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Research Article

Dietary Inclusion of Rapeseed Meal as Soybean Meal Substitute on Growth Performance, Gut Microbiota, Oxidative Stability and Fatty Acid Profile in Growing-Fattening Pigs

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Abstract

Due to the variability of feed cost and in order to maintain the competitiveness of pork products, the pig farmers of the European Union try to maximize the use of locally produced feeds, such as rapeseed meal which is a by-product of the oil and biofuel industries. Dietary rapeseed meal of Greek origin was evaluated as alternative for imported soybean meal on the performance, meat quality parameters and gut microbiota of growing-fattening pigs. A total of 120 pigs were allocated to two equal groups for a period of 90 days. The pigs of the control group (C) were fed with commercial soybean meal based growing and fattening rations. The pigs of the second group were fed with isocaloric and isonitrogenous rapeseed meal based rations. Body weight gain did not differ ($p>0.05$) during the growing and the fattening periods. Feed conversion ratio did not differ ($p>0.05$) during the growing period but was higher ($p\leq0.05$) for group R during the fattening period. Some differences ($p\leq0.05$) were found in the meat chemical composition (moisture, crude protein, crude fat) between the two groups. In the steak cuts, group R had higher ($p\leq0.05$) total monounsaturated fatty acids and lower ($p\leq0.05$) saturated fatty acids and polyunsaturated fatty acids, compared to group C. No differences ($p>0.05$) were found on the ham and steak meat lipid oxidative stability after 4 days or 7 days of refrigerated storage 4°C. Group R had higher ($p\leq0.05$) *Lactobacillus* spp., in the caecum and lower ($p\leq0.05$), *Clostridium perfringens* in the mid-colon.

Key words: Performance, meat quality, meat chemical composition, meat lipid oxidation, gut microbiota

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The pig industry in Europe relies heavily on imported protein feedstuffs and Mainly Soybean Meal (SBM). According to De Visser *et al*. (2014) SBM constitutes the 64% of the protein-rich feeds for livestock in the European countries, while the self-sufficiency is not more than 3%. Furthermore, Van Gelder *et al*. (2008) underlined the dependency of Europe in SBM since the amount of this feed used per unit of pork meat produced is 648 g kg^{-1}.

Soybean meal (SBM) is the major by-product of the oil extraction from soybeans. The SBM is often used as the primary protein feed material in pig nutrition due to the high protein content (43-53%) and the balanced amino acid profile (high amounts of lysine, tryptophane, threonine and isoleucine) (McDonald *et al*., 2011; De Visser *et al*., 2014) while, the apparent ileal digestibility of lysine was 84%, the highest of other oilseed feeds (canola meal, sunflower meal, linseed meal, etc.). However, in SBM sulfur amino acid levels are suboptimal for pig diets, so methionine supplementation will be necessary (McDonald *et al*., 2011). Nevertheless, SBM is a poor source of vitamins, while about 60-70% of its phosphorus content is bound to phytic acid, which reduces its availability, making necessary the inclusion of inorganic phosphorus or phytase (McDonald *et al*., 2011). The SBM contains oligosaccharides such as raffinose and stachyose, which cannot be digested by monogastric animals due to the lack of specific gastric enzymes (Parsons *et al*., 2000).

Soybean meal (SBM) cost and availability may change rapidly, because it has a strong relationship with fluctuation as an agricultural commodity in the global market (Florou-Paneri *et al*., 2014). Accordingly, due to the variability of feed cost, the pig producers in the European Union maximize the use of locally produced feeds, obtained from oil-based protein crops to reduce feeding cost in order to maintain the competitiveness of pork products (Trostle, 2008; Torres-Pitarch *et al*., 2014). Recently there is increased focus on home-grown protein crops and by-products like rapeseed and rapeseed meal (RSM), which induce less impact on the environment than those imported like SBM, particularly in relation to the long transport distances and the increasing demands of land use change (Dalgaard *et al*., 2008; Lehuger *et al*., 2009; Meul *et al*., 2012). This reduced impact can include reduced carbon dioxide and other gas emissions, limited use of fertilizers and less modification of the native flora and fauna. Therefore, it must be reported that pig-feeding reflects the increasing concern by the European Union about the vulnerability of current over-reliance on imported GM soybeans and the by-products for animal nutrition (De Visser *et al*., 2014). Thus there is a need to increase locally-produced meals, obtained from protein and oil crops, like RSM as a alternative proteinaceous feed for pig diets (Florou-Paneri *et al*., 2014).

