the presence of a sufficient amount of Na⁺ and K⁺ ions, a neutralization reaction occurs and lactic acid is transformed into lactate (sodium or potassium salt of lactic acid (Tomovic et al., 2014).

Under the influence of pre-slaughter stress, the release of aldosterone, which purpose is to maintain optimal water-salt homeostasis, is activated. The main target organ towards the action of the hormone is the kidney, where aldosterone causes an increase in sodium reabsorption in the body as well as an increase in urinary potassium excretion (Bazhov et al., 2013). Slaughter and the ageing of meat related to the excess of aldosterone production, which leads to a delay in the body's sodium and water, are determined by the neutralization of lactic acid, and pork is DFD defective (Soler et al., 2013; Babicz et al., 2016; Görres et al., 2016; Khan et al., 2013).

Table 1. The effect of lithium ascorbate on the indicators of freshness of pig meat.

Group	pH	Peroxidase	Reaction with CuSO ₄	VFA (mg KOH)
Control	5.61 ± 0.09	+	-	3.58 ± 0.05
Group1 (10 mg/kg)	5.78 ± 0.05*	+	-	3.47 ± 0.04*
Group2 (5 mg/kg)	5.73 ± 0.08	+	-	3.51 ± 0.06
Group3 (2 mg/kg)	5.68 ± 0.12	+	-	3.52 ± 0.08
Group4 (0.5 mg/kg)	5.61 ± 0.14	+	-	3.52 ± 0.12

*significant at p<0.05

Table 2. Organoleptic characteristics of meat and broth from meat of pigs that received lithium ascorbate (points)

Group	Appearance	Flavor	Taste	Consistency	Juiciness	Overall rating
Meat						
Control	7.3 ± 0.23	7.3 ± 0.11	7.1 ± 0.23	7.4 ± 0.23	7.2 ± 0.35	7.2 ± 0.3
Group 1	7.6 ± 0.11	7.0 ± 0.23	7.2 ± 0.35	7.4 ± 0.23	7.7 ± 0.35	7.2 ± 0.3
Group2	7.4 ± 0.21	7.2 ± 0.18	7.2 ± 0.24	7.4 ± 0.27	7.7 ± 0.37	7.2 ± 0.6
Group3	7.0 ± 0.18	6.8 ± 0.27	7.0 ± 0.19	7.0 ± 0.34	7.2 ± 0.28	7.0 ± 0.4
Group4	7.1 ± 0.18	7.0 ± 0.23	7.2 ± 0.21	7.2 ± 0.24	7.2 ± 0.18	7.14 ± 0.4
Broth						
Group	Appearance	Flavor	Taste	Transparency	Richness	Overall rating
Control	6.1 ± 0.23	6.1 ± 0.23	5.8 ± 0.47	5.7 ± 0.47	5.7 ± 0.47	5.7 ± 0.3
Group1	6.7 ± 0.23	6.5 ± 0.35	7.0 ± 0.25	7.0 ± 0.23	7.0 ± 0.23	6.7 ± 0.3
Group2	6.6 ± 0.17	6.4 ± 0.28	7.2 ± 0.39	7.0 ± 0.27	7.0 ± 0.17	6.7 ± 0.7
Group3	5.9 ± 0.31	5.8 ± 0.47	6.4 ± 0.28	7.0 ± 0.34	7.0 ± 0.46	6.7 ± 0.8
Group4	6.1 ± 0.21	6.0 ± 0.37	6.2 ± 0.28	6.9 ± 0.34	6.5 ± 0.46	6.34 ± 0.4

Table 3. The effect of lithium ascorbate on the categorization of carcasses during slaughter (n = 10)

Group	Categories					
	II		III		IV	
	Animals (n)	%	Animals (n)	%	Animals (n)	%
Control	4	40	5	50	1	10
Group1	8	80	2	20	0	0
Group2	8	80	2	20	0	0
Group3	6	60	2	20	2	20
Group4	4	40	5	50	1	10

The higher pH reading in the first experimental group was 3 % relative to the control group. When the pH balance is shifted to the acidic side, the acidosis occurs, that negatively affects muscle contraction. In the post-mortem period the acidosis prevented the contraction of muscle fibers, which increased the organoleptic qualities of meat products. The quality of NOR meat largely depends on the level of feeding and the negative external impact. The high content of lactic acid leads to the damage to the sarcoplasmic reticulum accompanied by the release of calcium ions (Ca²⁺). Calcium ions activate enzymes (proteases) that break down proteins, which cause a loss in meat quality (Lebret et al., 2006, 2011; Peeters et al., 2006).

