Replacing soybean meal with rapeseed meal and faba beans in pig diet: effect on growth performance, meat quality and metabolite changes

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Abstract

Rapeseed meal and faba beans (RSM/FB) can serve as an alternative to imported soybean meal (SBM). In this study, forty Norwegian crossbred ([Landrace x Yorkshire] x Duroc) growing-finishing pigs (108.7±4.2 kg final BW) were fed a diet with either SBM or RSM/FB as protein sources. RSM/FB increased feed conversion ratio (P=0.04) in the finishing period, reduced lightness (P=0.04) and yellowness (P=0.004) of meat, changed amounts of individual fatty acids, but not of total SFA, MUFA and PUFA. Importantly, RSM/FB reduced the glucose level ($P < 0.05$) in meat. Lower pyroglutamic acid ($P = 0.06$) in RSM/FB indicate lower oxidative stress in pre-rigor muscle cell. Increased abundance of free amino acids, sweet tasting metabolites, reduced warmed-over flavor and flavor attributes indicated desirable properties of RSM/FB meat. To conclude, RSM/FB in pig diet supported a high growth performance and carcass quality comparable to SBM and had a positive effect on meat quality.

Keywords

Local diet, carcass traits, pork meat, chilled storage, metabolites, sensory quality

1. Introduction

Modern pig production in Europe heavily relies on imported soybean meal (SBM) as the main protein supplement in animal diet (Jezierny, Mosenthin, & Bauer, 2010). Considering the often large price fluctuations for SBM, an overall economic pressure on pork production (Florou-Paneri et al., 2014), and also the high reliance of imported protein rich feedstuffs in Norway and Europe, an alternative is to increase the use of rapeseed meal (RSM) and faba beans (FB) as locally produced high-protein ingredients in Europe (Jezierny et al., 2010; Sobotka, Pomianowski, & Wójcik, 2012). In addition, the possibility of replacing SBM with protein ingredients from European agricultural systems can contribute to more sustainable pork production (Hanczakowska & Świątkiewicz, 2014; van Zanten, Bikker, Mollenhorst, Meerburg, & De Boer, 2015). Some studies showed no adverse effects of replacing SBM with alternative protein sources on the growth performance of growing-finishing pigs (Partanen et al., 2003; Skoufos et al., 2016). A meta-analysis showed that the use of up to 30% rapeseed meal in growing-finishing pig diets did not compromise growth performance compared to a SBM based diet when added to a nutritionally balanced diet (Hansen et al.,2020). However, limited information exists on the metabolic adaptation of pigs and metabolite changes in pork meat resulting from replacing SBM with RSM and FB.

Metabolite analysis are more often used in human interventions identifying metabolites associated with the intake of nutrients and different dietary patterns (Esko et al., 2017; Lécuyer et al., 2020), while it has not been commonly used to study the impact of metabolites on animal performance and meat quality in farm animals. Chen et al. (2018) reported that RSM in diets (200 g/kg of feed) for young pigs induced changes in metabolite profile of liver and plasma by increasing the level of oxidized metabolites (*e.g*. oxidized glutathione) and compromised redox balance. Metabolite analysis of meat, may show similar changes. However, Skugor et al. (2019) showed that feeing rapeseed based diets contains natural antioxidants that led to an increased expression of genes that gives protection against oxidative stress and removes free radicals in the muscle. Different energy sources, such as low starch, high fiber and fat content diets, can modulate physiological and nutritional condition of muscle, *i.e.* glycogen storage (Li et al., 2015), having indirect effect on early *post mortem* mechanisms that leads to conversion of muscle into meat (Li et al., 2017). However, diet-induced metabolite changes in early *post mortem* pork muscle tissue and during

storage have not been studied so far. Overall, both variations in nutritional and antinutritional composition of growing-finishing pig diets can cause the changes at metabolic level that can affect the sensory traits. Hanczakowska & Świątkiewicz (2014), however, reported that pigs fed a RSM and field beans based diet, replacing SBM resulted in a less tasty, but more tender pork compared with pigs fed a SBM based diet.

The aim of the current research was to address the effect of replacing SBM (142.6 g/kg) with RSM/FB (120 g/kg of RSM and 161.12 g/kg of FB) on growth performance, carcass quality, metabolite status and meat quality in Norwegian crossbred growing-finishing pigs. In addition, sensory attributes were evaluated to understand the effect of diet on flavor and texture development of pork meat.

2. Materials and methods

2.1. Experimental animals and housing

The study was conducted at the Center for Animal Research, Norwegian University of Life Sciences, Ås, Norway and the experimental protocol for the study was approved by the Norwegian Food Safety Authority (ID:8217). All animals were cared for according to laws and regulations controlling experiments with live animals in Norway (the Animal Protection Act of December 20th, 1974, and the Animal Protection Ordinance concerning experiments with animals of January 15th, 1996).

Forty Norwegian crossbred pigs ([Landrace x Yorkshire] x Duroc) from 5 litters with equal distribution of gender were included in the study. The mother line was Norwegian Landrace x Duroc (LY), and the sire line was Duroc (DD). The initial average body weight (BW) was 27.7 ± 2.9 kg and final average BW was 108.7 ± 4.2 kg. The pigs were split in groups of 4 pigs per pen assigned to 2 dietary treatments. Pigs were housed in an environmentally controlled barn with partially slotted concrete floor.

2.2. Feed composition and use

Animals were randomized and allocated to one of two dietary treatments: 1) a control diet based on barley, oat and soybean meal (SBM), and 2) a local diet based on barley, oat, 18% inclusion of commercial expeller pressed rapeseed (Mestilla, UAB, Klaipeda, Lithuania) and 16% inclusion of faba beans (RSM/FB). Level of Standardized ileal digestible (SID) lysine was adjusted to 8.4 g FUp^{-1} by using crystalline amino acids. The diets were formulated to be isonitrogenous, isoenergetic, and to contain equal levels of methionine + cysteine, and threonine. The diets were produced and pelleted to 3-mm diameter at a commercial feed factory (Felleskjøpet Kambo, Moss, Norway). Diets were formulated to meet or exceed the nutritional requirements (NRC, 2012). A cumulative feed sample from each dietary treatment was taken for chemical analysis. Chemical composition of diets was determined as previously described by Skugor et al. (2019) and presented in Table 1. The analyzed fatty acid composition of the two diets is shown in Table 2. All pigs were individually fed twice per day according to a restricted Norwegian feeding scale (Øverland et al., 2000). The pigs within the same dietary treatment received the same diet during the growing and finishing period.

2.3. Slaughter procedure and tissue sampling

All animals were transferred to a commercial slaughterhouse (Nortura Tønsberg, Norway). The last feeding was 2.5 – 3 hours before slaughter. Pigs were anesthetized using CO_2 followed by exsanguination. Five minutes after bleeding, 15 g samples of *longissimus thoracis* from the left side of carcass were excised, trimmed from adipose tissue and placed on dry-ice for subsequent fatty acid and metabolite analysis in *pre rigor* phase. The samples were taken from SBM (N = 20) and RSM/FB (N = 19) carcasses. One carcass was missed on the slaughtering line. The samples were transported on ice to the research laboratory at Norwegian University of Life Sciences, vacuum packed and stored at -80 ℃.

Hot carcass weight was recorded for dressing percentage determination (hot carcass weight/live weight × 100). Carcass lean percentage was determined using a GP7Q pistol (Hennessy System Ltd., Auckland, New Zealand) to measure the depth of *longissimus thoracis* and the back fat thickness at two sites (behind the last rib, 6 cm from the midline). The prediction of carcass lean percentage was done according to Gangsei et al. (2016). Carcasses were then chilled according to standard commercial routines at 1-3°C for 20 h until the core temperature in the ham was 7 °C.

Further, carcass and meat quality assessment was performed 24 hours *post mortem* on thirty-one carcasses (N = 15 from SBM and N = 16 from RSM/FB dietary treatment) that were selected based on dietary treatment, litter and hot carcass weight (65.9 – 74.9 kg) at the Norwegian Meat and Poultry Research Centre. The carcasses were subjected to commercial cutting procedure to determine primal cut yields of backpart, midpart and frontpart (Råvarebok, https://www.totalmarked.nortura.no) to measure percent carcass lean and fat as described by Øverland et al. (2000). Back fat thickness was measured behind the last rib, 6 cm from the midline using a ruler. In addition, *longissimus thoracis* muscle was removed from 31 carcasses for meat quality analysis. Based on similar carcass weight, carcass lean % (GP7) and fat % of the muscle, 24 *longissimus thoracis* muscles (N = 12/dietary treatment) were subjected to metabolite and warmed-over flavor analysis. Samples were stored for 7 days at 4 °C, vacuum packed and kept at -20 °C until analysis. Furthermore, to evaluate the effect of dietary treatment on sensory attributes (flavor and texture) of pork meat, napping test was performed on smaller subset of samples (N = 4/dietary treatment).

2.4. Meat quality measurements

The pH_u at 24 h *post mortem* was measured in *longissimus thoracis* muscle with a portable pH meter equipped with electrode (WTW 82362, pH 330i, Welheim, Germany) suitable for meat penetration.

The color of *longissimus thoracis* muscle prior to packaging (approximately 24 h p*ost mortem*, after 1 h blooming time at 4 °C) was measured using a Minolta CR400 (Minolta Co. Ltd., Osaka, Japan). CIELAB (L*, a*, b*) measurements were done in triplicate.

Drip loss was measured using gravimetric EZ-driploss method (Christensen, 2003) on the cutting day using a slice of 10 g *longissimus thoracis* (20 mm thickness). From the middle of slice along the fibre direction, two pieces were cut using a 25 mm diameter circular knife and placed in vertical fibre direction in the containers. After 24 h at 4 °C, the containers were reweighed to calculate drip loss.

Collagen, fat, protein and water content of meat were analysed using a FOSS FoodScan ™ (Anderson, 2007).

