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Meat and fat quality of Krškopolje pigs reared in conventional and organic production systems

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Short title: Krškopolje pigs: conventional vs. organic rearing

Abstract

Data on production traits of the only Slovenian autochthonous pig breed, the Krškopolje pig, is very scarce. Krškopolje pigs are reared in conventional and organic production systems, which were compared in the present study. After weaning, 24 barrows were assigned within litter to either conventional (**CON**) or organic (**ECO**) rearing system. Group CON (n=12) was housed indoors in two pens (7.5 m^2) with partly slatted floor. Group ECO (n=12) was held in a sty with sheltered area (concrete floor, bedded with straw, 16 m^2) and outdoor paddock area (100 m^2). The trial started when pigs had 68±8 kg BW and 157±6 days of age. Two diets were formulated with equivalent ingredients and composition. For ECO diet the ingredients used were ecological. Group ECO received a diet with 12.4 MJ metabolisable energy (ME) per kg and 12.9% CP and group CON a diet with 12.7 MJ ME per kg and 13.6% CP.

Feed distribution was limited to 3.5 kg per pig daily. In line with the rules for organic production, ECO pigs were additionally given alfalfa hay *ad libitum*. After 73 days on trial, the pigs were slaughtered and carcass, meat and fat quality was evaluated. Meat quality traits (pH, colour, water holding capacity), fatty acid composition, lipid and protein oxidation, collagen content and solubility were analysed in *longissimus lumborum* (**LL**) muscle. Fatty acid composition, lipid oxidation, and vitamin A and E concentrations were determined in backfat. There were no significant differences in growth rate and carcass traits between ECO and CON pigs, however ECO pigs tended ($P<0.10$) to have higher daily gain and lower dressing percentage, higher ($P<0.001$) pH 45 min and lower ($P<0.01$) pH 24 h *post mortem*, affecting ($P<0.10$) also water holding capacity and objective colour parameters ($P<0.05$) of LL muscle. There were no differences in intramuscular fat (**IMF**) content of LL muscle, however IMF of ECO pigs had lower ($P<0.05$) proportion of saturated and higher ($P<0.01$) proportion of monounsaturated fatty acids accompanied by higher ($P<0.001$) values of thiobarbituric reactive substances (**TBARS**). In backfat, ECO pigs showed lower ($P<0.05$) vitamin E content, higher ($P<0.001$) TBARS, higher ($P<0.01$) degree of unsaturation (percentage of polyunsaturated fatty acids), and also higher ($P<0.05$) vitamin A concentration than CON pigs, which can be related to alfalfa hay supplementation of ECO pigs. In brief, organic rearing of Krškopolje pigs did not affect performances but had an effect on meat and fat quality.

KeywordsLocal breed; Diet; Performance, Fatty acids; Oxidative stability;

Implications

Similar fattening performance of Krškopolje pigs can be expected in organic and conventional production system when nutrition is similar and adequate. Organically raised pigs experience outdoor activity and more diverse stimuli during life and consequently seem to confront better the pre-slaughter handling. Krškopolje pigs like to consume roughage feed (alfalfa hay) however this can affect fat tissue oxidative stability.

Introduction

Krškopolje pig is the only Slovenian autochthonous pig breed. The breed was abandoned in the second half of last century because of its low performance compared to modern, genetically improved pig breeds. Krškopolje pig is also reputed for its excellent meat quality, but scientifically based facts to support these claims are scarce (Kastelic and Čandek-Potokar, 2013). In the past years, owing largely to the preservation programme for genetic resources, the interest and use of this breed has been growing among farmers; however, the pigs of this breed remain predominantly reared on small-scale, non-intensive farms, very often on organic and agro-touristic farms (Kastelic and Čandek-Potokar, 2013). The legislation on organic production and labelling of organic products (OJ EU L250/1, 2008) recommends the use of breeds which are better adapted to local conditions with a preference for indigenous breeds. It has been shown that indigenous local pig breeds (e.g. Angler Sattelschwein and Schwäbisch-Hällisches; Brandt *et al.*, 2010) may be less affected by reduced dietary protein (and energy) intake in organic rearing system. Namely, the regulation on organic production and labelling of organic products precludes the use of synthetic amino acids (and genetically modified organisms) in organic feed. Additionally, roughage feed and larger space allowance including outdoor access

must be provided to organically reared pigs. These factors can exert an impact on growth, carcass and meat quality traits (Lebret, 2008). The limitations in amino acid supply could also affect intramuscular fat (**IMF**) content which is a key property of meat eating quality (Bonneau and Lebret, 2010). Until now, no scientific information on the effect of the rearing practice in Krškopolje pigs exists. In view of the mentioned circumstances, the aim of the present study was to evaluate meat and fat quality traits of this breed reared in conventional and organic system.