Changes in the pH of meat in experimental groups correlated with volatile fatty acids; moreover, the level of VFA was lower in experimental groups. Thus, in the group with a dosage of 10 mg/kg of lithium ascorbate, the level of VFA was 3.07 % lower than in control. Fatty, aromatic, and other acids including volatile are formed in meat as a result of deamination of amino acids (of direct, reductive, oxidizing, and hydrolytic types) during meat putrefaction. In addition, fatty acids can also be formed under the influence of certain anaerobic microorganisms. It was found that in the early stages of putrefactive decomposition, acetic acid is formed in the greatest amount, followed by butyric acid, and in the later stages formic and propionic acids appear (Pugliese et al., 2015). Therefore, the total amount of these acids can serve as one of the indicators of the freshness of meat. A decrease in this indicator in the experimental group indicates an increase in the quality of meat products.

Thus, the given scheme of the lithium ascorbate use will allow to achieve the best organoleptic and physicochemical qualities of pig meat products.

Conclusions

We registered that inclusion of lithium ascorbate in pig diet leads to an increase in the categorization of carcasses and post-mortem quality of meat. The lithium ascorbate prevented the activation of metabolic processes under the action of catecholamines. The obtained data indicate that classical glycolysis occurs only in pig meat of normal quality (NOR-pork) with high intravital stress resistance. This was achieved by using lithium ascorbate throughout the entire cycle of feeding and growing pigs. We observed certain deviations from the normal glycolysis process in the meat with PSE and DFD defects. Stress deviations created the most favorable conditions, under which severe meat acidosis or microbial putrefaction developed, caused a further decrease in its physicochemical and organoleptic properties. We recommend to include lithium ascorbate in a standard pig diet in a dosage of 10 mg per kg of body weight during entire feeding period to achieve the best organoleptic and physicochemical qualities of meat products.

Acknowledgements

The work was carried out as part of the State Assignment No. 0626-2014-0006 “The development of the method for applying a new normotimic to intensify growth, increase non-specific resistance and stress resistance of pigs, approved and supported by the Ministry of Science and Higher Education of the Russian Federation. It is published with the support of the Federal State Budget Scientific Institution “Federal Scientific Center for Livestock – Russian Institute of Livestock Breeding named after academician L.K. Ernst”, within the framework of the program “Improving feeding and fodder production systems, norms of animal and energy requirements for animals based on the study of metabolic processes in farm animals, the development of methods for physiological, biochemical and microbiological regulation in order to increase the realization of the genetic potential of productivity, reproduction function and the effectiveness of livestock industries” for the period of 2015–2020. State registration number of Scientific and Research, Development and Technological Work is AAAA-A18-118021590136-7.

Authors' contributions

OK developed a research program, conducted a comprehensive study, conducted morphometric and statistical analysis, and helped to compile a manuscript. OA participated in a comprehensive study, conducted organoleptic studies, analyzed the data and compiled the manuscript. LV conducted biochemical studies, performed data interpretation, and helped to compile a manuscript. All authors approved the final version of the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

- 1) Babicz, M., Szyndler-Nędza, M., Skrzypczak, E., Kasprzyk, A. 2016. Reproductive Performance of Native Pulawska and High Productivity Polish Landrace Sows in the Context of Stress During the Period of Early Pregnancy. *Reproduction in Domestic Animals*. 51(1):91–7. Doi: 10.1111/rda.12650.
- 2) Bazhov, G.M., Krishtop, E.A., Baranikov, A.I. 2013. Technological characteristics of pork with the defects of PSE and DFD. *Polythematic online scientific journal of Kuban State Agrarian University*. 89:973–984.
- 3) Cook, N.J., Schaefer, A.L., Lepage, P., Morgan, J.S. 1996. Salivary vs. serum cortisol for the assessment of adrenal activity in swine. *Canadian Journal of Animal Science*. 76:329–335.
- 4) Cook, N.J. 2012. Minimally invasive sampling media and the measurement of corticosteroids as biomarkers of stress in animals. *Canadian Journal of Animal Science*. 92:227–259.
- 5) Detmer, S.E., Patnayak, D.P., Jiang, Y., Gramer, M.R., Goyal, S.M. 2011. Detection of Influenza A virus in porcine oral fluid samples. *Journal of Veterinary Diagnostic Investigation*. 23:241–247.
- 6) Gajana, C.S., Nkukwana, T.T., Marume, U., Muchenje, V. 2013. Effects of transportation time, distance, stocking density, temperature and lairage time on incidences of pale soft exudative (PSE) and the physico-chemical characteristics of pork. *Meat Science*. 95:520–525.
- 7) Görres, A., Ponsuksili, S., Wimmers, K., Muráni, E. 2016. Genetic variation of the porcine NR5A1 is associated with meat color. *Journal of Applied Genetics*. 57(1):81–89. Doi: 10.1007/s13353-015-0289-2.
- 8) Khan, M., Ringseis, R., Mooren, F.C., Krüger, K., Most, E., Eder, K. 2013. Niacin supplementation increases the number of oxidative type I fibers in skeletal muscle of growing pigs. *BMC Veterinary Research*. 9:177–198. Doi: 10.1186/1746-6148-9-177.
- 9) Lebret, B., Meunier-Salaün, M.C., Foury, A., Mormède, P., Dransfield, E., Dourmad, J.Y. 2006. Influence of rearing conditions on performance, behavioral, and physiological responses of pigs to preslaughter handling, carcass traits, and meat quality. *Journal of Animal Science*. 84(9):436–447.
- 10) Lebret, B., Prunier, A., Bonhomme, N., Foury, A., Mormède, P., Dourmad, J.Y. 2011. Physiological traits and meat quality of pigs as affected by genotype and housing system. *Meat Science*. 2011. 88(1):14–22. Doi: 10.1016/j.meatsci.2010.11.025.