Measurement of thiobarbituric reactive substances (TBARS) was used to determinate the development of warmed-over flavor (WOF). TBARS was performed on defrosted and homogenized (IKA 11 basic Analytical mill, Germany) pork meat preheated to 71 ℃ and chill stored 24 h to accelerate oxidation. Then, 2 g of meat was mixed with 10 mL TBA stock solution (0.38% TBA and 15% TCA in 0.25 N HCl). The mixture was incubated for 10 min in boiling water bath. The tubes were quickly cooled on ice to reach room temperature. Solution (1.5 mL) was transferred to Eppendorf tube and centrifuged for 25 min at 21500 × *g* at 4 ℃. The absorbance was measured at 532 nm using Synergy H4 Hybrid Microplate Reader BioTek (ThermoFisher, Göteborg, Sweden). The calculation of malondialdehyde (MDA) concentration in meat sample was based on extinction coefficient of 1.56×10^5/M/cm. The results were expressed as mg MDA/kg of meat. Each sample was analyzed in duplicate.

2.5. Fatty acid analysis

Muscle samples (N = 31) obtained in *pre rigor* phase were used for fatty acid analysis. These samples were removed from carcasses that were later selected for primal cut and meat quality assessment. Prior to analysis, frozen meat samples were homogenized using laboratory homogenizer (IKA 11 basic Analytical mill, Germany). Samples of 0.25 g was extracted as described by Yi, Haug, Nyquist, & Egelandsdal (2013). Briefly, 1 mL of tridecanoic acid was added to meat samples as an internal standard dissolved in methanol (0.5 mg C13:0/mL MeOH). The samples were dissolved and hydrolysed using 0.56 mL of 10 N KOH in water and 4.2 mL of methanol. The tubes were incubated in water bath at 55 ℃ for 1.5 h with hand shaking for 5 s every 20 min. After cooling, 0.46 mL of 24 N sulphuric acid in water was added, tubes were incubated and cooled. The fatty acid methyl esters (FAME) were separated with 3 mL of hexane by mixing for 5 min and centrifuging at 653 × *g* for 10 min. The hexane layer with FAME was transferred to GC vials and kept at -20 ℃ until analysis. FAME analysis were carried out on Carlo Erba GC 8000 GC instrument equipped with a Carlo Erba EL 980 Automatic Sampler, flame ionization detector (Carlo Erba AS V570 FID, Carlo Erba Instruments, Milano, Italy) and CP 88

capillary column (length: 50 m, i.d.: 0.25 mm, film thickness: 0.20-µm; Varian, Agilent Technologies, Matriks, Norway) as reported by Inglingstad et al. (2017). The FA present in samples were identified and quantified using four standard solutions prepared from a FAME Mix (Supelco 37 component FAME Mix). Finally, the FA concentration was expressed as mg FA/100 g of meat.

2.6. Extraction, derivatization, and GC/MS analysis of meat metabolites

Metabolites were extracted from *pre rigor* muscle (N = 39) and chilled meat samples (N = 24). The extraction was done using 1g of homogenized lean meat with water: methanol: chloroform (1: 2.5: 1) and as internal standard ribitol (66 µg/mL) was added. The mixture was vortexed, incubated at 60 °C for 60 min in sonication bath and centrifuged for 10 min at 1469 × *g*. Finally, 1 mL of supernatant was transferred into a 1.5 mL Eppendorf tube, dried in a SpeedVac (SPD111V-230, Thermo Scientific, Gothenburg, Sweden) at room temperature. The dried samples were dissolved in 80 µL mixture containing methoxyamine hydrochloride in pyridine (20 mg/mL), incubated at 30 °C for 60 min and sonicated at 30 °C for 30 min. In addition, 80 µL of N-methyl-N-(trimethylsilyl) trifluoroacetamide were added and kept at 37 °C for 30 min. The derivatized samples were transferred into glass autosampler vials with insert and aliquot of 1 µL was injected. The analysis was performed on GC−MS (1310-ISQ QD single quadrupole GC−MS instrument from Thermo Fisher) equipped with a capillary column (CP9012 VF-5ms 30 m, ID 0.25 mm and 0.25 µm film thickness with 5m EZ-Guard, Agilent), at 1 mL/min flow rate of helium. The temperature of injector was held at 250 °C. The GC temperature program: 70 °C for 5 min, ramped at 5 °C/min until 310 °C. Analysis time was 60 min. Ions were generated in an electron ionization source by a 70 eV electron beam. The source temperature was 250 °C, and the recorded mass range was *m/z* 50−700. MS files from Chromeleon software (v7.2; Thermo Fisher Scientific) were exported in the netCDF format (Thermo Scientific Xcalibur, v4.1.50) to GC/MS Agilent Translator.

Identification of metabolites was performed in MassHunter Qualitative Analysis (B.07.00, Agilent) using NIST17 (National Institute of Standards and Technology, Gaithersburg, MD, USA) with ≥ 70% mass spectral match. Mass spectra of identified compounds were confirmed using GOLM metabolome database (Max-Planck Institute for Molecular Plant Physiology, Golm,

Germany). Compounds that were not present in at least 50% of samples from one dietary treatment were removed from the data set. The internal standard was used for data quality control and semiquantification of identified metabolites. External alkane standard mix (C_8-C_{20}) was used to calculate retention index (RI) of metabolites. RI of compounds that eluted after C_{20} was extrapolated.

2.7. Sensory profiling

Meat (n = 4/dietary treatment) for sensory testing was defrosted during 2 days at 2 ℃. Whole loins in vacuum bags were heated for 2 h at 70 ℃ in an oven (Rational CombiMaster, mod CM 101) with steam (100% moisture). Thirty min after cooking samples were sliced in 3 × 0.5 cm pieces and served to assessors. Serving temperature was 30 ± 5 ˚C. The sensory test was performed on 4 samples per diet in a standardised sensory laboratory at Nortura (ISO 8589:2007). The recruited assessors (N = 8; 5 males and 3 females, 32-60 years old) were Nortura's employees, semi-trained, but experienced in sensory evaluation (flavor and texture) of pork meat by the partial napping method (Perrin et al., 2007). Prior to testing, all assessors were re-introduced to the napping principles by using additional 4 samples. During training, they were given a set of words to describe the samples. The assessors were asked to place the samples on an A3 paper according to perceived sensory difference, followed by an Ultra-Flash Profiling (UFP) were the samples were described with sensory attributes (Perrin et al., 2008). X and Y coordinates were then recorded for each sample along with the frequencies of each attribute used to describe the samples. In order to measure the degree of reproducibility of the assessors, one replicate sample was included in each test.

2.8. Statistical analyses

Statistical analyses on animal performance and carcass characteristics were performed using the GLM procedure of SAS (1990) for a complete randomized block design with individual pig as the experimental unit. Fatty acid composition (mg/100 g of meat) and TBARS were analyzed using one-way ANOVA (Minitab, version 16 from Minitab Inc., State College, PA, USA) to define the differences between dietary treatments.

The effect of diet and chilled storage on metabolite abundance in pork meat from the two dietary treatments was investigated using one-way ANOVA, multivariate ANOVA (MANOVA), principal component analysis (PCA) and fold change analysis. One-way ANOVA was performed using Minitab 16 (Minitab Inc., State College, PA, USA) to show the effect of diet on metabolite abundance (mg/kg) in *pre rigor* muscle (N = 39 samples) and chilled stored meat (N = 24 samples). To investigate the effect of diet on overall metabolite profile, 50-50 MANOVA (Windows version, Prediktor, 2016) was applied. Further interpretation of metabolomics data from MassHunter Qual was carried out with Mass Profiler Professional 15.0 (Agilent Technologies). The abundance of identified metabolites was log2 transformed. Fold change analysis (threshold: FC > 2.0), applying Benjamini-Hochberg multiple test corrections, and principal component analysis were carried out to monitor the effect of storage on metabolite abundance in both diet groups. For comparison of pork muscle and chilled meat metabolite profiles, the same 24 carcasses were used to avoid individual differences between animals.

Napping analysis was done using the multiple Procrustean factor analysis function of the SensoMineR package (http://sensominer.free.fr/napping-data.html). Napping is a sensory technique used to obtain differences more easily although quantitative data, as in profiling, is sacrificed.

3. Results and discussion

3.1. Growth performance and carcass characteristics

In general, growth performance of pigs fed rapeseed meal and faba bean (RSM/FB) diets during the total period was not significantly (P > 0.05) different from pigs fed the SBM diet, except for a higher feed conversion ratio (P = 0.04) during the finishing period in pigs fed the RSM/FB diet (Table 3). Skugor et al. (2019) reported that the inclusion of 20% commercial expeller pressed RSM in pig diet reduced growth performance of growing-finishing pigs while Hansen et al. (2020) reported only minor effects on growth performance of growing-finishing pig fed increasing levels of RSM when fed a nutritionally balanced diets.

The poorer feed conversion ratio in the finishing period could be related to antinutritive factors present in RSM and FB. Both RSM and FB have a higher fibre content, compared with SBM and

309 they contain several antinutritive factors that might reduce feed utilization. RSM contain
310 glucosinolates and their breakdown products have shown to reduce feed intake and to alter thyroid
311 function by inhibiting production of thyroid hormones (Mejicanos et al., 2016) and consequently
312 affecting metabolism and performance. Phenolic compounds such as tannins in RSM and FB for
313 instance can reduce protein digestibility and interfere with protein metabolism (Jansman et al.,
314 1995) and thus reduce feed conversion ratio.
315
316 ***3.2. Cutting traits and meat quality***
317
318 There was no effect of dietary treatment ($P > 0.05$) on cutting traits of growing-finishing pigs and
319 physio-chemical properties of pork muscle (Table 4). These results are in agreement with previous
320 studies that examined the effect of legume and rapeseed cake in pig diet on carcass and meat
321 quality (Hanczakowska & Swiatkiewicz, 2013; Hanczakowska & Swiatkiewicz, 2014; Partanen et
322 al., 2003).
323 Replacing SBM with RSM/FB in diets for pig resulted in darker meat. Pigs fed RSM/FB had
324 significantly ($P < 0.05$) lower Minolta L* (lightness) and b* (yellowness) values than pigs fed
325 SBM. Reduced L* of pork meat could be associated with slightly lower fat % (Table 4). In
326 addition, lower values for lightness of meat may indicate abundance of deoxymyoglobin (DMb)
327 on the meat surface (Phung, 2012). DMb has a purple-red color immediately after cutting or in
328 vacuum packed meat (Renerre, 1990). Inclusion of RSM in pigs' diet increased haem pigment
329 level, darkness and redness of meat compared to SBM (Dransfield, Nute, Mottram, Rowan, &
330 Lawrence, 1985). Additionally, L* values lower than 48 could be considered as darker meat having
331 better visual scores (Partanen et al., 2003). In addition, the differences found in meat color (L* and
332 b* parameters) might not be detrimental for meat quality.
333 In general, RSM/FB diet reduced ($P = 0.13$) fat % of the *longissimus thoracis* muscle compared
334 with SBM (Table 4). The fatty acid composition of pork muscle from two dietary treatment is
335 given in Table 5. The diet supplemented with RSM/FB significantly reduced ($P < 0.05$) the content
336 of arachidic acid (C20:0) and eicosapentaenoic acid (EPA, C20:5n-3). In addition, a tendency for
337 decrease in linoleic acid (C18:2n-6; $P = 0.07$) content was observed in the muscle when feeding
338 RSM/FB. Skoufos et al. (2016) observed similar results when SBM (10%) was completely
339 replaced with RSM (14.7%) in growing-finishing pigs. In general, with increase of double-bounds

in fatty acids, oxidative stability of meat declines and induce flavor changes (Wood et al., 2008). Therefore, lower PUFA content in RSM/FB pork meat might have reduced susceptibility to lipid oxidation and off-flavor intensity in cooked meat.