Material and methods

Animals and treatments

Twenty-four barrows of Krškopolje breed originating from 12 litters and farms (2 pigs per litter/farm) and born within two weeks period were assigned within litter to either conventional (**CON**) or organic (**ECO**) rearing system. Pigs had a known genotype at ryanodine receptor 1 (***RYR1***) gene (i.e. 5 and 7 carriers of the *RYR1* mutation in CON and ECO group, respectively). Group CON (n=12) was housed indoors in two pens of 7.5 m^2 with partly slatted floor. Group ECO (n=12) was held in a sty with sheltered area (16 m^2, solid floor, bedded with straw) and outdoor area (100 m^2). Ambient temperature of the indoor and outdoor areas was monitored (Figure 1). Although all experimental animals were reared on the same farm, the pigs in group ECO were reared respecting the legislation on organic farming. Prior to the start of the trial (from weaning until the age of 157±6 days), the pigs were fed commercial diets *ad libitum* (organic or conventional for ECO and CON group, respectively). For the experiment, two barley based diets were formulated (Table 1); group ECO received a feed mixture composed of ingredients from organic agriculture, while

group CON was fed a diet composed of same ingredients from conventional agriculture. During the trial (73 days), daily feed distribution was limited to 3.5 kg in ECO and CON groups and provided in two portions (morning and late afternoon). Pigs in group ECO were supplemented with alfalfa hay on *ad libitum* basis. Feeding regime was planned to allow pigs to exhibit their growth potential, but to limit an excessive fat deposition in the last phase of fattening. The pigs were weighed every two weeks. At the same occasion their backfat thickness at the level of last rib was measured ultrasonically using 4Vet mini ultrasound scanner (Draminski S.A., Olsztyn, Poland).

Feed

Two barley based feed mixtures were formulated as already described by Škrlep et al. (2017). For the ECO group it was formulated according to the legislation on organic farming with all the ingredients originating from organic agriculture. A formulation as similar as possible, but with the conventional ingredients was used for CON feed with addition of synthetic amino acids and the mineral-vitamin premix (Table 1). Diets ECO and CON were similar with regard to fatty acid composition (Supplementary Table S1) and estimated digestible amino acid composition (exception being lysine) using EvaPig® software (Supplementary Table S2). Chemical analysis of feed mixtures and alfalfa hay (dry matter, crude protein, crude fat, crude fibre and crude ash) was determined according to the standard procedures (AOAC, 2000) and is given in Table 2.

Carcass and meat quality measurements

125 At the average age of 228 days, the pigs were transported to a nearby commercial
126 abattoir. The transport (30 km) lasted 40 min, after unloading the pigs rested for 1.5 h
127 and were slaughtered according to routine procedure (CO_2 stunning followed by
128 exsanguination). Last feeding of pigs was in the morning a day before slaughter.
129 After the slaughter, the pigs were eviscerated, leaf fat was removed and weighed,
130 carcasses were weighed and classified by official classification body using a method
131 approved for Slovenia (OJ EU L56/28, 2008). Backfat thickness was measured on
132 split carcasses at the level of withers and over *gluteus medius* muscle. The pH was
133 measured in the *longissimus lumborum* muscle (**LL**) at the level of the last rib 45
134 minutes *post mortem* (**pH45**) and a day after the slaughter (**pH24**) using the MP120
135 pH meter (Mettler-Toledo GmbH, Schwarzenbach, Switzerland) equipped with a
136 combined glass electrode (InLab427) and a temperature correction probe. The
137 carcasses were cut at the level of last rib perpendicularly to the spine. Measurements
138 of LL loin eye area, area of corresponding fat and marbling subjective and objective
139 colour (CIE L*,a*,b* colour parameters) were performed as described in Batorek *et*
140 *al*. (2012). Colour was evaluated on the LL surface immediately after cutting (no
141 blooming). Additionally, colour parameters chroma (numerically quantified as
142 $C^* = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($h^\circ = tan^{-1} b^*/a^*$) were calculated. Two LL
143 chops were trimmed of epimysium and external fat and used for the determination of
144 chemical composition, drip, thaw and cooking loss, and shear force. Chemical
145 composition (moisture, IMF and protein content) in minced samples was determined
146 by NIRS (NIR Systems 6500 Monochromator, Foss NIR System, Silver Spring, MD,
147 USA) using internal calibration with the following predictive ability based on R^2 (0.81,
148 0.82, 0.97, 0.81, for moisture, IMF and protein content, respectively) and *Sy.x*
149 (0.65%, 0.30%, 0.73%, for moisture, IMF and protein content, respectively). Drip loss