The RSM is the by-product remaining from the oil industry and recently from biofuel production (Lehuger *et al*., 2009). Rapeseed is the world's third leading source of oil yielding crops and the second leading source of protein meal (Lehuger *et al*., 2009; De Visser *et al*., 2014). Major producing areas include the European Union, Canada, USA, China, India and Australia (McDonald *et al*., 2011; Lomascolo *et al*., 2012). The RSM contains on average 31-38% crude protein, 10-12% crude fibre, 1-2% lipids, 6-8% ash, less than 1% calcium and 1.2% total phosphorus (Okrouhla *et al*., 2012; Florou-Paneri *et al*., 2014; Choi *et al*., 2015). The RSM compared to SBM has a higher content of sulfur amino acids and more available phosphorus and calcium which can reduce the cost of minerals in the feed (Okrouhla *et al*., 2012). Rapeseeds include some amounts of anti-nutritional agents such as glucosinolates (goitogenic), erucic acid (toxic), tannins and sinapine (phenols) (Okrouhla *et al*., 2012; Florou-Paneri *et al*., 2014). Due to considerable progress in plant breeding modern varieties of rapeseeds have different nutrition profiles, in addition to less glucosinates and erucic acid. This genetically modified rapeseed is often referred to as "00 rapeseed" (double zero RSM) or canola (Bell, 1993; Stanacev *et al*., 2013; Torres-Pitarch *et al*., 2014).

The present study aimed to assess the effect of dietary inclusion RSM of Greek origin as an alternative for SBM on the performance and the gut microbiota of growing-finishing pigs as well as the oxidative stability and the fatty acid profile of their meat.

MATERIALS AND METHODS

The experimental procedures described in this research were according to the principles of the Greek Directorate General of Veterinary Services for the care of animals in experimentation.

Animals and housing: A total of 120 pigs ([large white× landrace]×duroc) 3 months old were selected from a commercial pig herd (VIKI, S.A., Arta, Greece). They were randomly allocated to two equal groups with 4 replications of 15 pigs each. The whole experiment lasted 90 days and was divided into two periods: growing (1-30 days) and fattening (31-90 days). The pigs were housed in pens with slatted floor. The environmental conditions were controlled according to the age of the animals and access to feed and drinking water was *ad libitum* at all times.

Table 1: Ingredients and chemical composition of diets of pigs for the growing and fattening periods

	Growing period (0-30 days)		Fattening period (31-90 days)	
Parameters	C	R	C	R
Ingredients (g kg^{-1})				
Wheat	372.8	315.8	426.5	383.5
Barley	370	370.0	360	360.0
Wheat bran	100	100.0	120	120.0
Soybean meal	110	-	65	-
Rapeseed meal	-	167.0	-	106.0
Hemoglobin	10	10.0	-	-
Animal fat	5	10.0	-	4.0
Calcium carbonate	11	6.0	12	10.0
Dicalcium phosphate	4.1	4.1	1.5	1.5
Salt	6.1	6.1	6	6.0
Lysine	4.1	4.1	4.4	4.4
Methionine	0.2	0.2	-	-
Threonine	1.3	1.3	1.1	1.1
Thryptophan	1.9	1.9	-	-
Vitamin and mineral premix[1]	3.0	3.0	3.0	3.0
Enzyme premix[2]	0.5	0.5	0.5	0.5
Chemical analysis (g kg^{-1})				
Dry matter	880.0	881.0	880.0	882.0
Net energy (Mj kg^{-1})	9.5	6.5	9.6	9.6
Crude protein	160.0	160.0	143.0	143.0
Crude fat	23.7	29.0	19.3	23.1
Crude fiber	42.7	54.6	42.6	50.2
Ash	45.1	41.3	41.9	40.0
Lysine	10.5	10.5	8.8	8.8
Methionine	2.4	2.4	2.1	2.2
Methionine+Cystine	5.3	5.5	4.8	5.0
Threonine	6.6	6.7	5.5	5.5
Thryptophan	2.1	2.1	1.7	1.7
Ca	6.0	6.0	5.5	5.5
P (total)	4.9	4.9	4.6	4.6
Mg	1.7	1.7	1.7	1.7
Na	2.7	2.7	2.6	2.6
Cl	4.5	4.5	4.4	4.4

[1]Provided per kg of feed: Vitamin A: 10,000 IU, Vitamin D_3: 2,000 IU, Vitamin E: 60 mg, Vitamin B_1: 2 mg, Vitamin B_2: 7 mg, Vitamin B_6: 2.5 mg, Vitamin B_{12}: 30 μg, Vitamin K_3: 2 mg, Nicotinic acid: 20 mg, Pantothenic acid: 25 mg, Folic acid: 1.5 mg, Choline: 150 mg kg^{-1}, Zn: 100 mg, Mn: 30 mg, Fe: 40 mg, Cu: 60 mg, I: 0.2 mg, Se: 0.02 mg, [2]Provided per kg of feed: 0.05 g Phytase, 0.10 g Xylanase, 0.10 g Glucanase, Ca: Calcium, P: Phosphorus, Mg: Magnesium, Na: Sodium, Cl: Chlorine, C: Soybean meal, R: Rapeseed meal