In general, complete replacement of SBM with RSM/FB and lower inclusion of tallow in pig's diet, when digestible energy was balanced, had no significant effect on total content of SFA, MUFA and PUFA in the muscle. However, a tendency for increase of PUFA level in intramuscular fat of SBM fed pig is in agreement with Okrouhlá, Stupka, Čítek, Lebedová, & Zadinová (2018), that showed an increase of PUFA in pork back fat.

3.3. The effect of diet on metabolite status of muscle in pre rigor phase and meat after 7 d storage

Metabolite profiles of pork muscle from *pre rigor* phase and pork meat after 7 d chill storage were significantly affected by dietary treatment and storage time. In total, forty-six compounds were quantified in *longissimus thoracis* muscle obtained in *pre rigor* phase and 44 in meat after chilled storage (7 days at 4 °C) from the two dietary treatments (Table 6). "Multivariate analysis" of semi-quantified compounds showed clear separation between *pre rigor* metabolite profile and after chilled storage (Fig. 1). The PCA plot explained 46% of variance, where the first component separated the two sets of samples. Thus, overall metabolite profile of *pre rigor* muscle was significantly (50-50 MANOVA; $P < 0.001$) different compared with 7 d chilled meat.

Dietary treatment had an effect on metabolite status of pigs in growing-finishing phase as measured at the time of slaughter. β-alanine and glucose were enriched ($P = 0.022$ and $P = 0.003$, respectively) in SBM muscle of 39 animals (Fig.2A). To date, the metabolic benefit of β-alanine is attributed to carnosine biosynthesis (Quinn, Boldyrev, & Formazuyk, 1992). Carnosine synthesis in skeletal muscle cell is dependent on the uptake of L-histidine and β-alanine from blood stream into muscle cell (Drozak, Veiga-da-Cunha, Vertommen, Stroobant, & Van Schaftingen, 2010). Moreover, endogenous synthesis of carnosine is catalyzed by carnosine synthetase in skeletal muscle (Artioli, Gualano, Smith, Stout, & Lancha, 2010). Because the affinity of carnosine synthase is greater for L-histidine than for β-alanine, higher concentration of β-alanine is needed for carnosine *in vivo* synthesis (Harris et al., 2006). Therefore, we speculated that elevated concentration of β-alanine in muscle from SBM diet indicates failure of endogenous synthesis of carnosine. In addition, muscle of pigs fed with SBM containing higher content of rendered fat

showed two times higher glucose level in muscle compared with RSM/FB. There are multiple possible reasons for increased glucose level in muscle from SBM fed animals. Glucose storage and uptake, lipid and protein synthesis are regulated by a number of genes that control the insulin signaling pathway (Chang, Chiang, & Saltiel, 2004). Study by Park et al. (2012) demonstrated close relation of lipid metabolism with insulin regulation, the type of dietary fat affected insulin-related gene expression in pork muscle. Higher inclusion of rendered fat in rat diet reduced the efficiency of glucose metabolism due to decreased number of insulin receptors and activity of glucose transport system (Dobbins et al., 2002; Kraegen, James, Storlien, Burleigh, & Chisholm, 1986; Olefsky & Saekow, 1978). In addition, the difference in muscle glucose level between the two dietary treatments in our study may be partly attributed to the antinutrients present in FB. Fernández-Quintela, Barrio, Macarulla, & Martínez (1998) reported a lower glucose concentration in plasma of rats fed with faba beans compared with soybeans and peas. However, carbohydrate metabolism is to a large extent regulated by thyroid hormones (Brenta, 2011). Feeding the growing-finishing pigs with 6-10% of 00-RSM resulted in higher weight of thyroid gland, but normal concentration of thyroid hormones (Svetina, Jerković, Vrabac, & Ćurić, 2003). Depending on the nature and concentration, breakdown products of glucosinolates from RSM may inhibit hormone production by the thyroid gland (Mejicanos, Sanjayan, Kim, & Nyachoti, 2016). Pérez de Nanclares et al. (2017) reported increased weight of thyroid gland in pigs fed with RSM, with total glucosinolates below recommended limit of 2.1 mmol/kg. An increased intake of glucosinolates by castrated boars decreased thyroid hormones in blood having no effect on the growth rate (Spiegel, Bestetti, Rossi, & Blum, 1993). Furthermore, Skugor et al. (2019) showed upregulation of several muscle genes (ODK4, UCP3, ESRRG and ESRRB) involved in glucose metabolism in RSM pigs suggesting increased glucose uptake for energy homeostasis. However, we speculate that RSM/FB dietary treatment with lower amount of tallow has provided less available energy and possibly had an effect on upregulated glucose utilization. In addition, reduced abundance of oxidized metabolites (glycine and pyroglutamic acid) in muscle from RSM/FB group suggested lower oxidative stress in the muscle cell. The observed nominal decrease of glycine ($P = 0.332$) and pyroglutamic acid ($P = 0.058$) provided by RSM/FB could suggest glutathione synthesis efficiency and improved cellular defense system. This is in agreement with Skugor et al. (2019) that reported upregulation of genes involved in antioxidant defense and ROS reduction in the RSM pigs. On the contrary, Chen et al. (2018) found that metabolite markers of oxidative stress

(pyroglutamic acid and butanal) were upregulated in liver of RSM fed pigs. These results indicate that dietary treatment had different effect on muscle and liver metabolite profiles. Regarding meat eating quality the RMS/FB diet may actually have some beneficial effects. However, the present study demonstrates scarce effect of diet on metabolite pathways in the *pre rigor* muscle.

Metabolite profile of meat (n = 24) after chill storage for 7 d was significantly affected by diet. The level of glycine (P = 0.01), glyceric acid (P = 0.04), serine (P = 0.04), creatinine (P = 0.04), glutamine (P = 0.01) and myo-inositol (P = 0.04) increased in meat from animals fed the RSM/FB diet (Fig. 2B). Higher concentration of sugar-related compounds (glyceric acid, myo-inositol) in our study could be related with higher glycogen content and its degradation during optimal storage period (Koutsidis et al., 2008). In addition, accumulation of free amino acids (glycine, serine, glutamine) in the RSM/FB *pre rigor* muscle suggested increased proteolytic activity and hydrolysis of glycoproteins.

3.4 Metabolite profiles after chilled storage

Fold change analysis of identified metabolites showed changes that occurred during conversion of *pre rigor* muscle into meat (n = 24) under optimal storage conditions (Table 6). To the best of our knowledge, it has not been previously reported in the literature the effect of different diets on the abundance of metabolites in *pre rigor* pork muscle vs chill stored meat. The set of 24 metabolites were down-regulated ($P < 0.05$; FC > 2.0) in SBM chill stored meat and 23 were up-regulated compared to *pre rigor* muscle. In RSM/FB, 24 metabolites were down-regulated and 30 up-regulated in chilled meat compared to *pre rigor* muscle. In addition, semi-quantitative data of three metabolites (mannose, palmitic acid, 9,12-octadecadienoic acid) do not coincides with their log-fold-changes presented in Table 6. Thus, fold change analysis indicated differences between *pre rigor* muscle and chilled meat when metabolite abundance was used, but semiquantitation based on internal standard is possibly less precise and do not show clear differences of metabolite concentration. The content of lactic acid and sugar-related compounds (sugar and sugar-phosphates) significantly increased after chill storage. Conversion of muscle into meat and activation of glycolysis has been attributed to increase lactic acid in *pre rigor* phase. Furthermore, the increased lactic acid may be affected by the number of gram-positive bacteria in stored vacuum packed meat (Pothakos, Devlieghere, Villani, Björkroth, & Ercolini, 2015). On the other hand,

chill stored meat exhibited lower levels of glutamate, pyroglutamic acid, and galactose. This finding might reflect enhanced mitochondria related metabolism in muscle cell in early *post mortem* phase (Aguer et al., 2011). Storage had a negative effect on Krebs cycle substrates (*i.e.* fumaric acid, citric acid, malic acid), that may indicate the utilization of substrates to maintain cell function both in *pre rigor* and early *post mortem* phase. Succinic acid, a complex II substrate involved in formation and elimination of reactive oxygen species, increased in chill stored meat possibly due to an incomplete function of the Krebs cycle. Abraham, Dillwith, Mafi, VanOverbeke, & Ramanathan (2017) reported that increased succinic acid indicates either lower mitochondrial consumption or conversion of glutamic acid. In addition, RSM/FB chilled stored meat showed an increase in urea level compared with SBM, probably related to a higher *post mortem* production of ammonia through increased protein breakdown. An increase of free amino acids in RSM/FB chilled stored meat is possibly linked with antioxidants in RSM that assured suppression of reactive oxygen species stimulating prolonged endogenous protease activity in early *post mortem* conditions. As shown in Table 6, generally RSM/FB diet increased flavor precursors independent of thresholds and maintained proteolytic activity, i.e. as previously reported for RSM (Skugor et al., 2019). Metabolite data suggest adaption of RSM/FB muscle to meet essential biological functions and nominally induced transformation to a more oxidative metabolism.