- 11) Magomedaliev, I.M., Nekrasov, R.V., Chabaev, M.G., Dzhavakhiya, V.V., Glagoleva, E.V., Kartashov, M.I., Durnikin, D.A., Matsyura, A.V. 2019. Use of different concentrations of Enzymesporin probiotic in feeding of growing young pigs. *Ukrainian Journal of Ecology*. 9(4):704-708. Doi: 10.15421/2019_813
- 12) Moore, K.L., Mullan, B.P., D'Souza, D.N. 2012. The interaction between ractopamine supplementation, porcine somatotropin and moisture infusion on pork quality. *Meat Science*. 92(2): 125–131. Doi: 10.1016/j.meatsci.2012.04.022.
- 13) Niyazov, N.S.-A., Ostrenko, K.S., Lemiasheuski, V.O., Ovcharova, A.N. 2019. Efficiency of antioxidant action of vitamin C additive in pregnant and lactating sows. *Problems of Productive Animal Biology*. 3: 67–77. Doi: 10.25687/1996-6733.prodanimbiol.2019.3.67-77.
- 14) Ostrenko, K.S., Galochkina, V.P., Galochkin, V.A., Lencher, O.S. 2019. Increasing the productivity of pigs under the influence of a new generation of stress protectors. *Advances in agricultural and biological sciences*. 2: 5–14. Doi: 10.22406/aabs-19-5.2-5-14.
- 15) Peeters, E., Driessen, B., Geers, R. 2006. Influence of supplemental magnesium, tryptophan, vitamin C, vitamin E, and herbs on stress responses and pork quality. *Journal of Animal Science*. 84(7): 1827–1838.
- 16) Rey-Salgueiro, L., Martínez-Carballo, E., Fajardo, P., Chapela, M.J., Espiñeira, M., Simal-Gandara, J. 2018. Meat quality in relation to swine well-being after transport and during lairage at the slaughterhouse. *Meat Science*. 142:38–43. Doi: 10.1016/j.meatsci.2018.04.005.
- 17) Rey-Salgueiro, L., Martínez-Carballo, E., Simal-Gándara, J. 2015. Liquid chromatography–mass spectrometry method development for monitoring stress-related corticosteroids levels in pig saliva. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*. 990: 158–163.
- 18) Soler, L., Gutiérrez, A., Escribano, D., Fuentes, M., Cerón, J.J. 2013. Response of salivary haptoglobin and serum amyloid A to social isolation and short road transport stress in pigs. *Research in Veterinary Science*. 5: 298–302.
- 19) Tomovic, V.M. 2014. Technological quality and composition of the *M. semimembranosus* and *M. longissimus dorsi* from Large White and Landrace Pigs. *Agricultural and Food Science*. 23: 9–18.
- 20) Ustyantseva, I.M., Khokhlova, O.I. 2009. New perceptions about the role of lactate in shock. *Polytrauma*. 2: 70–73.
- 21) Vermeulen, L., Van de Perre, V., Permentier, L., De Bie, S., Verbeke, G., Geers, R. 2016. Pre-slaughter sound levels and pre-slaughter handling from loading at the farm till slaughter influence pork quality. *Meat Science*. 116:86–90. Doi: 10.1016/j.meatsci.2016.02.007.
- 22) Vermeulen, L., Van de Perre, V., Permentier, L., De Bie, S., Verbeke, G., Geers, R. Pre-slaughter handling and pork quality. *Meat Science*. 2015. 100:118–123.
- 23) Pugliese, C., Sirtori, F., Škrlep, M., Piasentier, E., Calamai, L., Franci, O., Čandek-Potokar, M. 2015. The effect of ripening time on the chemical, textural, volatile and sensorial traits of Biceps femoris and Semimembranosus muscles of the Slovenian dry-cured ham Kraški pršut. *Meat Science*. 2015. 100:58-68. doi: 10.1016/j.meatsci.2014.09.012