Table 2: Chemical composition of soybean meal and rapeseed meal

Chemical composition (g kg^{-1})	SBM	RSM
Dry matter	87.0	90.0
Crude protein	46.0	35.5
Crude fat	0.6	2.3
Crude fiber	6.0	14.5

SBM: Soybean meal, RSM: Rapeseed meal

Diets and performance measurements: Two experimental diets were formulated for each period. The diets for the control group (C) did not contain RSM but contained SBM at 11 and 10% during the growing and fattening periods, respectively. In the diets for the second group (R), RSM was included at 16.7 and 10.6%, completely replacing SBM. The diets for each period were formulated to be isoenergetic for Net Energy (NE) and isonitrogenous (Novus, 1992; AOAC., 2005; NRC., 2012), by modifying the inclusion of cereals (Table 1). Amino acid content for lysine, methionine, threonine and tryptophan as well as calcium and phosphorus content was also equalized for both diets. Analysis of major constituents of SBM and RSM is presented in Table 2. Total glucosinolates and erucic acid content of RSM were 35 μmol g^{-1} and 6 mg g^{-1}, respectively, according to the supplier (VIKI, S.A., Arta, Greece).

Pigs were individually weighed at the 1st, 30th and 90th day of the experimental period. In addition, food consumption was measured daily.

Slaughter and carcass quality measurements: At the end of the experiment all pigs were slaughtered at a commercial slaughter house. Representative samples (shoulder, pancetta, ham and steak) were collected from 4 animals per replicate and placed at -20°C pending analysis.

The chemical composition of the meat samples (moisture, crude protein and crude fat) were measured using a NITT (Near Infrared Transmittance) method. The stored samples were thawed at 4°C overnight and then portions of 200 g were separated, minced (Cutter K35, Electrolux) and placed in the device tray of a FoodScan™ Lab (FOSS, Denmark). For the steak the eye part i.e., longissimus dorsi muscle was used. For the leg parts, hams were cut, the biceps femoris muscles were removed and then all intermuscular fat and external connective tissue (perimysium) were trimmed. For the shoulder, the supraspinatus and the Infraspinatus muscles were used. For the pancetta, the rectus abdominis was removed and then all intermuscular fat and external connective tissue (perimysium) were trimmed.

The fatty acid composition of the steak and shoulder cuts was determined by gas chromatography. Fatty acids methyl esters were obtained from the frozen samples using the protocol described by O'Fallon *et al.* (2007). Then, the separation and quantification of the methyl esters was carried out with a gas chromatographic system (TraceGC model K07332, ThermoFinnigan, ThermoQuest, Milan, Italy) equipped with a flame ionization detector, a model CSW 1.7 chromatography station (CSW, DataApex Ltd., Prague, Czech Republic) and a fused silica capillary column, 30 m×0.25 mm i.d., coated with cyanopropyl polysiloxane (phase type SP-2380) with a film thickness of 0.20 μm (Supelco, Bellefonte, PA, USA). The chromatographic conditions were; carrier: N_2, flow: 1 mL min^{-1}, oven: temperature 70°C for 0.5 min, increase 30°C min^{-1} to 180°C for 10 min, increase 5°C min^{-1} to 225°C

for 15 min, Inlet temperature: 250°C, detector temperature: 250°C, injection: 1 μL with split 1/ 20. Fatty acid methylesters retention times and elusion order were identified using reference standards of the Supelco 'F.A.M.E Mix C8-C24' (C.N. 18918-1AMP), the Supelco '37 Component FAME Mix' (47885-U), the Supelco 'Linoleic acid methyl ester cis/trans isomers' (4-7791) and the Sigma 'Tridecanoic acid' (T0502-5G) as well as accompanying Supelco reference material for the procedure. Fatty acids were quantified by peak area measurement and the results are expressed as percentage (%) of the total peak areas for all quantified acids.

For the determination of the lipid oxidation of the samples, the previously frozen samples were thawed overnight at 4°C, minced using a commercial food processor, wrapped in oxygen-permeable film and placed in a nonilluminated refrigerated cabinet at 4°C for a total of 7 days. On the 4th and the 7th refrigeration days, from each sample, subsamples were taken and processed using the method described by Florou-Paneri *et al*. (2005). Absorbance was read at 532 nm against a blank sample using an UV-VIS spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Japan) 1,1,3,3 tetraethoxypropane was used as standard and results were expressed as ng of malondialdehyde (MDA) per g of sample.