was determined according to the EZ method (Christensen, 2003). Thaw and cooking losses were determined on LL chop (8×5×4 cm) which was weighed, vacuum packed and frozen at -20°C until analysis. To determine thaw loss, the samples were thawed overnight at 4°C, softly drained with a paper towel and reweighed. The same sample was afterwards used for cooking loss and shear force measurement. The samples were cooked in a thermostatic water bath (ONE 7-45, Memmert GmbH, Schwabach, Germany) until the internal temperature reached 72°C, cooled and weighed. On the next day, three to four 1.25 mm cylindrical cores were excised from cooked samples and shear force was measured perpendicular to the direction of fibres using a TA Plus texture analyser (Ametek Lloyd Instruments Ltd., Fareham, UK) equipped with a 60° V-shaped rectangular-edged blade and a crosshead speed set at 3.3 mm/s. The average value of the measurements within one sample was calculated and used for statistical analysis. Additional LL sample was homogenized to fine dust in liquid nitrogen and stored at -80°C to determine fatty acid composition, concentration of thiobarbituric reactive substances (**TBARS**), carbonyl groups, myoglobin, and collagen content.

Samples of backfat were also taken and objective colour (CIE L*,a*,b* colour parameters) was measured. The samples were homogenized to fine dust in liquid nitrogen and stored at -80°C before the analysis of fatty acid composition, TBARS, and vitamins A and E.

Fatty acid analysis

The fatty acid composition of feed, LL muscle and backfat samples was determined by gas chromatography following the transesterification of lipids (as described by Rezar et al. 2017). Approximately 0.5 g of sample was transmethylated *in situ* (Park

and Goins, 1994) using 0.5 M NaOH in methanol followed by 14% BF_3 in methanol. Fatty acid methyl esters (**FAME**) were extracted using hexane. For FAME separation, an Agilent 6890 GC (Agilent, Santa Clara, CA, USA) equipped with an Omegawax 320 column (30 m x 0.32 mm i.d. x 0.25 µm; Supelco, Bellefonte, PA, USA) and flame ionization detector (**FID**) was used. An Agilent GC ChemStation (Agilent, Santa Clara, CA, USA) was used for data acquisition and processing. The programmed initial temperature was 185 °C, subsequently increased to 215 °C at 1 °C/min. The injector and FID temperatures were set at 250 °C and 290 °C, respectively. Individual FAMEs were identified using standard mixtures (Nu Chek Prep Inc., Elysian, MN, USA). Adipose tissue fatty acid concentrations were determined using an internal standard (C 19:0) and the tissue lipid concentration was calculated by multiplying the values with the factor 1.049.

Intra-assay CV of fatty acid analyses in the present study was 2.2%. The laboratory results of inter-assay CVs resulting from interlaboratory comparison are 10.5, 5.1, 2.9, 2.5 and 2.3% for fatty acids with weight percentages of <1%, 1-5%, 6-10%, 11-20% and >20%, respectively.

Lipid and protein oxidation

Concentration of TBARS in LL and backfat samples was analysed according to the method described by Lynch and Frei (1993). Briefly, samples of 0.5 g were minced in 10 ml of 0.15 M KCl with 0.1 mM BHT, centrifuged and the supernatant (0.5 ml) incubated with 1% (w/v) 2-thiobarbituric acid in 50 mM NaOH and 2.8% (w/v) trichloroacetic acid in a thermostatic heating block for 10 min. After cooling to room temperature, the pink chromogen was extracted into n-butanol and its absorbance was measured at 535 nm (BioSpectrometer Fluorescence, Eppendorf, Hamburg,

Germany). Concentration of TBARS were calculated using 1,1,3,3-tetramethoxypropane as a standard and expressed in µg malondialdehyde (**MDA**)/kg. Inter-assay CV of TBARS analysis was 4.5%, and intra-assay CV was 2.7%.

