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Effect of dietary protein source on piglet meat quality characteristics.

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ABSTRACT.

An experiment was conducted to examine the effects of different dietary protein sources (soybean meal vs whey protein) on piglet meat quality characteristics. Eighteen castrated male Large White × Duroc × Landrace piglets were randomly assigned to 2 groups. Piglets were kept in individual metabolic cages and fed ad libitum over a period of 38 days the following 2 diets: diet SB, which was formulated to meet the nutrient requirements of piglets using soybean meal as the main crude protein source and diet WP, where SB was totally replaced by a mixture of whey proteins on equal digestible energy and crude protein basis. At the end of the experiment, piglets were weighed and slaughtered. After overnight chilling, samples of Longissimus dorsi muscle were taken and were used for meat quality measurements.

No significant differences were observed in the values of pH, color, water holding capacity, shear force and intramuscular fat content of L. dorsi muscle between the dietary treatments. Measurement of lipid oxidation values showed that dietary supplementation with different protein sources did not influence meat antioxidant properties during refrigerated storage. The SB piglets had lower C14:0 ($P<0.01$) and higher C18:3n-3 ($P<0.001$) levels in intramuscular fat in comparison with WP piglets. However, these changes were attributed to background differences in the dietary FA profile and not to a direct protein source effect. The results of this preliminary study indicate that the examined dietary protein sources do not have a significant effect on meat quality characteristics of piglets.

1 Introduction

Pork is one of the most commonly consumed meats worldwide. According to FAO databases, the last decade (2001-2011) the production of pig meat in the European Union has increased from 21454984 to 23066700 tn (FAO, 2013). During the last decades attempts have been made to improve pig meat products in line with the new dietary guidelines in order to make them more attractive to consumers. Additionally, there has been an increased interest in finding ways to manipulate the fatty acid composition of meat, since it is the major source of fat and especially saturated fatty acids, which have been implicated in several diseases associated with the contemporary life in the developed countries. Dietary interventions that influence lipid metabolism in farm animals have been therefore employed, since they represent a useful tool to modulate fat deposition and improve meat quality (Wood *et al.*, 2008). In pigs, an effort has been made to increase n-3 polyunsaturated fatty acids (PUFAs) and keep the favorable balance between n-6 and n-3 PUFA (ratio of less than 4) (Wood *et al.*, 2003).

Soybean meal and milk products, such as dried whey concentrates are two protein sources widely incorporated in swine diets (Mahan, 1993; Yun *et al.*, 2005; Yang *et al.*, 2007). Inclusion of whey proteins in weanling piglets' diets appears to have a beneficial effect on feed efficiency, nutrient digestibility and growth performance (Tokach *et al.*, 1989; Grinstead *et al.*, 2000). At the same time, soybean meal (40 %) could replace more expensive protein sources in nursery pig diets without affecting feed intake and growth performance (Lenehan *et al.*, 2007).

In addition to the meat quality importance, the investigation of such dietary treatments in pigs could serve as a valuable model for studying the intermediary lipid metabolism in humans (Carey, 1997). In this sense, numerous studies have focused in investigating the mechanisms underlying the influence of nutritional treatments on the lipogenic process. Recent studies have indicated that the source of dietary protein has been shown to affect lipid metabolism. Soy protein appears to reduce blood lipid levels and to have a positive effect in cholesterol levels and reduce the risk of diabetes, obesity, cardiovascular and coronary heart disease (Sadler, 2004; Torres *et al.*, 2006). In detail soy protein, when compared to casein reduces the gene expression of lipogenic enzymes in liver (Tovar *et al.*, 2002; Ascencio *et al.*, 2004) and this effect is thought to be mediated by the decreased insulin to glucagon ratio, which has been attributed to the lower lysine to arginine ratio and the higher glycine content of soy proteins (Tovar *et al.*, 2002). At the same time, soy proteins may stimulate proliferator-activated receptor α (PPAR-α) in liver and PPAR-γ in adipose tissue increasing, thus, fatty acid oxidation (Tovar *et al.*, 2005) and fatty acid uptake from plasma (Farmer, 2005), respectively.