3.5. Sensory properties of pork from two dietary treatments

The multiple factor analysis (MFA) plot maps 8 loin samples on two axes based on the terms used to describe their flavor and texture properties (Fig.3A and B). The plot showed clear separation between SBM and RSM/FB based on flavor attributes given by panelists in the napping session (Fig.3A). The first two dimensions explain 59% of variability between the two groups (41.44% PC1 and 18.04% PC2), after the panelist's tablecloths have been aligned by rotation and scaling. According to panellists, SBM pork was described as tame, with a weak (right amount of) smell, and a round aftertaste. Higher inclusion of rendered fat in SBM diet resulted in a nominally higher fat % that may have an impact on changes in fatty acid composition and possibly intensified round aftertaste note. Frank, Appelqvist, Piyasiri, Wooster, & Delahunty (2011) reported more balanced perception of flavor with increased inclusion of fat in the emulsion system. Moreover, SBM pork

was also described with the attributes liver taste, off taste and chemical/old attributes. The meat samples selected for metabolite analysis were subjected to an accelerated oxidation test (warmed-over flavor; WOF) development. SBM pork produced more WOF (0.34 mg MDA/kg) compared to RSM/FB (0.27 mg MDA/kg) that may reflect nominally higher oxidation of unsaturated fatty acids (i.e. C18:2n-6) that give characteristic aroma volatiles and enhance unpleasant flavor (Ramalingam et al., 2019). However, the oxidative flavor was below flavor threshold (0.5 mg MDA/kg of meat; Wood et al., 2008) indicating that taste-active metabolites were playing a key role in modulation of pork flavor. In general, free amino acids in meat are significant constituents that markedly affect the meat flavor (Ramalingam et al., 2019). RSM/FB pork was located on positive side of the first and second dimension of the MFA plot, described with attributes as fresh, powerful smell, long flavor, meaty flavor, round and sweet taste. Glycine and glutamine were present in a concentration above flavor threshold (see Table 6) while the other metabolites that increased during storage may also contribute to the flavor profile of RSM/FB meat. Bitter-related amino acids (valine, isoleucine, leucine and phenylalanine) were present at higher concentrations in meat from RSM/FB together with ornithine that might had a bitterness-suppression effect. The concentration of sweet-related glycine, serine, threonine, and sugar related compounds increased with storage and generally contributed to sweet taste of pork meat from RSM/FB. Moreover, these amino acids and sugars are important flavor-precursors in the development of meaty, roasty, and boiled flavor properties when meat is strongly heated (barbequed). Napping test showed large texture variations (Fig.3B) between the samples from RSM/FB group and metabolite analysis higher concentration of free amino acids indicating possible inconsistent proteolytic activity. The SBM samples clustered more clearly together.

4. Conclusion

Overall, feeding a local diet containing rapeseed meal and faba beans, when replacing soybean meal in pig diets gave no differences in growth rate, feed intake or carcass traits. Feeding the local diet improved color of pork meat and increased the concentration of free amino acids, and sweet tasting metabolites, leading to reduced warmed-over flavor and flavor attributes. The results, thus, show desirable properties of feeding rapeseed meal and faba beans on pork meat.

Conflict of interest

All authors declare that they have no conflict of interest.

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References

Abraham, A., Dillwith, J. W., Mafi, G. G., VanOverbeke, D. L., & Ramanathan, R. (2017). Metabolite profile differences between beef longissimus and psoas muscles during display. *Meat and Muscle Biology, 1*, 18-27.

Aguer, C., Gambarotta, D., Mailloux, R. J., Moffat, C., Dent, R., McPherson, R., & Harper, M. E. (2011). Galactose enhances oxidative metabolism and reveals mitochondrial dysfunction in human primary muscle cells. *PLOS ONE, 6*(12), 1-11.

Anderson S. (2007). Determination of fat, moisture, and protein in meat and meat products by using the FOSS FoodScan near-infrared spectrophotometer with FOSS artificial neural network calibration model and associated database: collaborative study. *Journal of AOAC International*, 90, 1073–1083.

Artioli, G. G., Gualano, B., Smith, A., Stout, J., & Lancha, A. H. J. (2010). Role of β-Alanine supplementation on muscle carnosine and exercise performance. *Medicine & Science in Sports & Exercise, 42*(6), 1162-1173.

Budavari, S. (1996). *The Merck Index.* (12th Ed.). Whitehouse Station, NJ, Merck & Co.

Brenta, G. (2011). Why can insulin resistance be a natural consequence of thyroid dysfunction? *Journal of Thyroid Research, 2011*, 1-9.

Brittain, H. G., Bogdanowich, S. J., Bugay, D. E., DeVincentis, J., Lewen, G., Newman, A. W. (1991). Lactose, Anhydrous. In K. Florey (Ed.). *Analytical profiles of drug substances* (pp. 369-398). Academic Press.

Burdock, G.A. (2005). *Fenaroli's Handbook of Flavor Ingredients*. (5th ed.). New York: Taylor & Francis

Burdock, A. G. (2016). *Fenaroli's handbook of flavor ingredients*. (6th ed.). New York: Taylor & Francis.

Chang, L., Chiang, S.-H., & Saltiel, A. R. (2004). Insulin signaling and the regulation of glucose transport. *Molecular medicine (Cambridge, Mass.), 10*(7-12), 65-71.

Chen, C., Pérez de Nanclares, M., Kurtz, J. F., Trudeau, M. P., Wang, L., Yao, D., Saqui-Salces, M., Urriola, P. E., Mydland, L. T., Shurson, G. C., & Overland, M. (2018). Identification of redox imbalance as a prominent metabolic response elicited by rapeseed feeding in swine metabolome1. *Journal of Animal Science, 96*(5), 1757-1768.

Christensen, L. B. (2003). Drip loss sampling in porcine m. longissimus dorsi. *Meat Science, 63*(4), 469–477.

Dobbins, R. L., Szczepaniak, L. S., Myhill, J., Tamura, Y., Uchino, H., Giacca, A., & McGarry, J. D. (2002). The composition of dietary fat directly influences glucose-stimulated insulin secretion in rats. *Diabetes, 51*(6), 1825-1833.

Dransfield, E., Nute, G. R., Mottram , D. S., Rowan, T. G., & Lawrence, T. L. J. (1985). Pork quality from pigs fed on low glucosinate rapeseed meal: Influence of level in the diet, sex and ultimate pH. *Journal of the Science of Food and Agriculture, 36*, 546-556.

Drauz, K., Grayson, I., Kleemann, A., Krimmer, H. P., Leuchtenberger, W., & Weckbecker C. (2007). *Amino acids, ullmann's encyclopedia of industrial chemistry*. (6th ed.) New York: John Wiley & Sons.

Drozak, J., Veiga-da-Cunha, M., Vertommen, D., Stroobant, V., & Van Schaftingen, E. (2010). Molecular identification of carnosine synthase as ATP-grasp domain-containing protein 1 (ATPGD1). *The Journal of biological chemistry, 285*(13), 9346-9356.

Dunkel, A., & Hofmann, T. (2009). Sensory-directed identification of β-alanyl dipeptides as contributors to the thick-sour and white-meaty orosensation induced by chicken broth. *Journal of Agricultural and Food Chemistry, 57*, 9867-9877.

Engan, S. (1974). Organoleptic threshold values of some organic acids in beer. *Journal of the Institute of Brewing, 80*, 162-163.

Esko, T., Hirschhorn, J. N., Feldman, H. A., Hsu, Y.-H. H., Deik, A. A., Clish, C. B., Ebbeling, C. B., & Ludwig, D. S. (2017). Metabolomic profiles as reliable biomarkers of dietary composition. *The American Journal of Clinical Nutrition, 105*(3), 547–554.

Faurion, A., & Vergneau, L. (1992). (cited by: A. Faurion, Le goût sucré, Sucre et Nutrition, DOIN, Paris (1992) 53-69). Unpublished results.

Fernández-Quintela, A., Barrio, A. S. d., Macarulla, M. T., & Martínez, J. A. (1998). Nutritional evaluation and metabolic effects in rats of protein isolates obtained from seeds of three legume species. *Journal of the Science of Food and Agriculture, 78*(2), 251-260.

Florou-Paneri, P., Christaki, E., Giannenas, I., Bonos, E., Skoufos, I., Tsinas, A., Tzora, A., & Peng, J. (2014). Alternative protein sources to soybean meal in pig diets. *Journal of Food, Agriculture & Environment 12*(2), 655-660.

Frank, D., Appelqvist, I., Piyasiri, U., Wooster, T. J., & Delahunty, C. (2011). Proton transfer reaction mass spectrometry and time intensity perceptual measurement of flavor release from lipid emulsions using trained human subjects. *Journal of Agricultural and Food Chemistry, 59*(9), 4891-4903.

Gangsei, L. E., Kongsro, J., Olsen, E. V., Røe, M., Alvseike, O., & Sæbø, S. (2016). Prediction precision for lean meat percentage in Norwegian pig carcasses using 'Hennessy grading probe 7': Evaluation of methods emphasized at exploiting additional information from computed tomography, *Acta Agriculturae Scandinavica, Section A — Animal Science 66(1)*, 17-24.

Gardner, W. H. (1977). Acidulants in food processing. In T. E. Furia (Ed.). *Handbook of food additives* (pp 225–87). Cleveland, Ohio: CRC Press.

Grabež, V., Bjelanović, M., Rohloff, J., Martinović, A., Berg, P., Tomović, V., Rogić, B., & Egelandsdal, B. (2019). The relationship between volatile compounds, metabolites and sensory attributes: a case study using lamb and sheep meat. *Small Ruminant Research, 181,* 12-20.

Graham, C. S., Graham, B. G., Bartlett, J. A., Heald, A. E., & Schiffman, S. S. (1995). Taste and smell losses in HIV infected patients. *Physiology & Behavior, 58,* 287-293.

Grosch, W., & Laskawy, G. (1984). Untersuchungen über die beteiligung von linolsäure am bittergeschmack von mohnsamen (Papaver somniferum). *Zeitschrift für Lebensmitteluntersuchung und -Forschung A, 178,* 257-259.

