

Origanum vulgare L. and *Rosmarinus officinalis* L. Aqueous Extracts in Growing-finishing Pig Nutrition: Effects on Antioxidant Status, Immune Responses, Polyphenolic Content and Sensorial Properties

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Abstract

The effects of an oregano and/or rosemary (*Origanum vulgare* L. and/or *Rosmarinus officinalis* L.) dietary supplementation to the diet of fattening pigs were investigated. Thirty-two grower-finisher pigs (45 kg) were divided into four dietary groups identified as: control diet (CTR); CTR+ 0.2% oregano (O); CTR + 0.2% rosemary(R), and CTR+ 0.1% oregano + 0.1% rosemary (OR). During the finishing period, all groups received a further supplementation of 0.5% of conjugated linoleic acids (CLA). Blood samples were collected after an adaptation period of 15 days to the new diet (T1) and at the end of the finishing period (T2) to evaluate antioxidant status (total antioxidant power and reactive oxygen metabolites) and immune responses (lymphocytic phenotyping and IgG levels). Pork meat samples were evaluated for glutathione peroxidase activity (GSHPx), total phenolic content and preference rating. A significant increase in B lymphocytes (CD79⁺) and a higher IgG level was observed in the R and O groups (P<0.05). Furthermore, there were significant effects of dietary supplementation on meat GSHPx activity and total phenolic contents (P<0.001 and P<0.005, respectively). Preference rating showed that pork derived from group R was the most preferred by the consumers.

Keywords: CLA, immune modulation, redox status, rosemary, oregano, pork, polyphenols, preference rating

1. Introduction

Healthy eating is a fundamental prerequisite for the health and well being of human beings. In the Western world, life expectancy has increased due to an adequate supply of nutrients. In recent years, there has been increasing awareness of the close association between health and diet, which has led consumers to make more conscious choices, and hence, the quality of food is receiving increased attention. It is now well established that some unsaturated fatty acids (UFA) like oleic and α -linolenic acids can improve human health and help prevent disease (Bauman, Mather, Wall, & Lock, 2006; FAO/WHO Expert Consultation, 2009; Bhattacharya, Banu, Rahman, Causey, & Fernandes, 2006). Consumption of pig meat and its processed foods is currently equal to 40.4 % of total world meat consumption (OECD/FAO, 2018). Therefore, to raise the image of pork as a healthy meat and hence increase this percentage, it could be a good strategy for pork producers to attempt to increase the concentration of polyunsaturated fatty acids (PUFA) in the intramuscular fat. Indeed, it is well known that the FA composition in pig meat greatly depends on genotype, physiological state and environmental/rearing factors among which the feeding regimen is the most important. In particular, the composition of the fat tissue greatly reflects the quality of the dietary FA (Boselli, Pacetti, Lucci, Di Lecce & Frega, 2008; Cameron et al., 2000; Temperán, Lorenzo, Castiñeiras, Franco, & Carballo, 2014; Wood et al., 2008), which, in turn, influences the tenderness, aroma and juiciness of the meat (Janz et al., 2004). However, the reduction of intra-muscular fat

together with its shift towards a higher PUFA concentration may corresponds to healthier meat, but it also confers lower sensory properties. Indeed, the higher oxidative instability given by PUFA contributes to worsening of the aroma (due to rancidity development) and makes the color of the meat darker. Pork meat with a fatty acid profile mainly composed of mono-unsaturated or saturated fatty acids is the most appreciated from the sensory point of view, and it also more suitable for the meat processing industry (Hardon, Eberhard, Guggisberg, Piccinalli, & Schlictherle-Cerny, 2008) due to its higher oxidative stability and fat firmness, but the health properties are not satisfied.

In view of this, several animal feeding strategies have been investigated to improve the nutritional characteristics of pork, as well as its antioxidant stability and sensorial or technological qualities (Boselli, Pacetti, Lucci, Di Lecce, & Frega, 2008; Echegaray, Domínguez, Franco, Lorenzo, & Carballo, 2018; Pacetti, Balzano, Gagliardi, Mozzon, & Frega, 2014; Ranucci et al., 2015).