As it has been already stated, the diet has a direct effect on muscle composition and meat quality in pig, being a monogastric species. Sensory properties in turn, such as flavor intensity, tenderness, juiciness and fat tissue firmness significantly influence the shelf life and the acceptability of pig meat products (Wood *et al.*, 2003; 2008). Whey proteins and, to a lesser extent, soy proteins seem to be particularly attractive ingredients for the inhibition of lipid oxidation processes and the quality improvement in processed muscle foods (Peña-Ramos and Xiong, 2003). However, little is known about the effect of these dietary protein sources on pig meat quality. The objective of this preliminary study was therefore to evaluate the effects of the above dietary protein sources; soybean meal or whey proteins on piglet meat quality characteristics.

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2 Materials and Methods

2.1 Animals and diets

Eighteen castrated male Large White × Duroc × Landrace piglets weaned at 29±2 days of age were selected from a commercial farm located near the city of Athens. Upon arrival at the experimental facilities, they were allowed an adaptation period of 4 days and were then allotted into 2 groups (9 pigs/group) balanced for body weight (average BW of 8.4 ± 0.68 kg; mean ± sd). Piglets were kept in individual metabolic cages (1.2 × 0.5 m) and fed *ad libitum* the following 2 diets, over a period of 38 days: diet SB, which was formulated to meet the nutrient requirements of piglets (NRC, 1998) using soybean meal (SB) as the main crude protein (CP) source and diet WP, where SB was totally replaced by a mixture of whey proteins [WP, 70% WheyPro65 (650 g CP/kg) + 30% WheyPro 80 (800 g CP/kg); Hellenic Proteins S.A., Veria, Greece], on equal digestible energy (DE) and CP basis. The replacement of SB by WP aimed to alter the dietary lysine/arginine ratio (2.12 vs. 0.85; diet WP and SB, respectively) and glycine content (5.5 vs. 8.6 g/kg; diet WP and SB, respectively). The ingredient and chemical composition of the diets are summarized in Table 1.

At the end of the experiment (72 days of age) piglets were stunned and killed by exsanguination. After overnight chilling, a 10 cm loin section was removed from both sides of carcasses and was used for the determination of meat quality characteristics. Handling and care of the experimental animals conformed to the guidelines of the Faculty of Animal Science and Aquaculture of Agricultural University of Athens.

2.2 Meat quality measurements

2.2.1 pH24 and color

pH was measured using a Sentron 1001 pH System (Roden, Netherlands) model, with the electrode inserted into the centre of the *Longissimus dorsi* muscle at the last rib 24 h after slaughter. The part of the muscle between 12th and 13th ribs was sliced across the fibers, left exposed to the air at room temperature for 30 min and meat color measured (3 measurements per sample) on the cut surface using a Miniscan XE (HunterLab, Reston, USA) chromameter set on the L*, a* and b* system (CIE 1976, Commission International de l' Eclairage).

2.2.2 Shear force value

Samples (80 ± 2 g, 2 cm thickness) of *L. dorsi* muscle from each pig were placed in plastic bags and cooked in a water bath at 80°C for 50 min (internal temperature 72 ± 1°C), left under running water for 30 min and then placed in a refrigerator at about 4°C for 24 h. Five sub samples with a cross section of 1 cm^2 were cut parallel to the muscle fibers and shear force value of the *L. dorsi* muscle was measured using a Warner Bratzler (WB) shear blade fitted to a Zwick Testing Machine Model Z2.5/TN1S (Zwick GmbH & Co., Germany). Peak force values in Newton were recorded.