Protein oxidation was measured in myofibril isolates according to description by Rezar *et al*. (2017). Two aliquots of myofibrillar suspension were treated with 2 N HCl (to determine the concentration of proteins), while two aliquots were treated with 0.2% (w/v) 2,4-dinitrophenylhydrazine (**DNPH**) in 2 N HCl to determine the concentration of carbonyl groups. Samples were incubated for 1 h at room temperature under shaking and afterwards precipitated by 50% trichloroacetic acid. After centrifugation (4000×*g* for 15 min at 4°C), the pellets were washed three times with ethanol and ethyl acetate (1:1) to eliminate traces of residual DNPH. The pellets were afterwards dissolved in 6 M guanidine HCl with 20 mM sodium phosphate buffer (pH 6.5) and centrifuged for 15 min at 4000×*g*. The protein and carbonyl group concentrations were determined spectrophotometrically (BioSpectrometer Fluorescence, Eppendorf, Hamburg, Germany). The concentration of protein was calculated from absorbance at 280 nm according to the standard concentrations of BSA in 6 M guanidine HCl while the concentration of carbonyl groups was measured at 370 nm in the samples treated with DNPH (considering that the extinction coefficient for DNPH at 370 nm is 21 mM^{-1} cm^{-1}) and expressed in nmol/mg proteins. Inter-assay CVs for chemical analysis of carbonyl groups was 7.7%, and intra-assay CV was 6.0%.

Muscle pigment

Myoglobin concentration was analysed spectrophotometrically (BioSpectrometer Fluorescence, Eppendorf, Hamburg, Germany) according to the method described

by Trout (1991). Briefly, 2 g of LL samples were homogenized in 20 ml 0.04 M potassium phosphate buffer (pH 6.5) and filtered; 4 ml of filtrate was mixed with 1.4 ml 10% (v/v) Triton X-100 and 0.1 ml 0.065 M sodium nitrite. After 60 min incubation at 22 °C, the absorbance at 370 and 409 nm was measured and used for calculation of myoglobin concentration (mg/g) in the samples. Inter-assay CV of myoglobin analysis was 4.2% and intra-assay CV was 2.5%.

Collagen content

For the determination of total collagen, hydroxyproline was determined according to the ISO 3496 standard (1994). Briefly, cooked sample (at 77°C for 90 minutes) was incubated with 3 M H_2SO_4 for 16 hours at 105°C. After the hydrolysis, the samples were filtered, diluted with deionized water, neutralized with 1 M NaOH and 4 ml of this solution was transferred to a glass tube, mixed with chloramine T and incubated at room temperature for 20 minutes. After the incubation, 2 ml of colour reagent (p-dimethylaminobenzaldehyde dissolved in perchloric acid and propan-2-ol) was added and the samples were incubated in a water bath for 20 min at 60°C. The cooled samples were then incubated at room temperature for 30 min. The absorbance was measured spectrophotometrically at 558 nm (BioSpectrometer Fluorescence, Eppendorf, Hamburg, Germany) and hydroxyproline content was determined according to a standard calibration curve. For insoluble collagen fraction, LL sample was heated (to 77 °C for 90 min) in Ringer's solution and centrifuged. The supernatant was discarded and the pellet was then further processed as in the case of total collagen. Soluble collagen was determined from the difference between total and insoluble collagen content. Inter-assay CV of collagen analysis was 4.7% while intra-assay CV was 3.4%.

Vitamins A and E

Vitamins E and A in feed and fat were determined in a commercial laboratory (Nutricontrol, Veghel, The Netherlands) according to the accredited ISO 17025 method. Briefly, after saponification under nitrogen environment and extraction, the vitamins were separated by liquid chromatography and detected by fluorescence spectrophotometry. Inter-assay CV was 10.0% for vitamin E and 5% for vitamin A analysis, while intra-assay CV was 2% and 2%, respectively.

Statistical analysis

Data were analysed using one-way ANOVA (GLM procedure of SAS; SAS Institute Inc., Cary, USA) with fixed effect of treatment group (equation 1). In the case of carcass and meat quality traits (Table 3, 4 respectively) *RYR1* genotype was added as main effect in the model (equation 2). Interaction T×*RYR1* was always insignificant and therefore not considered. Not being the objective of this study, the data on *RYR1* effect are not further shown or discussed. The differences between treatment groups were considered significant when P<0.05.

$$Y_{ij} = \mu + T_i + e_{ij} \qquad \textit{Equation 1}$$

where: μ = intercept; T_i = effect of treatment group; i = CON, ECO; e_{ij} = residual error.

$$Y_{ij} = \mu + T_i + RYR1_j + e_{ijk} \qquad \textit{Equation 2}$$

where: μ = intercept; T_i = effect of treatment group, i=CON, ECO; *RYR*1_j = effect of *RYR1* genotype, j = *NN, Nn*; e_{ij} = residual error.