Among these, supplementation of the pig diet with a commercial source of conjugated linoleic acids (CLA) during the finishing period has been shown to influence the rate of fat deposition in the adipose tissue (Corino, Magni, Pastorelli, Rossi, & Mourot, 2003) and the immune variables (Bontempo et al., 2004).

Natural antioxidant molecules could be a significant resource in this context because not only are they able to counteract oxidative instability, possibly worsened by CLA, but also to exert antibacterial and antifungal activities and to modulate the animal immune responses (Cullen, Monahan, Callan, & O'Doherty, 2005; Middleton & Kandaswami, 1992). Their cost is similar to that of other synthetic antioxidants without affecting the normal running costs of pig breeding. The aim of the present research was to evaluate the influence of an oregano and/or rosemary (*Origanum vulgare* L. and/or *Rosmarinus officinalis* L.) dietary supplementation on the antioxidant status and immune response in fattening pigs receiving a CLA supplement in the finishing period as well as to evaluation the total phenolic content, GSHPx activity and sensory perceptions of the pork meat.

2. Materials and Methods

2.1 Experimental Design and Treatment

Thirty-two Duroc x Large White pigs (45.8 ± 2.3 kg at the beginning of the trial) were divided into four groups, each composed of four barrows and four gilts. Experimental diets were supplemented with an oregano (*Origanum vulgare* L.; O) and/or rosemary (*Rosmarinus officinalis* L.; R) aqueous leaf extract (AEs. Phenbiox[®], Bologna, Italy) obtained by enzymatic water extraction (Setti & Zanichelli, 2009). They were identified as: (CTR) control diet; (O) diet supplemented with 0.2% oregano; (R) diet supplemented with 0.2% rosemary, and (OR) diet supplemented with 0.1% oregano + 0.1% rosemary. According to the producer's indications the AEs provided an antioxidant action equal to 17780 ± 260 vs 10460 ± 160 ORAC/L, a total polyphenol content of 2.5 ± 0.2 vs 2.3 ± 0.2 g/L and reducing sugars of 3.3 ± 0.2 vs 2.9 ± 0.1 g/L, in O and R, respectively.

The feeding program consisted of three stages identified as early grower (from the initial live weight (LW) to 90 kg LW), late grower (from 90 to 120 kg LW) and finisher (from 170 to 180 kg LW, the typical slaughter weight of Italian heavy pigs). During finishing, all animals received a degermed corn-barley-soybean-based diet further supplemented with a 1% commercial source of conjugated linoleic acids (LodeStarTMCLA, Berg+Schmidt Functional lipids, Hamburg, Germany) containing a minimum of 50% CLA, including c9 t11 (min. 24%) and t10 c12 (min. 24%) isomers in equal parts. All diets were wet fed (water to feed ratio of 3:1) and all pigs had free access to water. The pigs were reared until they reach the slaughter weight typical of heavy pigs (170-180 kg) after which they were slaughtered in a local slaughterhouse (final weight 183.2 ± 11.4 kg).

This study was conducted in accordance with the European recommendations for the protection of animals used for scientific purposes (EU Directive 2010/63/EU, September 22, 2010).

2.2 Blood Antioxidant Status and Immune Response Evaluation

Peripheral blood (PB) samples (5 ml) were withdrawn by jugular vein puncture in tubes without anticoagulants after 15 days of adaptation to the new diet (Time 1) and at the end of the finishing period (Time 2). Two aliquots of sera were obtained by centrifuging blood samples. One was used to determine the antioxidant species (AOP) and reactive oxygen metabolites (ROMs) using commercial kits supplied by Diacron International (Grosseto, Italy), according to the methodology previously described (Ranucci et al., 2015). Whereas, the second aliquot was used for immune globulin (IgG) titration using a commercial ELISA kit (Bethyl Laboratories Inc, Montgomery, TX, USA). A further blood sample was collected from each animal at Stage 2 in tubes with heparin (5 ml). The latter samples were used for lymphocytic phenotyping by flow cytometry analysis. Blood cells were labeled with a different combination of monoclonal antibodies (FITC labeled mouse anti-pig CD4a mAb, clone 74-12-4; PE labeled mouse anti-pig CD8a mAb, clone 76-12-11 and APC labeled mouse anti-human CD79a

mAb, clone HM47 cross reacting with pig; BD Biosciences, New Jersey, USA). For CD79a staining, cells were permeabilized with 0.1% saponin blocking buffer. Samples were acquired using a standard FACSCalibur™ flow cytometer (Becton Dickinson, Mountain View, CA) using CELLQuestPro™ software.