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Table 1. Ingredient and chemical composition of the experimental diets

		Diet*	
		SB	**WP**
Ingredients (g/kg)	Maize	621.0	754.0
	Soybean meal (440 g CP/kg)	342.0	-
	Whey proteins† (660 g CP/kg)	-	210.0
	L-Lysine 80%	2.0	2.0
	DL-Methionine 99%	1.0	1.0
	Sodium chloride	5.0	4.0
	Calcium carbonate	13.0	14.0
	Monocalcium phosphate	13.0	12.0
	Mineral-vitamin premix‡	3.0	3.0
Analyzed chemical composition (g/kg DM)	Dry matter (g/kg)	888.0	903.0
	Crude protein	232.0	228.0
	Ether extract	33.1	40.4
	Total weights of FA (mg/100 g DM)	31.4	38.7
Individual FA (% of total FA)	C14:0	0.08	2.91
	C16:0	14.40	17.87
	C16:1 *cis*9	0.16	0.40
	C18:0	2.63	4.42
	C18:1 *cis*9	21.71	24.27
	C18:2*n*-6	54.28	41.42
	C18:3*n*-3	3.35	1.32
	C20:0	0.33	0.34
	C20:1*n*-9	0.22	0.20
	C22:2	0.60	0.77
Calculated chemical composition (g/kg DM) §	Digestible energy (MJ/kg DM)	15.5	15.4
	Metabolisable energy (MJ/kg)	14.7	14.6
	SID§§crude protein	166	177
	SID§§ methionine+cystine	6.9	8.6
	SID§§ lysine	11.2	14.2
	SID§§ arginine	12.1	5.4
	SID lysine/SID arginine ratio	0.93	2.63
	SID§§ threonine	7.2	11.2
	Total glycine	9.7	6.1
	Calcium	9.2	9.1
	Total phosphorus	7.4	8.4
	Sodium	2.4	2.4

***** SB, soybean meal as protein source; WP, whey proteins as protein source.

† Mixture of whey proteins (Hellenic Proteins S.A., Veria, Greece); 70% WheyPro 65 (650 g CP/kg) + 30% WheyPro 80 (800 g CP/kg) designed to have a content of 660 g CP/kg.

‡ Mineral-vitamin premix (Nuevo S.A., N. Artaki, Greece) provided per kg of diet: 15000 IU vitamin A (retinyl acetate), 2000 IU vitamin D3 (cholecalciferol), 100 mg vitamin E (DL-α-tocopheryl acetate), 3.5 mg menadione (vitamin K3), 2.5 mg vitamin B1, 6 mg vitamin B2, 3 mg vitamin B6, 25 μg cyanocobalamin, 25 mg nicotinic acid, 20 mg pantothenic acid, 2 mg folic acid, 250 μg biotin, 2 mg Co, 4 mg I, 600 μg Se, 300 mg Fe, 100 mg Mn, 100 mg Mg, 320 mg Cu and 240 mg Zn.

§ Digestible and net energy, macro-element, total amino acid and standardized ileal digestible (SID) protein and amino acid values for maize, soybean meal and whey proteins were adapted from tabulated data (FEDNA, 2003; NRC, 1988).

§§ Standardized ileal digestible nutrients. No data were available on glycine SID values.

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2.2.3 Drip loss

The left part of *L. dorsi* muscle was sampled in two positions for each experimental animal and the drip loss was measured applying the EZ-DripLoss method (Christensen, 2003). In brief, the *Longissimus* muscle of each experimental animal rested 4 h at approximately 7°C and was then cut into slices, each with a thickness of 2.5 cm. Two cylindrical cuts were made with a fixed blade knife of diameter 25 mm. Each sample was placed in a special EZ-DripLoss container and remained for storage at 4°C for eight days. All containers were tared before use. The meat samples were then removed from the EZ-DripLoss containers and each container with exudated meat juice was weighed on the scale used for the taring procedure.

2.2.4 Measurement of total lipids and lipid oxidation – MDA assay

Measurement of intramuscular total lipids (IMF) was made according to the method first described by Folch et al. (1957). Tissue samples were homogenized with 2:1 chloroform–methanol mixture to a final dilution 20-fold the volume of the tissue sample. The crude extract was mixed with 0.2 its volume of water and it was separated into two phases. The lower phase contained the tissue lipids.