Haefeli, R., & Glaser, D. (1990). Taste response and thresholds obtained with the primary amino acids in humans. *Lebensmittel-Wissenschaft und -Technologie, 23,* 523-527.

Hahn, H. & Ulbrich, L. (1948). Eine systematische untersuchung der geschmacksschwellen. *Pflüger's Archiv für die gesamte Physiologie des Menschen und der Tiere. 250,* 357-384.

Hahn, H., Kuckulies, G., & Taeger, H. (1938). Eine systematische untersuchung der geschmacksschwellen. *I. Mitteilung. Z. Sinnesphysiol., 67,* 259-306.

Hanczakowska, E., & Swiatkiewicz, M. (2013). Legume seeds and rapeseed press cake as substitutes for soybean meal in sow and piglet feed. *Agricultural and Food Science, 22*(4), 435-444.

Hanczakowska, E., & Świątkiewicz, M. (2014). Legume seeds and rapeseed press cake as replacers of soybean meal in feed for fattening pigs. *Annals of Animal Science, 14*(4), 921-934.

Hansen, J. Ø., Øverland, M., Skrede, A., Anderson, D. M., & Collins, S. A. (2020). A meta-analysis of the effects of dietary canola / double low rapeseed meal on growth performance of weanling and growing-finishing pigs. *Animal Feed Science and Technology, 259,*114302.

Harris, R. C., Tallon, M. J., Dunnett, M., Boobis, L., Coakley, J., Kim, H. J., Fallowfield, J. L., Hill, C. A., Sale, C., & Wise, J. A. (2006). The absorption of orally supplied β-alanine and its effect on muscle carnosine synthesis in human vastus lateralis. *Amino Acids, 30*(3), 279-289.

Harrison, G. A. F., & Collins, E. (1968). Determination of taste thresholds for a wide range of volatile and nonvolatile compounds in beer. *Proceedings American Society of Brewing*, 83-87.

Haseleu, G., Lubian, E., Courter, C., Mueller, & Chapter, S. (2014). Quantitative mapping of taste-active compounds. In V. Ferreira & R. Lopez (Eds.), *Flavour Science* (pp. 539-544). Academic Press.

Hidaka, I., Zeng, C., & Kohbara, J. (1992). Gustatory responses to organic acids in the yellowtail *Seriola quinqneradiata*. *Nippon suisan gakkaishi, 58*(6), 1179-1187.

Hofmann, T., Soldo, T., Ottinger, H., Frank, O., Robert, F., & Blank, I. (2005). Structural and functional characterization of a multimodal taste enhancer in beef bouillon. In C. Frey & R.L. Rouseff (Eds.), Natural flavors and fragrances (pp. 173-188). ACS Symposium Series, 908.

http://www.thegoodscentscompany.com

https://www.vcf-online.nl/VcfCompounds.cfm

Hufnagel, J. C., & Hofmann, T. (2008). Quantitative reconstruction of the nonvolatile sensometabolome of a red wine. *Journal of Agricultural and Food Chemistry, 56*, 9190-9199.

Inglingstad, R. A., Skeie, S., Vegarud, G. E., Devold, T. G., Chilliard, Y., & Eknæs, M. (2017). Feeding a concentrate rich in rapeseed oil improves fatty acid composition and flavor in Norwegian goat milk. *Journal of Dairy Science, 100*(9), 7088-7105.

Ishibashi, N., Ono, I., Kato, K., Shigenaga, T., Shinoda, I., Okai, H., & Fukui, S. (1988). Role of the hydrophobic amino acid residue in the bitterness of peptides. *Agricultural and Biological Chemistry, 52*, 91-94.

Jansman, A. J. M., Verstegen, M. W. A., Huisman, J., van den Berg, J. W. O. (1995). Effects of hulls of faba beans (Vicia faba L.) with a low or high content of condensed tannins on the apparent ileal and fecal digestibility of nutrients and the excretion of endogenous protein in ileal digesta and feces of pigs. *Journal of Animal Science, 73*(1), 118–127.

Jezierny, D., Mosenthin, R., & Bauer, E. (2010). The use of grain legumes as a protein source in pig nutrition: A review. *Animal Feed Science and Technology, 157*(3), 111-128.

Kawai, M., Sekine-Hayakawa, Y., Okiyama, A., & Ninomiya, Y. (2012). Gustatory sensation of l- and d-amino acids in humans. *Amino Acids, 43*(6), 2349–2358.

Kirk-Othmer (1992-1998). *Kirk-Othmer Encyclopedia of Chemical Technology*. (4th ed). New York: Wiley Interscience.

Koutsidis, G., Elmore, J. S., Oruna-Concha, M. J., Campo, M. M., Wood, J. D., & Mottram, D. S. (2008). Water-soluble precursors of beef flavour: I. Effect of diet and breed. *Meat Science, 79*(1), 124-130.

Kraegen, E. W., James, D. E., Storlien, L. H., Burleigh, K. M., & Chisholm, D. J. (1986). In vivo insulin resistance in individual peripheral tissues of the high fat fed rat: assessment by euglycaemic clamp plus deoxyglucose administration. *Diabetologia, 29*(3), 192-198.

Kuchiba-Manabe, M., Matoba, T., & Hasegawa, K. (1991). Sensory changes in umami taste of inosine 5'-monophosphate solution after heating. *Journal of Food Science, 56*, 1429-1432.

Lécuyer, L., Dalle, C., Micheau, Pétéra, M., Centeno, D., Lyan, B., Lagree, M., Galan, P., Hercberg, S., Rossary, A., Demidem, A., Vasson, M.-P., Partula, V., Deschasaux, M., Srour, B., Latino-Martel, P., Druesne-Pecollo, N., Kesse-Guyot, E., Durand, S., Pujos-Guillot, E., Manach, C., & Touvier, M. (2020). Untargeted plasma metabolomic profiles associated with overall diet in women from the SU.VI.MAX cohort. *European Journal of Nutrition*.

Ledahudec, J., & Pokorný, J. (1991). Effect of free fatty acids on the flavour of frying oil. *Nahrung, 35*, 1071-1075.

Lewis, R. J. (1997). *Hawley's Condensed Chemical Dictionary*. (13th ed.). New York: John Wiley & Sons, Inc.

Li, Y., Li, J., Zhang, L., Yu, C., Lin, M., Gao, F., Zhou, G., Zhang, Y., Fan, Y., & Nuldnali, L. (2015). Effects of dietary energy sources on post mortem glycolysis, meat quality and muscle fibre type transformation of finishing pigs. *PLOS ONE, 10*(6), 1-16.

Li, Y., Yu, C., Li, J., Zhang, L., Gao, F., & Zhou, G. (2017). Effects of dietary energy sources on early postmortem muscle metabolism of finishing pigs. *Asian-Australas Journal of Animal Science, 30*(12), 1764-1772.

Mejicanos, G., Sanjayan, N., Kim, I. H., & Nyachoti, C. M. (2016). Recent advances in canola meal utilization in swine nutrition. *Journal of animal science and technology, 58*, 1-13.

Meilgaard, M. C. (1982). Prediction of flavor differences between beers from their chemical composition. *Journal of Agricultural and Food Chemistry, 30*, 1009-1017.

Mosciano's Flavor Library, https://www.perfumerflavorist.com/flavor/library/

Nagai, M., Saitoh, J., Ohno, H., Hitomi, C., & Wada, M. (2006). Pentavalent vanadium at concentration of the underground water level enhances the sweet taste of glucose in college students. *BioMetals, 19*, 7-12.

NRC, National Research Council. (2012). *Nutrient Requirements of Swine*. Eleventh Revised Edition. National Academic Press, Washington, D.C. 20418 USA.

Okrouhlá, M., Stupka, R., Čítek, J., Lebedová, N., & Zadinová, K. (2018). Effect of duration of dietary rapeseed and soybean oil feeding on physical characteristics, fatty acid profile, and oxidative stability of pig backfat. *Animals, 8*(11), 1-11.

Olefsky, J. M., & Saekow, M. (1978). The effects of dietary carbohydrate content on insulin binding and glucose metabolism by isolated rat adipocytes. *Endocrinology, 103*(6), 2252-2263.

Øverland, M., Granli, T., Kjos, N. P., Fjetland, O., Steien, S. H., & Stokstad, M. (2000). Effect of dietary formates on growth performance, carcass traits, sensory quality, intestinal microflora, and stomach alterations in growing-finishing pigs1. *Journal of Animal Science, 78*(7), 1875-1884.

Pangborn, R. M. (1963). Relative taste intensities of selected sugars and organic acids. Journal of Food Science, 28, 726-733.

Park, J. C., Kim, S. C., Lee, S. D., Jang, H. C., Kim, N. K., Lee, S. H., Jung, H. J., Kim, I. C., Seong, H. H., & Choi, B. H. (2012). Effects of dietary fat types on growth performance, pork quality, and gene expression in growing-finishing pigs. *Asian-Australasian journal of animal sciences, 25*(12), 1759-1767.

Partanen, K., Alaviuhkola, T., Siljander-Rasi, H., & Suomi, K. (2003). Faba beans in diets for growing-finishing pigs. *Agricultural and food science in Finland, 12*, 35-47.

Pérez de Nanclares, M., Trudeau, M. P., Hansen, J. Ø., Mydland, L. T., Urriola, P. E., Shurson, G. C., Piercey Åkesson, C., Kjos, N. P., Arntzen, M. Ø., & Øverland, M. (2017). High-fiber rapeseed co-product diet for Norwegian Landrace pigs: Effect on digestibility. *Livestock Science, 203*, 1-9.

Perrin, L., Symoneaux, R., Maître, I., Asselin, C., Jourjon, F., & Pagès, J. (2007). Comparison between a free profiling carried out by wine professionals and a conventional profiling. *American Journal of Enology and Viticulture, 58*, 508–517.

Perrin, L., Symoneaux, R., Maître, I., Asselin, C., Jourjon, F., & Pagès, J. (2008). Comparison of three sensory methods for use with the Napping® procedure: Case of ten wines from Loire valley. *Food Quality and Preference, 19*(1), 1-11.

Phung, V. T. (2012). *Mitochondrial oxygen consumption and myoglobin redox stability in beef.* PhD Thesis, University of Life Sciences, Ås, Norway.