Microbiological analysis: To determine bacteria populations, during slaughter fresh digesta samples from jejunum, mid colon and caecum were collected from 3 animals per replication. These samples were weighted and then mixed homogeneously at a ratio of 1 g sample with 9 mL of peptone water (0.1% v/v) in the universal bottle for bacterial enumeration such as total aerobes, total anaerobes, *Lactobacilli* spp. and total coliforms by conventional microbiological techniques using selective agar media (Barrow and Feltham, 1993). Subsequently, serial decimal dilutions were made, avoiding aeration. Aerobes were enumerated using plate count agar; the inoculated plates were incubated aerobically for up to 48 h at 37°C. Anaerobes were enumerated by using plate count agar; the inoculated plates were incubated anaerobically (in jar) for up to 48 h. For the determination of *Lactobacillus* spp., the samples plated onto de Man Rogosa Sharpe (MRS) agar and incubated under anaerobic conditions at 37°C for 48 h. *Bifidobacterium* spp., were anaerobically assayed using Reinforced Clostridial Agar (RCA). *Enterococcus* spp., were enumerated using Slanetz and bartley agar (aerobial incubation at 37 °C for 48 h). *Clostridium perfringens* enumeration was based on tryptone sulfite agar with cyclocerine. For the detection and enumeration of Enterobacteriaceae Vilet Red Bile Glucose (VRBG) agar was used. Samples incubated under aerobic conditions at 37°C for 24 h on MacConkey agar for the determination of total coliform numbers. These processes were repeated twice and the results were expressed as Colony Forming Unit (CFU) per gram of sample (CFU g^{-1}).

Statistical analysis: For the statistical analysis the IBM SPSS Statistics 20 statistical package (SPSS Inc., Chigaco, IL, USA) was used. In every case the individual replication (cage) was regarded as the experimental unit. For the one-way analysis of variance (ANOVA) the groups were examined as fixed factors. Moreover, the homogeneity of the measurements was examined with Levene's test (Levene, 1960).

RESULTS

The effects of dietary SBM and RSM on the performance parameters are given in Table 3. Body Weight Gain (BWG) did not differ significantly ($p>0.05$) between the groups during the growing and the finishing periods. Feed Conversion Ratio (FCR) did not differ ($p>0.05$) during the growing period but was significantly ($p\leq0.05$) poorer for the group R during the finishing period, compared to the group C. No deaths were recorded and accordingly mortality was zero for both groups.

Table 4 presents the results of dietary SBM and RSM on the chemical composition of pork shoulder, pancetta, ham and steak. In the shoulder cut significantly ($p\leq0.01$) lower crude fat was measured in group R compared to group C but no differences were found for the other parameters (moisture and crude protein). In the pancetta cut, group R had significantly higher ($p\leq0.001$) crude fat and lower ($p\leq0.05$) crude protein and moisture content, compared to group C. In the ham cut group R had significantly ($p\leq0.001$) lower crude protein and significantly higher ($p\leq0.001$) crude fat content, compared to group C. In the steak cut, group R had significantly ($p\leq0.01$) higher moisture and crude protein and significantly ($p\leq0.01$) lower crude fat content compared to group C.

Table 3: Effect of dietary soybean meal and rapeseed meal on performance parameters of growing-fattening pigs

Groups	Growing period (1-30 days)	Fattening period (31-90 days)
Body weight gain (kg±SD)		
C	13.9±0.9	46.1±3.0
R	12.9±1.3	43.4±2.1
p-value	ns	ns
Feed conversion ratio (Mean±SD)		
C	02.674±0.174	3.141±0.208[a]
R	02.887±0.298	3.601±0.171[b]
p-value	ns	≤0.05

C: Soybean meal, R: Rapeseed meal, Column values with different superscripts differ significantly ($p\leq0.05$), ns: Not significant

Dietary SBM and RSM effects on the fatty acid composition of pork steak and shoulder meat are given in Table 5. In the steak cut group R had significantly ($p \leq 0.05$) higher C16:1 (palmitoleic), C18:1n-9c (oleic) and total monounsaturated fatty acids (MUFA), whereas, lower($p \leq 0.05$) C17:0 (heptadecanoic), C18:2n-6c (linoleic), C18:3n-3 (alpha-inolenic), total Saturated Fatty Acids (SFA) and total polyunsaturated fatty acids (PUFA), compared to the group C. Furthermore, in the shoulder cut group R had significantly lower C18:3n-3 (alpha-linolenic) and C20:2 (eicosadienoic) fatty acids compared to the SBM group but no significant ($p > 0.05$) differences were noticed for the total SFA, MUFA and PUFA.