Lipid oxidation was assessed on the basis of the malondialdehyde (MDA) formed during storage. In the present study, MDA concentration in *L. dorsi* muscle samples was determined 1, 3, 6 and 9 days after storage at 4°C using a selective third-order derivative spectrophotometric method, previously developed by Botsoglou et al. (1994). Derivative versus conventional spectrophotometry was adopted because it offers improved sensitivity, specificity and reliability of the measurements, since it eliminates potential interferences from other reactive compounds. In brief, 2 g of each sample (2 samples per pig) were homogenized (Edmund Buehler 7400 Tuebingen/H04, Germany) in the presence of 8 ml aqueous trichloroacetic acid (TCA) (50 g/l) and 5 ml butylated hydroxytoluene (BHT) in hexane (8 g/l), and the mixture was centrifuged for 3 min at 3000g. The top hexane layer was discarded and a 2.5 ml aliquot from the bottom layer was mixed with 1.5 ml aqueous 2-thiobarbituric acid (TBA) (8 g/l) to be further incubated at 70°C for 30 min. Following incubation, the mixture was cooled under tap water and submitted to third-order derivative (3D) spectrophotometry (Hitachi U3010 Spectrophotometer) in the range of 500–550 nm. The concentration of MDA (ng/g wet tissue) in analyzed samples was calculated on the basis of the height of the third-order derivative peak at 521.5 nm by referring to slope and intercept data of the computed least-squares fit of standard calibration curve prepared using 1,1,3,3-tetraethoxypropane (TEP), the malondialdehyde precursor.

2.2.5 Fatty acid methylesters synthesis and determination

The FA of diets and muscle tissue were hydrolyzed (with methanolic KOH) and methylated (sulphuric acid catalysis) directly, according to O'Fallon et al. (2007) in duplicate 1 g ground samples. The FA methylesters (FAME) were extracted with clear n- hexane and transferred into gas chromatograph (GC) vials. The FAME were subsequently analyzed in a temperature programmed run using a Perkin Elmer Autosystem XL GC equipped with a 30m×0.25mm i.d.×0.25μm film thickness HP-Innowax capillary column (Agilent Technologies, J&W GC

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columns) and a flame ionisation detector (FID). The oven temperature was programmed for 1 min at 140°C, raised by 2.5°C/min to 200°C, then to 230°C by 1°C/min and held for 1 min, and finally to 240°C by 4°C/min and held for 10 min. Helium was the carrier gas at a constant pressure of 18 psi and the temperature of both the injector and FID was set at 250°C. Fatty acids were identified by comparison with FAME 37 Component Mix (Supelco, Sigma-Aldrich Co., USA) and quantification was achieved using the internal standard (13:0) added prior to hydrolysis. Total weights of FA (mg/100g) in diets were calculated as the sum of areas for all FA peaks compared to area for 0.5 mg internal standard. Individual FA were expressed as % by weight of total FA.

2.2.6 Statistical analysis

Body weight (BW), intramuscular fat (%), fatty acids profile and meat quality characteristics, such as pH24, color parameters (L, a* and b*), cooking loss (%) and shear force value (N) measurements for the *Longissimus dorsi* muscle were analyzed using the mixed model procedure which contained the fixed effect of the diet. Malondialdehyde (MDA) concentration and drip loss (%) were also analyzed using a mixed model appropriate for repeated measurements per subject, which included the diet as fixed effect. Finally, Power (1-β) analysis for all tests was performed using α=0.05 (Zar, 1996). All model analyses were performed by SAS/STAT (2005).

3 Results

No significant differences were observed in body weight at slaughter (kg), carcass weight (kg) and dressing out (%) between the two experimental groups (P>0.05). No effects of dietary protein source on feed intake and BW gain were also observed over the whole experimental period (30–72 days of age). Average feed intake was 1.16±0.12 and 1.14±0.14 kg/day, and BW gain was 0.51±0.08 and 0.53±0.04 kg/day, resulting in an FCR of 2.41±0.20 and 2.10±0.17 for pigs fed the WP and SB diets, respectively. At the same time, meat quality characteristics did not significantly differ between SP and WP piglets (Table 2). In detail, although instrumental pH and color values did not indicate meat of excessive acidity or darkness, respectively, and were within the normal range, these parameters were also not significantly influenced by the dietary protein source (P>0.05). Moreover, dietary soybean meal or whey protein did not result in different values for shear force, intramuscular fat content, cooking loss and drip loss (Table 2).