Results

All the pigs finished the experiment and were slaughtered at an average age of 230 days and 122.4 kg BW. As previously reported by Škrlep et al. (2017) average daily feed intake in the experimental period was 3.38 and 3.37 kg per pig for CON and ECO groups, respectively and there were no differences between the two groups in starting and final BW or backfat gain, only average daily gain tended to be higher (P=0.10) in ECO than CON pigs. Pigs in group ECO had lower dressing percentage than CON pigs (P=0.02), while all the other carcass traits (lean meat percentage, backfat thickness measured at different locations, loin eye area, loin eye fat area) did not differ between ECO and CON pigs (Table 3).

The two groups differed in some of the quality traits of LL muscle (Table 4). Pigs of ECO group had higher pH45 than CON pigs (P<0.001), whereas pH24 was lower in ECO than CON pigs (P=0.003). Consequently, the LL muscle of ECO pigs tended to have lower water holding capacity as evidenced by somewhat higher drip (P=0.08), cooking (P=0.09) losses and different objective colour parameters; ECO pigs had higher CIE L* (P=0.04), CIE a* (P=0.002) and CIE b* values (P=0.003) in addition to higher chroma (P<0.001) and hue angle (P=0.018). There were no differences in subjective colour and marbling score or mechanical resistance (WBSF) of cooked LL samples.

Results of chemical analyses (Table 5) showed no differences in water and IMF content of LL muscle between ECO and CON group, but a higher LL muscle protein content (P=0.03) in ECO pigs. No differences were either noted for collagen (content and solubility), myoglobin and carbonyl groups concentrations. The only significant differences were noted for TBARS and fatty acid composition of LL muscle (Table 5,

Supplementary Table S3). In IMF of LL muscle, pigs of ECO group had lower proportion of saturated fatty acids (**SFA**) (P=0.02) and a higher proportion of monounsaturated fatty acids (**MUFA**) (P=0.02) mostly due to stearic (C18:0) and oleic acids (C18:1), respectively. Oxidation of muscle lipids, measured by concentration of TBARS, was also higher in ECO than CON pigs (P<0.001).

Chemical analyses of backfat (Table 6) showed a more pronounced lipid oxidation in ECO than CON pigs as depicted by higher TBARS values (P<0.001). Backfat of ECO pigs had a higher concentration of vitamin A (P=0.03), and a lower concentration of vitamin E (P=0.01), was more unsaturated as backfat of CON pigs, than demonstrated by higher concentration of polyunsaturated fatty acids (**PUFA**) (P=0.004), n-6 PUFA (P=0.007) and n-3 PUFA (P<0.001) due to higher concentrations of linoleic (C18:2n-6), linolenic (C18:3n:3), arachidonic (C20:4n-6) and eicosapentaenoic (C20:5n-3) acids (Supplementary Table S4). The n-6 to n-3 PUFA ratio of backfat was lower in ECO than CON pigs (P<0.001).

Discussion

Overall, the daily gain of Krškopolje pigs in the present study (745 g/day) shows a moderate growth potential compared to modern pig genotypes, however relatively good if compared with growth rates generally reported for local pig breeds (Čandek-Potokar *et al*., 2017). Organically reared pigs demonstrated 13% higher daily gain in spite of similar feed use and absence of differences in BW, backfat gain or carcass traits/composition and the fact that their assumed energy expenditure was higher as they could move around and be more active. Two circumstances could explain this result, one being a substantial consumption of alfalfa hay of pigs in ECO group, and

the other that less feed was wasted in ECO group. Under the given conditions, it was not possible to estimate the intake of alfalfa hay, but it was observed, pigs liked it and consumed it a lot (substantial usage). It is likely that lower ambient temperatures, in particular outdoor, stimulated the appetite in ECO pigs (Lebret *et al*., 2008). Usually the pigs reared outdoors exhibit lower growth rate compared to their indoor reared peers due to increased energy demands for exercise and thermoregulation (Bee *et al*., 2004; Hansen *et al*., 2006). However, faster growth of organically reared pigs (due to higher feed intake) was also reported (Millet *et al*. 2005). In the present study, there was no effect of rearing system on growth rate or carcass traits, only a tendency of lower dressing percentage in ECO pigs was noted which can be attributed to heavier digestive tract due to higher intake of fibrous feed (alfalfa hay), in agreement with observation of Rey *et al.* (2006) for free range Iberian pigs. Fatness of pigs was generally high (average lean meat percentage ≈43%) which is typical for local pig breeds, nowadays primarily used for the production of high value-added processed products and less for fresh meat consumption. In the present study, higher pH45 along with lower pH24 of LL muscle of ECO pigs is indicative of lower stress sustained during the pre-slaughter handling and higher muscle glycogen reserves at slaughter, in agreement with Terlouw *et al*. (2005). As reviewed by Millet (2004) effect of housing on meat quality can be mainly related to differences in physical training and pre-slaughter stress. Exercised pigs seem more resistant to pre-slaughter handling, as demonstrated by higher pH45 of ECO pigs. On the other hand with regard to pH24, higher glycogen stores in exercised pigs (Bonneau and Lebret 2010) can explain their lower pH24, whereas any effort before slaughter can lead to a depletion of muscle glycogen (Fernandez and Tornberg, 1991) which explains higher pH24 in CON pigs. Moreover, in the last period of the experiment