Table 4: Effect of dietary soybean meal and rapeseed meal on the chemical composition of pork meat cuts

Groups	Moisture	Crude protein	Crude fat
Shoulder (Percentage±SD)			
C	72.8±0.7	21.3±0.3	8.1±0.1[a]
R	72.8±0.5	21.4±1.1	7.2±0.2[b]
p-value	ns	ns	≤0.01
Pancetta (Percentage±SD)			
C	58.2±0.3[a]	15.3±0.1[a]	25.4±0.2[a]
R	57.7±0.1[b]	15.2±0.1[b]	26.2±0.1[b]
p-value	≤0.05	≤0.05	≤0.001
Ham (Percentage±SD)			
C	72.7±1.3	21.5±0.3[a]	3.3±0.3[a]
R	70.6±0.3	20.6±0.6[b]	4.2±0.3[b]
p-value	ns	≤0.001	≤0.001
Steak (Percentage±SD)			
C	69.9±0.3[a]	18.5±0.3[a]	5.1±0.1[a]
R	71.3±0.1[b]	19.1±0.5[b]	4.4±0.3[b]
p-value	≤0.01	≤0.01	≤0.01

C: Soybean meal, R: Rapeseed meal, Column values with different superscripts differ significantly ($p \leq 0.05$), ns: Not significant

Table 6 presents the effect of dietary SBM and RSM on the pork ham and steak meat lipid oxidative stability (4°C). No significant ($p > 0.05$) differences were noticed in the measured MDA after 4 days or 7 days of refrigerated storage at 4°C.

The effects of dietary SBM and RSM on the gut microflora are shown in Table 7. In the jejunum, total coliform were significantly ($p \leq 0.05$) higher and total aerobes were significantly ($p \leq 0.05$) lower for group R, compared to group C. In the caecum, the group R had significantly ($p \leq 0.05$) higher total anaerobes and *Lactobacillus* spp. and lower ($p \leq 0.05$)

Table 5: Effect of dietary soybean meal and rapeseed meal on the fatty acid profile of pork steak and shoulder meat cuts (total fatty acid percentage)

		Steak					Shoulder				
		C		R			C		R		
Fatty acids	Common names	%	±SD	%	±SD	p-value	%	±SD	%	±SD	p-value
C12:0	Lauric	0.072	±0.005	0.070	±0.014	ns	0.073	±0.011	0.079	±0.002	ns
C14:0	Myristic	1.147	±0.040	1.006	±0.116	ns	1.098	±0.132	1.052	±0.120	ns
C14:1	Myristoleic	0.018	±0.004	0.017	±0.005	ns	0.019	±0.007	0.016	±0.005	ns
C16:0	Palmitic	22.399	±0.936	20.775	±1.502	ns	21.383	±1.172	22.100	±1.448	ns
C16:1	Palmitoleic	2.636[a]	±0.079	3.180[b]	±0.228	≤0.01	2.576	±0.141	2.838	±0.314	ns
C17:0	Heptadecanoic	0.317[a]	±0.047	0.231[b]	±0.012	≤0.05	0.281	±0.048	0.245	±0.036	ns
C17:1	Heptadecenoic	0.257	±0.029	0.252	±0.104	ns	0.233	±0.026	0.185	±0.031	ns
C18:0	Stearic	13.306	±1.363	12.294	±0.317	ns	11.811	±1.149	12.505	±0.611	ns
C18:1n-9t	Trans-Oleic	0.242	±0.111	0.307	±0.206	ns	0.273	±0.073	0.264	±0.070	ns
C18:1n-9c	Cis-Oleic	40.693[a]	±0.609	45.555[b]	±1.253	≤0.001	41.200	±1.688	40.914	±2.263	ns
C18:2n-6t	Trans-Linoleic	0.063	±0.010	0.053	±0.012	ns	0.058	±0.008	0.050	±0.005	ns
C18:2n-6c	Cis-Linoleic	12.008[a]	±0.553	9.236[b]	±1.210	≤0.01	12.646	±1.145	11.031	±2.000	ns
C18:3n-6	γ-Linolenic	0.082	±0.019	0.074	±0.029	ns	0.074	±0.019	0.073	±0.041	ns
C20:0	Arachidic	0.156	±0.034	0.150	±0.025	ns	0.136	±0.029	0.122	±0.006	ns
C18:3n-3	α-Linolenic	0.582[a]	±0.052	0.424[b]	±0.103	≤0.05	0.569[a]	±0.093	0.441[b]	±0.021	≤0.05
C20:1n-9	Eicosenoic	0.824	±0.058	0.820	±0.099	ns	0.916	±0.149	0.751	±0.105	ns
C21:0	Heneicosylic	0.049	±0.025	0.046	±0.012	ns	0.041	±0.010	0.026	±0.018	ns
C20:2	Eicosadienoic	0.535	±0.134	0.527	±0.093	ns	0.567[a]	±0.064	0.414[b]	±0.053	≤0.01
C20:3n-3	Eicosatrienoic	0.260	±0.059	0.252	±0.066	ns	0.285	±0.081	0.328	±0.116	ns
C20:4n-6	Arachidonic	1.345	±0.464	1.494	±0.473	ns	1.780	±0.512	2.329	±0.917	ns
C22:1n-9	Erucic	0.021	±0.007	0.035	±0.011	ns	0.018	±0.008	0.029	±0.024	ns
C20:5n-3 EPA	Eicosapentenoic	0.045	±0.010	0062	±0.020	ns	0.057	±0.013	0.065	±0.035	ns
C24:0	Lignoceric	0.356	±0.090	0.357	±0.066	ns	0.444	±0.104	0.521	±0.171	ns
C22:5n-3 DPA	Docosapentaenoic	0.204	±0.052	0.232	±0.058	ns	0.743	±1.133	0.310	±0.127	ns
C22:6n-3 DHA	Docosahexaenoic	0.045	±0.018	0.050	±0.019	ns	0.054	±0.013	0.061	±0.018	ns
Σ SFA	Total Saturated	38.013[a]	±1.852	35.134[b]	±1.530	≤0.05	35.526	±2.317	36.962	±1.909	ns
Σ MUFA	Total Monounsaturated	44.690[a]	±0.637	50.166[b]	±1.120	≤0.001	45.235	±1.713	44.995	±2.581	ns
Σ PUFA	Total Polyunsaturated	15.170[a]	±0.976	12.403[b]	±0.674	≤0.01	16.833	±0.848	15.102	3.244	ns