Pothakos, V., Devlieghere, F., Villani, F., Björkroth, J., & Ercolini, D. (2015). Lactic acid bacteria and their controversial role in fresh meat spoilage. *Meat Science, 109*, 66-74.

Prediktor. (2016). 50 : 50 MANOVA.

Quinn, P. J., Boldyrev, A. A., & Formazuyk, V. E. (1992). Carnosine: Its properties, functions and potential therapeutic applications. *Molecular Aspects of Medicine, 13*(5), 379-444.

Ramachandran, S., Fontanille, P., Pandey, A., & Larroche, C. (2006). Gluconic Acid: properties, applications and microbial production. *Food Technolology and Biotechnology, 44*(2), 85–195.

Ramalingam, V., Song, Z., & Hwang, I. (2019). The potential role of secondary metabolites in modulating the flavor and taste of the meat. *Food Research International, 122*, 174-182.

Råvarebok (in Norwegian). Available at: https://www.totalmarked.nortura.no.

Renerre, M. (1990). Factors involved in the discoloration of beef meat. *International Journal of Food Science & Technology, 25*(6), 613-630.

Rothe, M. (1963). Zur definition der begriffe aroma und aromastoff. *Ernährungsforschung, 7*, 639-646;

Rothe, M., Wölm, G., Tunger, L., Siebert, H. J. (1972). Schwellenkonzentrationen von aromastoffen und ihre nutzung zur auswertung von aromaanalysen, *Nahrung, 16*, 483-495.

Rudrum, M., & Shaw, D. (1965). The structure and conformation of some monosaccharides in solution. *Journal of the Chemical Society*, 52–57.

Scharbert, S., & Hofmann, T. (2005). Molecular definition of black tea taste by means of quantitative studies, taste reconstitution, and omission experiments. *Journal of Agricultural and Food Chemistry, 53*, 5377-5384.

Schiffman, S. S., Hornack, K., & Reilly, D. (1979). Increased taste thresholds of amino acids with age. *The American Journal of Clinical Nutrition, 32*, 1622-1627.

738 Schiffman, S., Sennewald, K., & Gagnon, J. (1981). Comparison of taste qualities and thresholds
739 of D- and L-amino acids. *Physiology & Behavior, 27*(1), 51-59.
740 SensoMineR package. Available at: http://sensominer.free.fr/napping-data.html.
741 Skoufos, I., Tzora, A., Giannenas, I., Bonos, E., Papagiannis, N., Tsinas, A., Christaki, E., &
742 Florou-Paneri, P. (2016). Dietary inclusion of rapeseed meal as soybean meal substitute
743 on growth performance, gut microbiota, oxidative stability and fatty acid profile in
744 growing-fattening pigs. *Asian Journal of Animal and Veterinary Advances, 11*(2), 89-97.
745 Skugor, A., Kjos, N. P., Sundaram, A. Y. M., Mydland, L. T., Ånestad, R., Tauson, A.-H., &
746 Øverland, M. (2019). Effects of long-term feeding of rapeseed meal on skeletal muscle
747 transcriptome, production efficiency and meat quality traits in Norwegian Landrace
748 growing-finishing pigs. *PLOS ONE, 14*(8), 1-27.
749 Sobotka, W., Pomianowski, J. F., & Wójcik, A. (2012). Wpływ zastosowania genetycznie
750 zmodyfikowanej poekstrakcyjnej śruty sojowej oraz poekstrakcyjnej śruty rzepakowej
751 „00" na efekty tuczu, właściwości technologiczne i sensoryczne mięsa świń. *Żywność:*
752 *nauka - technologia - jakość, 1*(80), 106-115.
753 Sonntag, T., Kunert, C., Dunkel, A., & Hofmann, T. (2010). Sensory-guided identification of N-
754 (1-methyl-4-oxoimidazolidin-2-ylidene)-α-amino acids as contributors to the thick-sour
755 and mouth-drying orosensation of stewed beef juice. *Journal of Agricultural and Food*
756 *Chemistry, 58*, 6341-6350.
757 Spiegel, C., Bestetti, G., Rossi, G., & Blum, J. W. (1993). Feeding of rapeseed presscake meal to
758 pigs: Effects on thyroid morphology and function and on thyroid hormone blood levels,
759 on liver and on growth performance. *Journal of Veterinary Medicine Series A, 40*(1-10),
760 45-57.
761 Stark, T., Bareuther, S., & Hofmann, T. (2006). Molecular definition of the taste of roasted cocoa
762 nibs (Theobroma cacao) by means of quantitative studies and sensory experiments.
763 *Journal of Agricultural and Food Chemistry,* 54, 5530-5539.
764 Stevens, J.C. (1997). Detection of very complex taste mixtures: generous integration across
765 constituent compounds. *Physiology & Behavior, 62*, 1137-1143.
766 Svetina, A., Jerković, I., Vrabac, L., & Ćurić, S. (2003). Thyroid function, metabolic indices and
767 growth performance in pigs fed 00-rapeseed meal. *Acta Veterinaria Hungarica, 51*(3),
768 283-295.

Tanimura, S., & Mattes, R. D. (1993). Relationship between bitter taste sensitivity and consumption of bitter substances. *Journal of Sensory Studies, 8*, 31-41.

van Zanten, H., Bikker, P., Mollenhorst, H., Meerburg, B., & De Boer, I. (2015). Environmental impact of replacing soybean meal with rapeseed meal in diets of finishing pigs. *Animal, 9*(11), 1866-1874.

Watkins, P. J., Frank, D., Singh, T. K., Young, O. A., & Warner, R. D. (2013). Sheepmeat flavor and the effect of different feeding systems: A Review. *Journal of Agricultural and Food Chemistry, 61*(15), 3561-3579.

Woo, A. H., & Lindsay, R. C. (1983). Statistical correlation of quantitative flavor intensity assessments and individual free fatty acid measurements for routine detection and prediction of hydrolytic rancidity off-flavors in butter. *Journal of Food Science, 48*, 1761-1766.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., & Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science, 78*(4), 343-358.

Yi, G., Haug, A., Nyquist, N. F., & Egelandsdal, B. (2013). Hydroperoxide formation in different lean meats. *Food Chemistry, 141*(3), 2656-2665.

Yimdee, T., & Wang, X. C. (2016). Comparison of odor and taste of commercial brand fish sauces from East and South East Asian countries. *International Journal of Food Properties, 19*(4), 873-896.

Yoshida, M., Ninomiya, T., Ikeda, S., Yamaguchi, S., Yoshikawa, T., & Ohara, M. (1966). Studies on the taste of amino acids. Part I. Determination of threshold values of various amino acids. *Journal of the Agricultural Chemical Society of Japan, 40*, 295-299.

Figure Captions

Fig. 1. PCA scores plot of metabolites ($P < 0.05$; FC > 2.0) identified in *pre rigor* muscle (n =24) and chilled meat at 4 ℃ during 7 d (n = 24) from SBM and RSM/FB dietary treatments.

Fig. 2. Replacement of SBM with RSM/FB showed significant ($P < 0.05$) effect on metabolite profile of *longissimus thoracis* muscle. A) metabolites isolated from *pre rigor* muscle (N = 20 from SBM; N = 19 from RSM/FB). B) metabolites isolated from chilled meat (n = 12/ diet). Orange bars for SBM and green bars for RSM/FB group. The significant difference detected by ANOVA ($P < 0.05$; Tukey's test) is marked with *.

Fig. 3. Partial napping evaluation of SBM and RSM/FB pork meat by semi-trained panellists. A) Flavor individual factor map. B) Texture individual factor map. C) Attributes used for at least 2 samples/diet to describe the flavor of pork meat.

Table 1.

Ingredient and chemical composition (g/kg) of Control diet based on soybean meal (SBM) and Local diet produced from rapeseed meal/ faba bean (RSM/FB) produced in Norway and Europe

Ingredients	Diet	
	SBM	RSM/FB
Barley	585.4	456.0
Oats	150.0	150.0
Soybean meal (SBM) (45% CP)	142.6	0.0
Rapeseed meal (RSM) (Mestilla)	60.0	180.0
Faba beans (Columbo)	0.0	161.12
Rendered fat[a]	23.0	16.5
Molasses	10.0	10.0
Limestone	9.7	8.7
Monocalcium phosphate	3.2	1.4
Salt	6.31	6.37
L-lysine HCl (98%)	3.25	3.04
L-threonine	1.28	1.39
L-methionine	0.98	0.97
L-tryptophan	0.07	0.31
Axtra phytase 5000T	0.02	0.02
Vitamin E (50%)	0.15	0.15
Premix[b]	3.87	3.86
Calculated contents		
Net energy, MJ/kg (FUn/kg)	9.42 (1.07)	9.42 (1.07)
SID[c] lysine, g/kg	8.99	8.99
SID methionine + cysteine, g/kg	5.56	5.57
SID threonine, g/kg	5.93	5.93
SID tryptophan, g/kg	1.75	1.75
Calcium, g/kg	6.65	6.54
ATTD[d] phosphorus, g/kg	4.46	4.76
Analyzed content, g/kg		
DM	868	870
Crude protein	153	158
Crude fat	40	51
Starch	424	422
Neutral detergent fibre (NDF)	165	173
Ash	45	44
Gross energy, MJ/kg	16.4	16.6

[a] In Norway this fat source often consists of 65-70% lard and 30-35% tallow, with possible variations with the season

[b] Provided the following amounts per kg of diet: Zn 72 mg, Fe 96 mg, Mn 48 mg, Cu 17 mg, I 20.48 mg, Se 0.27 mg, Vitamin A 6500 IU, Cholecalciferol 1500 IU, dl-α-tocopheryl acetate 75 mg, menadione 4.63 mg, Riboflavin 5.625

816 mg, D-pantothenic acid 15 mg, Cyanocobalamine 15 μg, Niacin 45 mg, Biotin 0.30 mg, Folic acid 1.69 mg, Choline
817 2300 mg (SBM) and 1605 mg (RSM/FB).
818 [c] Standardized ileal digestible.
819 [d] Apparent total tract digestible.