(Figure 1) the temperatures (esp. outdoor) to which ECO pigs were submitted were lower which also stimulates glycogen reserves (Lebret, 2008). As the nature of pH decline in meat is related to water-holding capacity (Huff-Lonergan and Lonergan, 2005) and colour parameters (Mancini and Hunt, 2005), it explains slightly lower water holding capacity and higher objective colour parameters in ECO than CON pigs. Chemical analyses of LL muscle showed no major effect of rearing system, exception being higher protein content, which is in agreement with Olsson *et al*. (2003), and a higher degree of lipid unsaturation and oxidation in ECO pigs. The main factors influencing fatty acid composition of porcine tissues are diet, breed, sex and the level of adiposity (Wood *et al.*, 2003). Pigs used in our study were of the same breed and sex, and siblings from 12 litters were assigned to treatment group within litter. Moreover, no differences in carcass adiposity and IMF content between groups were observed; therefore, the differences in fatty acid composition can be attributed to rearing/feeding system. Fatty acid composition of ECO and CON diets were similar since they were composed of equivalent/same ingredients, thus the observed differences in fatty acid composition could be related to alfalfa hay supplementation of ECO pigs, which agrees with reports where organically or outdoor reared pigs had access to green feed (Nilzén *et al.*, 2001; Hansen *et al.*, 2006). Moreover, exercise may increase the content of linoleic and linolenic acids and total PUFA in the neutral lipid fraction as shown in *psoas major* muscle of exercised Iberian pigs in comparison to pigs fed the same diet under sedentary rearing system (Daza *et al*., 2009). Ambient temperature is another factor influencing the fatty acid composition (as reviewed by Lebret, 2008). Namely, lower temperatures may increase MUFA and decrease PUFA. Fatty acid composition of IMF can be affected also by dietary lysine (Wang *et al*., 2018), which could also

375 explain the higher MUFA observed in LL muscle of ECO pigs and their higher
376 (though not significant) IMF content.
377 Although high PUFA content in meat is beneficial in terms of human health, it also
378 has a potential for higher lipid oxidation during storage, with adverse effects on
379 organoleptic quality (Edwards, 2005). Although TBARS as a marker of lipid oxidation
380 determined in muscle and backfat of pigs was well below the threshold for detection
381 of off flavour (0.5 mg MDA/kg) in both groups, higher concentrations were observed
382 in ECO pigs that were supplemented with alfalfa hay. Vitamin E is one of the main
383 factors preventing lipid oxidation (Jensen *et al*., 1998). Its concentration was lower in
384 backfat of ECO pigs despite its slightly higher concentration in organic feed, and can
385 be related to its depletion for antioxidant protection of PUFA (as demonstrated by
386 Fritsche and McGuire (1996) in plasma of rats fed highly unsaturated fish oil versus
387 lard). Contrary to vitamin E, vitamin A concentration was higher in backfat of ECO
388 pigs, despite being almost two-fold lower in organic diet which can be ascribed to
389 alfalfa hay, a good source of carotenes which are converted to vitamin A in the pig
390 intestine and absorbed (McDowell, 2000).
391 In conclusion, organic rearing of Krškopolje pigs provided with the diet composed of
392 organic ingredients and alfalfa hay did not lead to any major differences in productive
393 performance compared to their conventionally reared and fed peers. Differences in
394 dynamics of LL muscle pH decline (reflected also in water holding capacity and
395 colour of meat) are indicative of higher glycolytic potential of organically reared pigs
396 which can be related to more exercise and lower ambient temperature. The provision
397 of alfalfa hay to the pigs reared in organic system generated higher content of
398 unsaturated fatty acids in muscle and fat tissue leading to higher lipid oxidation, and
399 also to higher vitamin A content of backfat.

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Declaration of interest

The authors declare that there is no conflict of interest.