C: Soybean meal, R: Rapeseed meal, Row values with different superscripts differ significantly ($p \leq 0.05$)

Enterococcus spp., compared to group C. Moreover, in the mid-colon, group R had significantly higher ($p \leq 0.05$) total anaerobes and Enterobacteriaceae and lower ($p \leq 0.05$) total aerobes and *Clostridium perfringens* compared to group C.

DISCUSSION

The present study deals with the possibility of exchanging soybean meal with rapeseed meal in pig during the growing and fattening. A main issue that is in favour of soybean replacement is the high cost of soybean meal. Economic benefit was increased when 9% of RSM was supplemented in growing-finishing diets of pigs (Quiniou *et al*., 2012). Generally cost of RSM is significantly lower compared to SBM (Index Mundi, 2014).

Another concern of the end users of the meat industry is that a large amount of the soybeans produced in the world are Genetically Modified (GM), along with its environmental impacts (Swiatkiewicz *et al*., 2014). Production and processing of soya bean is associated with recent land use changes, which cause increased greenhouse gas emissions from deforestation and conversion of native land to arable land (Guo and Gifford, 2002). Substitution of SBM in pig diets is expected to reduce the environmental impacts of produced pork meat as production processing and transport of soyabean meal has been found to be one of the main sources of greenhouse gas emissions in the livestock industry (Eriksson *et al*., 2005). In addition, rapeseed is an important source of biofuel, which are a renewable source of energy and have lower carbon dioxide emission when burned, compared to conventional fossil fuels (Swiatkiewicz *et al*., 2014).

Table 6: Effect of dietary soybean meal and rapeseed meal on the lipid oxidative stability of pork ham and steak meat cuts after 4 and 7 days of storage (4°C)

	Storage (MDA (ng g^{-1}))	
Groups	4 days	7 days
Ham (Mean MDA ng g^{-1}±SD)		
C	55.3±7.1	36.3±9.4
R	45.6±6.7	32.9±6.6
p-value	ns	ns
Steak (Mean MDA ng g^{-1}±SD)		
C	45.8±7.1	32.0±5.8
R	56.3±9.6	33.2±5.1
p-value	ns	ns

C: Soybean meal, R: Rapeseed meal, MDA: Malondialdehyde, No significant differences ($p>0.05$) were found

Table 7: Effect of dietary soybean meal and rapeseed meal on the microbial populations of the pig jejunum, caecum and mid-colon