Refrigerated storage increased lipid oxidation in stored (4 °C) compared to fresh meat (P<0.001). However, dietary protein source appeared not to significantly influence lipid oxidation (MDA formation) values in raw pork *L. dorsi* muscle after storage at 4°C for up to 9 days (Table 3). With respect to the muscle fatty acid profile, lauric (C12:0) and myristic (C14:0) percentages were lower (*P*<0.05), whilst α-linolenic (C18:3*n*-3) and arachidonic (C22:4*n*-6) were higher (*P*<0.001 and *P*<0.05, respectively) for SB compared to WP fed piglets. However, total saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) were not affected by diet (Table 4).

ISSN: 2283-3927

Table 2. Effect of dietary protein source (whey proteins or soybean meal) on piglets' meat quality characteristics (LS means ± s.e.m.)

	Diet[1]		S.E.M.
	WP	SB	
Body Weight at slaughter (kg)	28.33	30.63	2.57
Cold Carcass Weight (kg)	18.99	19.82	1.75
Dressing out (%)	67.28	64.17	1.34
Intramuscular fat content (%)	1.50	1.39	0.10
pH (24 h)	5.44	5.42	0.17
Color Parameters			
L	51.33	50.08	1.10
a*	4.08	4.90	0.39
b*	9.91	9.88	0.19
Cooking loss (%)	36.40	36.21	0.48
Shear Force (N)	35.16	37.08	1.36
Drip loss (%) (days)[2]			
1st	5.02	6.55	0.71
2nd	6.79	8.78	0.70
3rd	7.81	9.74	0.73
4th	8.75	10.45	0.73
5th	9.33	10.98	0.74
6th	9.87	11.34	0.76
7th	10.23	11.62	0.79
8th	10.74	12.04	0.79

1 SB, soybean meal as protein source; WP, whey proteins as protein source.

2 Time effect was significant (P<0.001), but the effect of interaction of time with treatment (P=0.210) was not significant.

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4 Discussion

No effect of dietary protein source on piglet meat quality was found. In detail, pH, color, shear force, intramuscular fat content, cooking loss and drip loss values were not significantly different between the WP and SB piglet group. Data referring to the effects of the dietary protein source on pig meat quality are scarce. In general, soy proteins are more commonly used in processed meat products for their functional properties (water- and fat-binding ability, enhancement of emulsion stability) and relatively low cost compared with lean meat (Chin *et al.*, 2000). For example, soy protein isolates can be incorporated to chorizo raw sausage (Porcella *et al.*, 2001) or low-fat bologna (Chin *et al.*, 2000) without any detrimental effect on the organoleptic, physicochemical and microbiological properties of these meat products. Moreover, dietary protein source (soybean meal, sunflower meal, pea and fish meal) had no effect on pig meat characteristics (intramuscular fat content, pH_{45} and pH_{24}, drip loss and color) (Szabo *et al.*, 2001). Dietary soy isoflavones also appear not to influence pH_{24}, color parameters, drip loss, firmness-wetness and marbling of pig meat (Payne *et al.*, 2001).

At the same time, the dietary protein sources used in the present study (soybean meal or whey protein) appeared not to affect differently lipid oxidation values in raw pork *longissimus* muscle after storage up to 9 days at 4 °C (Table 3). These findings are in contrast with the already demonstrated *in vitro* antioxidant activity of whey proteins (Peña-Ramos and Xiong, 2003); hence, the lack of such a positive effect on muscle tissue (*in vivo*) is unclear. Nevertheless, MDA values for WP tended to be numerically lower compared to the SP group.

Finally, the limited differences in muscle FA profile; lower lauric and myristic and higher α-linolenic and arachidonic percentages for SB compared to WP fed piglets mainly depicted the incorporation of dietary FA into muscle lipids, i.e. lower C14:0 and higher C18:3*n*-3 in SB fed pigs and not a protein source effect. Any other differences in FA observed in muscle between groups were more likely due to endogenous FA metabolism (Wood and Enser, 1997).