Ethics statement

The research was undertaken on a family farm respecting the Slovenian law on animal protection (Zakon o zaščiti živali, 2007), rules of protection of farm animals (Pravilnik o zaščiti rejnih živali, 2010), and legislation on organic production and labelling of organic products (OJ EU L250/1, 2008). No procedures on animals were conducted which would demand ethical protocols according to Directive 2010/63/EU (2010).

Software and data repository resources

The data of this study are not deposited in any official repository.

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533 **Table 1:** *Ingredient composition of finishing diets for conventionally (CON) and*

534 *organically (ECO) reared Krškopolje pigs (Škrlep et al., 2017)*

Ingredients (%)	CON	ECO
Barley	74.49	76.45
Soybean cake	5.98	8.35
Sunflower cake	8.0	10.0
Wheat feed flour	5.38	/
Molasses	3.00	3.00
Calcium carbonate	0.96	1.10
Salt	0.34	0.34
Monocalcium phosphate	0.24	0.69
Vitamin and trace mineral mixture	0.500	/
L-Lysine HCl	0.546	/
Methionine[1]	0.146	/
L-Threonine	0.132	/
L-Tryptophan	0.044	/
Lignosulphonate	0.250	/
Iron sulphate	/	0.028
Copper sulphate	/	0.006
Zinc sulphate	/	0.022
Manganese oxide	/	0.007
Organic selenium	/	0.003
Vitamin A	/	0.001
Vitamin E	/	0.100

535 [1]Methionine hydroxy analogue, 80% efficiency.

536

537 **Table 2:** *Chemical composition of diets for conventionally (CON) and organically*

538 *(ECO) reared Krškopolje pigs*

	CON	ECO	Alfalfa hay
Metabolisable energy, MJ/kg	12.7	12.4	6.9
Dry matter, %	88.0	87.6	80.9
Crude ash, %	4.3	4.6	7.7
Crude protein, %	13.6	12.9	14.2
Crude fat, %	2.7	3.0	1.0
Crude fibre, %	6.1	6.4	25.6
Nitrogen free extract, %	61.3	60.7	32.3
Lysine, %	1.2	0.7	1.2
Fatty acids, g FA/100 g fat			
SFA	19.0	17.7	37.3
MUFA	18.0	19.6	4.3
PUFA	63.1	62.7	58.4
n-3 PUFA[1]	4.69	4.69	36.9
n-6 PUFA[2]	58.4	58.1	21.5
n6:n3 PUFA	12.5	12.4	0.58
Vitamin A, mg/kg	1.917	1.068	0.520
Vitamin E, mg/kg	246	268	22.9

539 FA = fatty acids; SFA = saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA.

540 [1]Includes C18:3 n-3.

541 [2]Includes C18:2 n-6 cc and C20:2 n-6.

542

543 **Table 3:** *Carcass traits of conventionally (CON) and organically (ECO) reared*

544 *Krškopolje pigs*

	CON	ECO	RMSE	P-value
Number of pigs	12	12		
Warm carcass weight, kg	97.6	98.8	10.63	0.58
Dressing, %[a]	81.1	79.6	1.71	0.019
Lean meat %[1, a]	42.5	43.3	4.39	0.97
Leaf fat, kg[a]	2.6	2.4	0.43	0.39
Fat thickness over *m. gluteus medius,* mm[a]	36.4	35.4	5.85	0.99
Fat thickness at withers, mm	54.0	51.9	7.77	0.60
Loin eye area, cm^2	37.0	35.6	5.78	0.41
Loin eye fat area, cm^{2a}	28.7	27.3	4.06	0.67

545 [1]% of lean meat in the carcass assessed with the official method approved for Slovenia.

546 [a]Traits significantly affected by ryanodine receptor 1 (*RYR1*) genotype.

547 **Table 4:** *Meat quality (LL muscle) traits of conventionally (CON) and organically*

548 *(ECO) reared Krškopolje pigs*

	CON	ECO	RMSE	P-value
Number of pigs	12	12		
pH 45 min p.m.[a]	6.33	6.65	0.193	<0.001
pH 24 h p.m.	5.71	5.47	0.163	0.003
Colour				
Subjective score (1-6)[1]	4.5	4.2	0.62	0.33
CIE L*	50.4	53.7	3.36	0.047
CIE a*	6.6	8.1	1.00	0.001
CIE b*	0.9	2.2	0.83	0.003
C*	6.7	8.5	1.04	<0.001
h°	8.0	14.8	6.03	0.011
Drip loss after 24 h, %[a]	4.2	6.1	2.64	0.082
Thaw loss, %[a]	11.5	14.2	3.79	0.141
Cooking loss, % [a]	26.9	29.7	3.37	0.092
Marbling score (1-7)[2]	4.3	3.8	1.07	0.23
WBSF, N	53.2	57.7	9.04	0.348

549 LL= *longissimus lumborum;* p.m. = *post mortem*; WBSF = Warner-Bratzler shear force ; C* = chroma;
550 h° = hue angle.
551 [1]Visually assessed on a freshly cut LL using a scale from 1 (for light, non-intensive) to 6 (for dark,
552 intensive colour).
553 [2]Visually assessed on a freshly cut LL using a scale from 1 (extremely lean) to 7 (extremely marbled
554 sample).
555 [a]Traits significantly affected by ryanodine receptor 1 (*RYR1*) genotype.