	C		R		
Microbial population	log CFU	±SD	log CFU	±SD	p-value
Jejunum					
Total aerobes	$6.59\times10^{8\,a}$	$\pm2.95\times10^{8}$	$1.45\times10^{8\,b}$	$\pm1.12\times10^{8}$	≤0.05
Total anaerobes	3.39×10^{8}	$\pm3.19\times10^{8}$	5.53×10^{8}	$\pm3.88\times10^{8}$	ns
Total coliforms	$1.02\times10^{6\,b}$	$\pm5.64\times10^{5}$	$5.58\times10^{6\,a}$	$\pm2.69\times10^{6}$	≤0.05
Clostridium perfringens	1.28×10^{4}	$\pm1.00\times10^{4}$	1.24×10^{4}	$\pm5.71\times10^{3}$	ns
Enterococcus spp.	2.62×10^{8}	$\pm2.31\times10^{8}$	3.08×10^{8}	$\pm2.96\times10^{8}$	ns
Enterobacteriaceae	5.62×10^{6}	$\pm2.20\times10^{6}$	1.48×10^{7}	$\pm2.63\times10^{6}$	ns
Lactobacillus spp.	4.33×10^{8}	$\pm2.34\times10^{8}$	2.70×10^{8}	$\pm1.12\times10^{8}$	ns
Bifidobacterium spp.	9.23×10^{7}	$\pm6.41\times10^{7}$	1.28×10^{8}	$\pm8.22\times10^{7}$	ns
Caecum					
Total aerobes	5.32×10^{8}	$\pm3.82\times10^{8}$	2.19×10^{8}	$\pm1.01\times10^{8}$	ns
Total anaerobes	$5.33\times10^{8\,b}$	$\pm4.22\times10^{8}$	$2.46\times10^{9\,a}$	$\pm1.93\times10^{9}$	≤0.05
Total coliforms	8.13×10^{5}	$\pm7.77\times10^{5}$	2.41×10^{5}	$\pm1.55\times10^{5}$	ns
Clostridium perfringens	5.69×10^{5}	$\pm5.38\times10^{5}$	1.06×10^{5}	$\pm7.91\times10^{4}$	ns
Enterococcus spp.	$1.16\times10^{8\,a}$	$\pm5.38\times10^{7}$	$1.26\times10^{7\,b}$	$\pm7.87\times10^{6}$	≤0.05
Enterobacteriaceae	4.79×10^{6}	$\pm6.14\times10^{6}$	4.44×10^{6}	$\pm1.41\times10^{6}$	ns
Lactobacillus spp.	$1.07\times10^{9\,b}$	$\pm4.93\times10^{8}$	$3.38\times10^{9\,a}$	$\pm2.16\times10^{9}$	≤0.05
Bifidobacterium spp.	1.98×10^{8}	$\pm1.77\times10^{8}$	3.62×10^{8}	$\pm3.12\times10^{8}$	ns
Mid-colon					
Total aerobes	$1.04\times10^{9\,a}$	$\pm8.01\times10^{8}$	$1.96\times10^{8\,b}$	$\pm1.26\times10^{8}$	≤0.05
Total anaerobes	$1.49\times10^{9\,b}$	$\pm8.27\times10^{8}$	$9.97\times10^{9\,a}$	$\pm8.67\times10^{9}$	≤0.05
Total coliforms	7.15×10^{5}	$\pm6.54\times10^{5}$	1.61×10^{5}	$\pm1.52\times10^{5}$	ns
Clostridium perfringens	$5.90\times10^{5\,a}$	$\pm4.67\times10^{5}$	$4.97\times10^{5\,b}$	$\pm3.30\times10^{5}$	≤0.05
Enterococcus spp.	1.15×10^{8}	$\pm6.40\times10^{7}$	7.64×10^{7}	$\pm3.00\times10^{7}$	ns
Enterobacteriaceae	$2.10\times10^{6\,b}$	$\pm1.88\times10^{6}$	$8.17\times10^{6\,a}$	$\pm5.32\times10^{6}$	≤0.05
Lactobacillus spp.	2.42×10^{9}	$\pm1.49\times10^{9}$	1.19×10^{9}	$\pm7.50\times10^{8}$	ns
Bifidobacterium spp.	4.92×10^{8}	$\pm5.53\times10^{8}$	1.72×10^{8}	$\pm8.04\times10^{7}$	ns

CFU: Colony forming units, C: Soybean meal, R: Rapeseed meal, Row values with different superscripts differ significantly ($p \leq 0.05$)

Another issue that remains when the soybean meal is totally replaced by the rapeseed meal is the balance of amino acids, protein and energy in the diet. In our study, we used supplementary quantities of commercial amino acids, extra fat and different quantities of minerals in order to equalize diets in both experimental groups (Table 1). In the past, several research trials have been conducted with partial substitution of soybean meal by rapeseed meal in pig diets (McDonnell *et al*., 2010; Okrouhla *et al*., 2012; Xie *et al*., 2012; Choi *et al*., 2015).