820 **Table 2.**

821 Fatty acid composition of experimental diets

Fatty acids (mg/kg)	Diet	
	SBM	RSM/FB
C12:0	17.94	13.17
C14:0	213.07	146.29
C15:0	37.94	29.11
C16:0	3142.33	2569.65
C17:0	69.24	49.58
C18:0	1302.24	931.21
C20:0	50.14	20.32
C21:0	9.25	9.64
C22:0	23.92	28.98
C24:0	21.73	16.53
C14:1n-3	16.98	12.27
C16:1n-7	186.35	146.61
C17:1n-7	4.27	5.05
C18:1n-9t	197.38	136.46
C18:1n-9	4777.36	5602.44
C20:1n-9	455.83	606.98
C22:1n-9	72.92	74.03
C24:1n-9	8.60	16.17
C18:2n-6t	19.42	12.16
C18:2n-6	4579.01	4846.05
C18:3n-6	3.39	3.01
C18:3n-3	300.28	399.85
C20:2n-6	32.02	25.58
C20:3n-3	4.81	4.89
C20:4n-6	12.16	8.68
SFA (g/100 g)	4.89	3.81
MUFA (g/100 g)	5.71	6.58
PUFA (g/100 g)	4.96	5.32

822 SBM = soybean meal; RSM/FB = rapeseed meal and faba beans

823

Table 3.

The effect of SBM and RSM/FB diets on growth performance and carcass characteristics

Item	Diet		SEM	P-value
	SBM (n = 20)	RSM/FB (n = 19)		
Initial weight, kg	27.69	27.82	0.66	0.89
Final weight, kg	108.93	108.53	1.03	0.78
Growing period				
ADG[a], kg	0.90	0.93	0.02	0.13
ADFI[b], kg	1.55	1.61	0.02	0.08
F:G[c], kg/kg	1.73	1.72	0.02	0.87
Finishing period				
ADG, kg	1.30	1.24	0.02	0.09
ADFI, kg	3.02	3.03	0.04	0.82
F:G, kg/kg	2.33	2.44	0.04	0.04
Overall period				
ADG, kg	1.09	1.08	0.01	0.71
ADFI, kg	2.24	2.27	0.02	0.25
F:G, kg/kg	2.06	2.12	0.02	0.12
Hot carcass weight, kg	70.39	70.31	0.69	0.94
Dressing percentage	64.62	64.79	0.40	0.76
Percentage carcass lean, %	59.75	59.91	0.47	0.80

[a] ADG: daily weight gain.

[b]ADFI: average daily feed intake.

[c] F:G: feed conversion ratio.

Table 4.

The effect of SBM and RSM/FB diets on cutting traits and physical characteristics of meat

Item	Diet		SEM	P-value
	SBM (n = 15)	RSM/FB (n = 16)		
Carcass characteristics				
Hot carcass weight, kg	70.41	70.59	0.68	0.85
Dressing percentage, %	64.57	65.04	0.49	0.50
Lean percentage, GP7	59.49	60.19	0.50	0.35
Backfat thickness, mm	13.05	12.14	0.63	0.32
Carcass lean, primal cuts, %	61.91	61.61	0.45	0.63
Frontpart, %	31.24	31.32	0.24	0.82
Midtpart, %	34.87	34.47	0.27	0.30
Backpart, %	33.88	34.21	0.21	0.28
Meat characteristics				
Fat, %	1.81	1.66	0.07	0.13
Protein, %	23.47	22.88	0.44	0.34
Collagen, %	0.36	0.35	0.01	0.65
Moisture, %	74.39	74.39	0.10	0.98
Drip loss, %	5.85	5.40	0.36	0.38
pH_u	5.41	5.45	0.02	0.10
Meat color				
Minolta L*	49.26	47.93	0.39	0.02
Minolta a*	7.26	7.11	0.15	0.47
Minolta b*	2.63	1.84	0.18	0.004

Table 5.

Fatty acid composition of *longissimus thoracis* muscle from pigs fed with SBM and RSM/FB.

	Fatty acids	SBM	RSM/FB	SEM[a]	P-values[b]
		(n = 15)	(n = 16)		
SFA (mg/100 g)	C12:0	9.8	7.9	0.84	0.13
	C14:0	144.3	110.0	15.26	0.12
	C16:0	2570.3	2009.6	257.91	0.13
	C17:0	33.2	25.3	3.12	0.08
	C18:0	1530.9	1135.7	156.87	0.08
	C20:0	18.2	11.9	1.67	0.01
MUFA (mg/100 g)	C16:1n-7	248.0	211.3	26.38	0.33
	C17:1n-7	44.1	42.1	1.71	0.42
	C18:1n-9t	30.2	24.2	3.08	0.18
	C18:1n-9	3884.6	3117.1	393.73	0.18
	C20:1n-9	35.0	26.9	3.45	0.10
	C22:1n-9	1.2	1.1	0.13	0.46
PUFA (mg/100 g)	C18:2n-6	1182.2	945.5	90.38	0.07
	C18:3n-6	8.2	7.4	0.39	0.17
	C18:3n-3	29.4	22.6	2.89	0.10
	C20:2n-6	35.8	28.2	3.37	0.12
	C20:3n-6	21.0	19.0	1.07	0.19
	C20:3n-3	6.8	5.2	0.67	0.09
	C20:4n-6	153.9	144.8	6.78	0.35
	C20:5n-3	14.7	12.6	0.69	0.03
	C22:6n-3	12.8	13.3	0.71	0.62
Total (g/100 g)	SFA[c]	4.32	3.31	0.44	0.11
	MUFA[d]	4.25	3.43	0.43	0.18
	PUFA[e]	1.46	1.20	0.10	0.08
	n-6 PUFA	1.40	1.14	0.01	0.08
	n-3 PUFA	0.06	0.05	0.004	0.09
	n-6/n-3[f]	22.01	21.22	0.55	0.32

[a] SEM = standard error of mean

[b] Probability of significant effect due to type of diet

[c] SFA = C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0

[d] MUFA = C14:1 cis 9+C16:1 cis 9+C17:1 cis 9+C18:1 trans 9+C18:1 cis 9+C20:1 cis 11+ C22:1 cis13

[e] PUFA = C18:2 cis 9,12+C18:3 cis 6,9,12+C18:3 cis 9,12,15+C20:2 cis 11,14+C20:3 cis 8,11,14+C20:3 cis 11,14,17+C20:4 cis 5,8,11,14+C20:5 cis 5,8,11,14,17+C22:6 cis 4,7,10,13,16,19 cis

[f] n-6 : n-3 = n-6 PUFA : n-3 PUFA

842 **Table 6.**

843 The semi-quantified metabolites (mg/kg) in *pre rigor* (n = 24) and 7 d chill stored (n = 24) *longissimus thoracis* pork muscle from

844 animals fed with SBM and RSM/FB

RI	RT	Items	SBM			RSM/FB			Flavor threshold in water (mg L^{-1}) / fat-oil (mg kg^{-1})	Odor / Taste
			Pre-rigor [a]	7 d [b]	Fold change 7 d vs pre-rigor [c]	Pre-rigor	7 d	Fold change 7 d vs pre-rigor		
		Amino acids								
1098.13	11.67	alanine	26.98	12.87		14.75	16.64	↑	600[1] / 135[2]	sweet[2]
1205.87	15.19	valine	1.81	1.11		1.16	1.24	↑	188[3]/ n.a.	sweet, bitter[4]
1246.3	17.47	isoleucine	0.44	0.32		0.29	0.43	↑	900[1]/ n.a.	bitter[5]
1248.14	17.59	proline	1.73	0.31		1.00	0.43	↓	> 1,000[6]	characteristic odor with slight sweet taste[7]
1352.02	17.81	glycine	61.36	17.37	↓	43.18	31.21	↓	3.9 – 35.67[8] / n.a.	odorless[2]/oily, bitter with a fatty, nutty aftertaste, slightly sweet[9]
1378.62	19.32	serine	2.13	0.66	↓	1.70	1.02	↓	0.2[10]/ n.a.	sweet, umami, sour[11]
1390.42	19.99	threonine	3.53	1.12	↓	1.87	1.52	↓	1250[12]/ n.a.	sweet[13]
1423.18	21.13	β-alanine	16.12	19.33	↑	6.85	22.32	↑	107[14]/ n.a.	slight sweet taste[15]
1615.66	25.88	glutamic acid	10.53	1.11	↓	6.72	1.50		9.2[16]/ n.a.	high strength, roasted type[17] /umami, sour[17]
1621.35	26.01	phenylalanine	0.91	0.66	↓	0.27	0.67	↑	530[18]/ n.a.	slight bitter taste[15]
1769.18	29.31	glutamine	35.94	1.85	↓	23.07	5.66	↓	0.14[10]/ n.a.	sweet, umami[11]
1809.23	30.17	ornithine	n.a.	0.38	↑	n.a.	0.42	↑	460[5] / n.a.	sweet[5]
1913.76	32.32	lysine	3.17	1.04	↓	1.37	1.53		104-327[19]/n.a.	odorless[15] / bitter[14]
1932.05	32.68	tyrosine	0.20	0.23		0.11	0.26	↑	725[5]/ n.a.	odorless/bland taste[15]
		Acids								
1058.83	10.41	lactic acid	57.00	69.13	↑	68.37	78.13	↑	10[20]	odourless, sour[21]
1074.42	10.91	glycolic acid	n.a.	0.09	↑	n.a.	0.09	↑	> 2000[22]/n.a.	odourless, very mild buttery[21]
1157.88	13.54	3-hydroxybutyric acid	0.12	0.36		0.13	0.48	↑	n.a. / n.a.	n.a.
1212.56	15.57	4-hydroxybutanoic acid	0.08	n.a.	↓	0.13	n.a.	↓	n.a.	n.a.
1357.13	18.10	succinic acid	1.82	8.76	↑	1.07	10.85	↑	200[23]	odourless, sour[21]
1363.11	18.44	glyceric acid	n.a.	1.44	↑	n.a.	2.26	↑	10.6[24] / n.a.	n.a.
1387.07	19.80	fumaric acid	0.37	n.a.	↓	0.25	n.a.	↓	n.a.	odourless[21]
1483.86	22.69	malic acid	11.14	1.63	↓	10.03	3.46	↓	9.6-99[25]/ n.a.	nearly odorless, sometimes a faint, acrid[15]/tart, acidic, nonpungent[15]
1497.47	23.04	aspartic acid	5.36	n.a.	↓	0.70	n.a.	↓	30[26] /n.a.	n.a / umami [26]
1497.86	23.05	2-aminomalonic acid	0.37	n.a.	↓	0.09	n.a.	↓	n.a.	n.a.
1973.48	31.50	citric acid	0.99	n.a.	↓	0.60	n.a.	↓	n.a.	n.a. / tart, delivers a “burst” of tartness [27]
1983.85	33.70	gluconic acid	n.a.	0.41	↑	n.a.	0.53	↑	75[28] / n.a.	refreshing sour[29], bitter, metallic[28]
		Alcohols								
1798.48	30.05	9H-purin-6-ol	0.79	1.21	↑	0.85	1.72	↑	n.a.	n.a.
1901.57	32.08	sorbitol	0.41	15.31	↑	0.33	13.16	↑	n.a.	odourless, sweet, caramel[21]