Table 3. Effect of dietary protein source (whey proteins or soybean meal) and duration of refrigerated (at 4°C) storage on lipid oxidation (MDA , ng/g) of raw piglet *Longissimus dorsi* muscle (LS means ± s.e.m.)

Storage period (days, at 4°C)	Diet[1]		S.E.M.
	WP	SB	
1	16.18	20.90	1.01
3	22.37	25.53	1.54
6	25.75	31.34	1.62
9	36.92	37.30	1.96

1 SB, soybean meal as protein source; WP, whey proteins as protein source.

Time effect was significant (P<0.001), but the effect of interaction of time with treatment (P=0.120) was not significant. Higher levels of MDA indicate higher rates of lipid oxidation.

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Table 4. Effect of diet on total fatty acids (mg/100 g wet muscle) and fatty acid (FA) composition (% of total FA) of *Longissimus dorsi* in piglets (LS means ± s.e.m.).

FA	Diet[1]		S.E.M.	P-value[5]
	SB	WP		
Total FA	1027	1179	84.7	ns
C12:0	0.08	0.11	0.007	*
C14:0	1.12	1.34	0.049	*
C16:0	22.31	22.73	0.261	ns
C16:1 *cis*9	2.10	2.45	0.147	ns
C18:0	11.54	11.41	0.208	ns
C18:1 *cis*9	25.50	27.77	1.249	ns
C18:1 *cis*11	3.32	3.27	0.074	ns
C18:2*n*-6	16.66	14.48	0.863	ns
C18:3*n*-3	0.40	0.20	0.027	***
C20:3*n*-6	0.53	0.46	0.028	ns
C20:4*n*-6	3.78	3.19	0.237	ns
C20:5*n*-3	0.33	0.32	0.044	ns
C22:4*n*-6	0.51	0.40	0.025	*
C22:5*n*-3	0.71	0.68	0.067	ns
C22:6*n*-3	0.66	0.60	0.046	ns
SFA[2]	35.74	36.21	0.437	ns
MUFA[3]	32.52	34.98	1.368	ns
PUFA[4]	24.19	20.86	1.295	ns

[1] SB, soybean meal as protein source; WP, mixture of whey proteins [WP, 70% WheyPro65 (650 g CP/kg) + 30% WheyPro 80 (800 g CP/kg); Hellenic Proteins S.A., Veria, Greece] as protein source.

[2] SFA, total saturated FA, including individual FA which are not presented (C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0).

[3] MUFA, total monounsaturated FA, including individual FA which are not presented (C14:1 + C16:1 *cis*9 + C16:1 *cis*7 + C17:1 + C18:1 *cis*9 + C18:1 *cis*11 + C20:1*n*-9).

[4] PUFA, total polyunsaturated FA, including individual FA which are not presented (C18:2*n*-6 + C18:3*n*-6 + C18:3*n*-3 + C20:2 + C20:3*n*-6 + C20:4*n*-6 + C20:5*n*-3 + C22:4*n*-6 + C22:5*n*-3 + C22:6*n*-3).

[5] ns: not significant, *P<0.05, ***P<0.001

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In conclusion, no significant differences between dietary soybean meal or whey protein diets on piglet meat quality characteristics (color parameters, pH_{24}, cooking loss, intramuscular fat content and shear values) were observed. The extent of lipid oxidation in raw *L. dorsi* muscle stored at 4°C for up to 9 days was also not influenced by the dietary protein source. The SB pigs had lower C14:0 ($P<0.05$) and higher C18:3*n*-3 ($P<0.001$) levels in intramuscular fat in comparison with WP pigs. However, these changes were attributed to background differences in the dietary FA profile and not to a direct protein source effect. The present results suggest that the two examined dietary protein sources (soybean meal or whey protein) do not have a different effect on meat quality characteristics of piglets. Further investigation is warranted to elucidate the mechanisms underlying the potential effects of dietary protein source on meat quality in piglets and pigs.

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