Table 5: *Chemical analysis and fatty acid (FA) composition of LL muscle of conventionally (CON) and organically (ECO) reared Krškopolje pigs*

	CON	ECO	RMSE	P-value
Number of pigs	12	12		
Intramuscular fat, %	2.81	3.23	0.831	0.23
Water, %	72.6	72.3	0.75	0.29
Protein, %	23.2	23.6	0.47	0.032
TBARS, µg MDA/kg	25.6	28.1	0.72	<0.001
Carbonyl groups, nmol/mg protein	1.49	1.32	0.307	0.18
Myoglobin, mg/g	1.39	1.32	0.187	0.41
Total collagen, mg/g	2.67	2.68	0.219	0.85
Soluble collagen, mg/g	0.52	0.48	0.099	0.30
Insoluble collagen, mg/g	2.14	2.20	0.155	0.36
Collagen solubility, %	19.6	17.9	2.67	0.13
Fatty acids, g FA/100 g fat				
SFA	41.0	39.4	1.60	0.021
MUFA	47.5	50.5	2.09	0.002
PUFA	11.6	10.1	2.34	0.15
n-3 PUFA[1]	0.698	0.653	0.1289	0.56
n-6 PUFA[2]	10.8	9.4	2.23	0.14
LC PUFA	2.74	2.31	0.717	0.15
LC n-3 PUFA	0.349	0.321	0.0932	0.46
LC n-6 PUFA	2.39	1.99	0.629	0.13
n-6/n-3 PUFA	15.7	14.3	1.13	0.006

LL= *longissimus lumborum;* TBARS = thiobarbituric reactive substances; MDA = malondialdehyde; FA= fatty acids; SFA = saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA; LC = long chain.

[1]Includes C18:3 n-3, C20:3 n-3, C20:5 n-3, C22:5 n-3 and 22:6 n-3.

[2]Includes C18:2 n-6, C20:2 n-6, 20:3 n-6, 20:4 n-6 and 22:4 n-6.

564 **Table 6:** *Chemical analysis and fatty acid (FA) composition and colour of backfat of*

565 *conventionally (CON) and organically (ECO) reared Krškopolje pigs*

	CON	ECO	RMSE	P-value
Number of pigs	12	12		
TBARS, µg MDA/kg	24.4	28.5	1.17	<0.001
Vitamin E, mg/kg	14.3	11.9	2.13	0.012
Vitamin A, mg/kg	0.600	0.675	0.0805	0.033
Fatty acids, g FA/100 g fat				
SFA	42.2	41.4	1.82	0.29
MUFA	43.6	42.6	1.50	0.15
PUFA	14.7	16.0	1.33	0.004
n-3 PUFA[1]	1.02	1.27	0.107	<0.001
n-6 PUFA[2]	13.2	14.7	1.22	0.007
LC PUFA	1.31	1.36	0.119	0.26
LC n-3 PUFA	0.244	0.275	0.0263	0.007
LC n-6 PUFA	1.06	1.09	0.096	0.54
n-6/n-3 PUFA	12.9	11.6	0.36	<0.001
Lipid content, %	80.1	78.4	3.34	0.228
Objective colour parameters				
CIE L*	78.3	78.6	0.99	0.50
CIE a*	3.3	3.1	0.73	0.45
CIE b*	2.5	2.4	0.81	0.85

566 TBARS = thiobarbituric reactive substances; MDA = malondialdehyde; FA= fatty acids; SFA =
567 saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA; LC = long chain.
568 [1]Includes C18:3 n-3, C20:3 n-3, C22:5 n-3 and C22:6 n-3.
569 [2]Includes C18:2 n-6, C20:2 n-6, 20:3 n-6, 20:4 n-6 and 22:4 n-6.
570

571 **Figure captions**

572

573 **Figure 1**: Outdoor and indoor ambient temperature recorded during the experiment in

574 organic (ECO) and conventional (CON) group of Krškopolje pigs