2112.80	34.27	scyllo-inositol	0.67	0.09	↓	0.42	0.16	↓	n.a.	n.a.
2172.73	35.45	*myo*-inositol	40.48	11.30		27.92	17.94	↓	3190[30]/ n.a.	sweet[30]
		Sugars								
1858.99	31.20	fructose	0.32	6.39	↑	0.24	7.22	↑	160[31] / n.a.	sweet[32]
1873.96	31.51	mannose	3.80	8.77	↑	4.85	4.42	↑	n.a.	sweet with a bitter aftertaste[33]
1884.11	31.72	glucose	70.87	59.73		30.42	73.81	↑	700 – 1000[34] / n.a.	sweet[35]
1969.63	33.42	glucopyranose	n.a.	1.42	↑	n.a.	2.29	↑	n.a.	n.a.
1976.74	33.56	galactose	7.48	n.a.	↓	6.60	n.a.	↓	n.a.	n.a.
2688.22	45.60	maltose	n.a.	1.42	↑	n.a.	2.29	↑	15103[6]/ n.a.	sweet [22]
2699.90	45.83	lactose	0.15	5.41	↑	3.89	5.20	↑	n.a.	sweet [36]
		Phosphates								
2365.72	39.25	fructose-6-phosphate	n.a.	25.86	↑	n.a.	25.89	↑	570[13] / n.a.	Sweet[13]
2375.88	39.45	mannose-6-phosphate	3.54	34.23	↑	1.72	40.15	↑	25[28]/ n.a.	sweet, metallic tomatoes[28]
2436.31	40.64	glucose-6-phosphate	4.21	25.90	↑	0.27	34.66	↑	360[13] / n.a.	sweet[13]
		Lipids								
2143.27	34.87	palmitic acid	6.56	6.57	↓	5.81	5.90		n.a./ 10 000[38]	rancid, wax odor[37], waxy, creamy fatty, soapy with a crisco like fatty, lard and tallow like mouth feel and a dairy nuance[39]
2295.63	37.87	octadecadienoic acid, 9,12-(*Z*,*Z*)-(18:2)	1.06	1.06	↑	1.71	0.90	↓	n.a. / 4000[40]	bitter[41]
2322.04	38.39	stearic acid	3.73	4.14		4.98	3.68	↓	n.a./15 000[38]	odourless, mild, fatty[21]
2390.60	39.74	oleic acid	0.06	n.a.	↓	0.88	n.a.	↓	n.a.	n.a.
		Other N-containing compounds								
842.48	4.35	2-butanedioic acid (Z)-, dimethyl ester	0.94	n.a.	↓	0.82	n.a.	↓	n.a.	n.a.
1115.99	12.23	hydroxylamine	0.03	n.a.	↓	0.04	n.a.	↓	n.a.	n.a.
1174.51	14.06	phosphoric acid, monomethyl ester	n.a.	5.46	↑	n.a.	7.58	↑	n.a.	n.a.
1230.53	16.59	urea	19.08	11.32		9.85	14.31	↑	0.19 – 6610[42]/ n.a.	almost odorless[43]/ cooling, saline taste[44]
1481.52	22.63	niacin	n.a.	1.74	↑	n.a.	2.25	↑	n.a.	n.a.
1517.17	23.52	niacinamide	1.67	n.a.	↓	1.31	n.a.	↓	n.a.	odorless [21]/ n.a.
1518.82	23.56	pyroglutamic acid	41.55	6.04	↓	23.12	9.13	↓	n.a.	n.a.
1547.79	24.26	creatinine	16.94	4.62	↓	19.77	9.84	↓	n.a.	bitter[45]
1752.60	28.95	phosphoric acid, 2,3-propyl ester	n.a.	0.09	↑	n.a.	0.10	↑	n.a.	n.a.
1836.28	30.73	phosphorylethanolamine	0.05	n.a.	↓	0.07	n.a.	↓	n.a.	n.a.
2163.08	35.26	panthotenic acid	0.18	n.a.	↓	0.10	n.a.	↓	n.a.	n.a.
2591.21	43.69	inosine	10.61	47.86	↑	5.68	48.19	↑	60-250[46] / n.a.	bitter[47]
2936.57	50.49	adenosine-5-phosphate	n.a.	29.33	↑	n.a.	27.73	↑	n.a.	n.a.

845

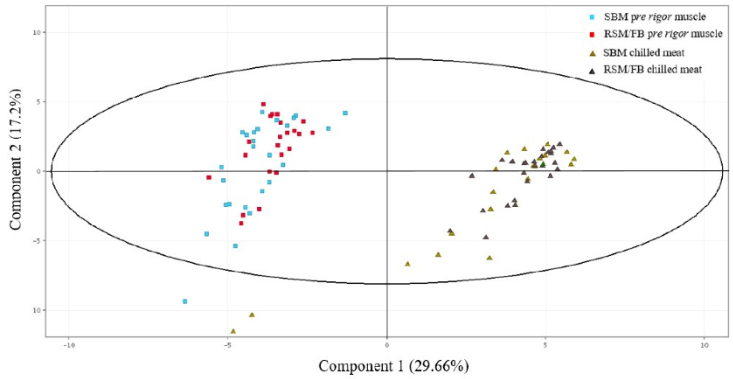
[a] Significantly different ($P < 0.05$) metabolites between SBM and RSM/FB in *pre rigor* muscle are presented in Fig.2A.

[b] Significantly different ($P < 0.05$) metabolites between SBM and RSM/FB in 7 days chilled meat are presented in Fig.2B.

[c] Fold changes of metabolites ($P < 0.05$; $FC > 2.0$) between *pre rigor* muscle and 7 days chilled meat.

[1]Yoshida et al. (1966); [2]Hofmann et al. (2005); [3]Haefeli et al. (1990); [4]Ishibashi et al. (1988); [5]Scharbert et al. (2005); [6]Harrison et al. (1968); [7]Burdock (2016); [8]Hahn et al. (1948); [9]Drauz et al. (2007); [10]Schiffman et al. (1981); [11]Kawai et al. (2012); [12]Faurion et al. (1992); [13]Sonntag et al. (2010); [14]Stark et al. (2006); [15]Burdock (2005); [16]Graham et al. (1995); [17]Kirk-Othmer Encyclopedia of Chem. Technology (1992); [18]Tanimura et al. (1993); [19]Schiffman et al. (1979); [20]Rothe et al. (1972); [21]http://www.thegoodscentscompany.com; [22]Meilgaard (1982); [23]Engan (1974); [24]Hidaka et al. (1992); [25]Stevens (1997); [26]Yimdee et al. (2016); [27]Gardner (1977); [28]Grabež et al. (2019); [29]Ramachandran et al. (2006); [30]Hufnagel et al. (2008); [31]Pangborn (1963); [32]Watkins et al. (2013); [33]Rudrum et al. (1965); [34]Rothe et al. (1963); [35]Nagai et al. (2006); [36]Brittain et al. (1991); [37]https://www.vcf-online.nl/VcfCompounds.cfm; [38]Woo et al. (1983); [39]Mosciano et al. (2001); [40]Ledahudec et al. (1991); [41]Grosch et al. (1984); [42]Hahn et al. (1938); [43]Lewis (1997); [44]Budavari (1996); [45]Haseleu et al. (2014); [46]Kuchiba-Manabe et al. (1991); [47]Dunkel et al. (2009).

Fig.1.


SBM *pre rigor* muscle
RSM/FB *pre rigor* muscle
SBM chilled meat
RSM/FB chilled meat
Component 2 (17.2%)
Component 1 (29.66%)

858
859

Fig.2A

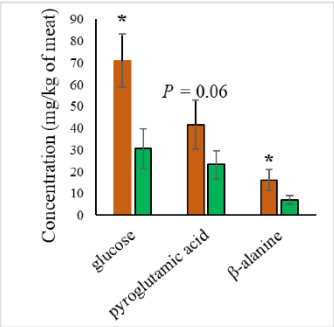


Fig.2B

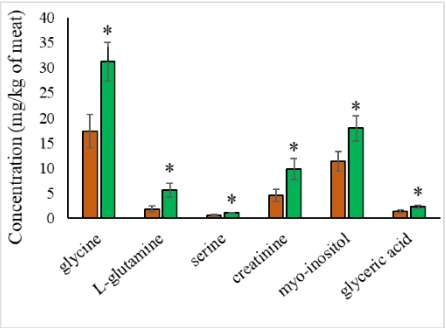


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Fig.3A

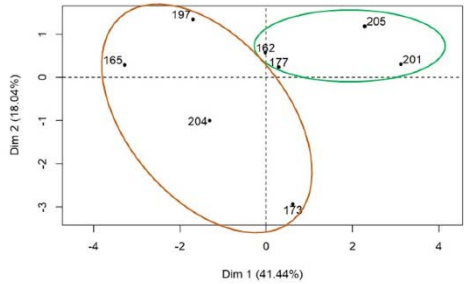
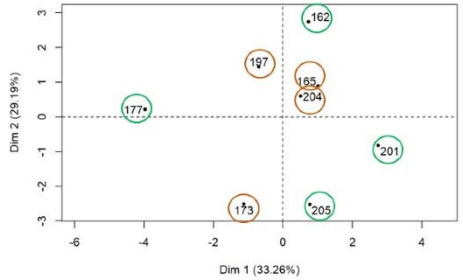


Fig.3B



862