The results showed that complete replacement of 11% SBM by 14.5% RSM and 10% SBM by 14.7% RSM for grower and finisher pigs, respectively had no significant effect on BWG during the growing and the finishing periods. This is in agreement with a range of researches showing no effect of dietary RSM on pig performance (McDonnell *et al*., 2010; Okrouhla *et al*., 2012; Xie *et al*., 2012). Contrary to these studies, other researchers (Castaing *et al*., 1998; Seneviratne *et al*., 2010; Sobotka *et al*., 2012; Choi *et al*., 2015; Gjerlaug Enger *et al*., 2015) showed that BWG of the pigs was decreased when RSM was incorporated in their diets. Moreover, some researchers (Moset *et al*., 2012; Torres-Pitarch *et al*., 2014) reported that grower pigs fed with RSM had lower BWG, a difference that disappeared in the finisher pigs. According to Choi *et al*. (2015), the main factor that limits the use of RSM in pig diets is the their anti-nutritional factor, mainly glucosinolates and erucic acid. Choi *et al*. (2015) concluded that RSM could be supplemented up to 9% in growing-finishing pig diets without any detrimental effect on performance, based on glucosinolates and erucic acid values of the examined RSM. Also, the likelihood of tannin-induced metabolic disorders affecting growth of young animals feed on a RSM diet is very low, provided that the inclusion of RSM is around 10% and that the other dietary constituents do not possess high tannin content.

In addition, the variability among RSM sources, the processing technologies used in oil extraction, the RSM inclusion levels in the diets, the fibre levels of RSM, in addition to the race, age and weight of the pigs used in the trials could explain the variability of results between different studies (Messerschmidta *et al*., 2014; Torres-Pitarch *et al*., 2014; Choi *et al*., 2015). In our work, due to changes in feed raw materials and addition of amino acids, final diets were similar in chemical composition, despite the differences in SBM and RSM (Table 1 and 2).

The total substitution of SBM by RSM in pig diets led to an increase of the protein percentage in the meat of the steak but to a decrease of this percentage in the pancetta and the ham. Additionally, it was found that dietary RSM, decreased the fat content in the meat of the shoulder and the steak but increased of this percentage in the pancetta and the ham. In a previous study, Okrouhla *et al*. (2012) noticed decrease in crude protein of loin and increase of ham moisture in pigs that were fed with diets containing extruded rapeseed meal, compared pigs that were fed with soybean meal diets. Other researchers (Torres-Pitarch *et al*., 2014; Gjerlaug Enger *et al*., 2015) did not observe any differences in the carcass fat of pigs fed RSM. However, it must be noted that in most of these studies SBM was only partially replaced by RSM.

Furthermore, some differences were found in the fatty acid profile of the steak for the animals fed RSM. In the steak cut, lower concentrations of SFA and PUFA were noted, while MUFA were found in higher amounts. In contrast, in the shoulder cut this modification of the fatty acid composition was not so pronounced. Similar results were reported by an other researcher, Torres-Pitarch *et al*. (2014) reported increase of unsaturated fatty acids and decrease of saturated fatty acids in the meat and subcutaneous fat, after when partially replacing SBM with RSM. Indeed, the reduction of saturated and the increase of unsaturated fatty acids in the meat can be considered beneficial, since it is linked to lower risk of cardiovascular diseases in consumers (Simopoulos, 2002).

In terms of the influence of dietary RSM on oxidative stability of pork meat, there is lack of reference data. Rapeseed meal (RSM) contains an important amount of antioxidant substances and has been under examination as a food additive for the protection of lard (Kreps *et al*., 2012) and meat (Salminen *et al*., 2006) against oxidation. In the present study no difference was observed between the RSM and SBM diets in steak and ham lipid oxidation after 4 days or 7 of refrigerated storage. Therefore, it can be hypothesized that the dietary RSM use did not increase the total antioxidant amounts in the meat, compared to the SBM diets.

Regarding the possible effects of RSM in gut function and microbial balance in has been reported that some of included substances, for examples non-starch polysaccharides fibre, glucosinolates, tannins and sinapine can modify the gastrointestinal tract fermentation process, directly affecting the digesta composition and the microflora balance in monogatric animals, i.e., swine (Metzler-Zebeli *et al*., 2010), poultry (Johnson *et al*., 2008; Zdunczyk *et al*., 2013) and fish (De Paula Silva *et al*., 2011). In the present study, RSM increased *Lactobacillus* spp., populations in the caecum, which are considered as beneficial intestinal bacteria and are often used as probiotics in animal nutrition (Cho *et al*., 2011). Also, in the caecum in the RSM group lower *Clostridium perfringens* populations were noticed, which are the etiologic agent of various enteric diseases in pigs, for example, hemorrhagic necrotic enteritis and clostridial diarrhea (Songer and Glock, 1998; Songer and Uzal, 2005).

CONCLUSION

Results from the present research showed that the total replacement of soybean meal by rapeseed meal in nutritionally balanced diets of growing-finishing pigs did not have any detrimental effects on their performance parameters, from growing to slaughter. Dietary rapeseed meal modified some quality parameters of the pork meat such as the chemical composition and the fatty acid profile but not the oxidative stability. In addition, dietary rapeseed meal modified some populations of the microbial balance in the digestive tract of the pigs. Therefore, rapeseed meal of Greek origin could potentially be a viable cheaper and eco-friendly alternative to imported soybean meal protein source in pig diets.

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