2.3 Meat Sampling, Total Phenolic Content Measurement and GSHPx Activity Evaluation

At the slaughterhouse, a tissue cube (1 cm × side) from one slice (1.5 cm thick) of the *Longissimus lumborum* muscle (LL) from 3 carcasses/group (barrows) was taken and transported (in a refrigerated bag at 4 °C) to the laboratory for analyzing antioxidant (GSHPx activity) and phenolic content; whereas, the entire ‘capocollo’ (neck pork meat) from two barrows/group was taken and transported (refrigerated at 4 °C) to the Sensorial Analysis Center for sensorial analysis.

The former samples were lyophilized and then frozen at -20 °C until analyses. Before analyses, the pork cubes were thawed (on ice), transferred to a Petri dish - on ice - and minced into smaller pieces with a surgical scissor. One hundred micrograms of each sample were homogenized (with a Potter-Elvehjem tissue grinder) in 3 mL of cold assay buffer containing Hepes 10 mM NaOH at pH 7.4, 1.7 mg PMSF, 8.56 g of saccharose and 37.2 mg of EDTA and then centrifuged to remove the insoluble material. The supernatants were used to measure the GSHPx activity (Ranucci et al., 2015) and the total amount of phenolics according a modified Folin-Ciocalteu method. The total phenolic content is expressed as gallic acid equivalents (GAE) per g of dry meat. The results are expressed as the average of three determinations/animal (Caprioli et al., 2016). Protein concentrations of tissue lysates were determined by the Bradford protein assay (Compton & Jones, 1985) to express the GSHPx activity as U/mg of protein (Stables & Gilroy, 2011).

At the Sensorial Analysis Centre, the lean part of the “capocollo” was separated from the fat and each was then ground separately. The cartilaginous part that could influence the consistency was discarded and, to ensure the genuineness of each sample, the meat grinder was cleaned between the grinding of each sample. To standardize samples, each sample was presented to the judges in the form of a burger. Furthermore, to respect the complete standardization of the samples, each burger was made up of 74% lean meat, 25% fat, and 1% salt. The burgers weighed 90 ± 5 grams (10 cm in diameter and 1 cm in thickness) and were made using a manual roller and stored at -18 °C until judgment. Burgers were cooked on an electric hotplate until reaching 75 ± 1 °C at the center of the product (measured with a thermometer probe). Close to the time of the tasting session, the meat was cooked, cut and wrapped in aluminum foil, both to prevent the meat from taking on odors or anomalous aromas, and to keep it warm until tasting. The tasters carried out the analysis in individual tasting booths (ISO8589: 1988) with red light illumination, to avoid that any perceived differences could be attributed to visual characteristics.

Burger samples were classified on the basis of preference through the ORDER TEST analysis (ISO 8587: 2006). Therefore, samples were compared with each other: CTR vs O vs R vs OR. The method consists in summing the value assigned to each sample of the burgers (from 1 = less preferred to 4 = most preferred), by 60 consumers (30 women and 30 men aged between 23 and 55 years) used to eating pork on an average of 3 times/week.

2.4 Statistical Analysis

In order to evaluate out the effects of the different dietary plant aqueous extracts on antioxidant status and immune responses, the results were statistically analyzed by GLM (General Linear Model) procedures carried out with the BM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp, where the independent variables were the dietary treatment (four levels), sampling time (two levels) and their interactions. Only the phenotypic lymphocyte analysis was performed by a one-way ANOVA for comparison of means between dietary treatments. Furthermore, Dunnett’s *t* test was used for comparing the means derived from animals whose diets were supplemented with plant extracts and the CTR group ($P < 0.05$). Results of the pork GSHPx activities and phenolic content were analyzed by Student’s *t*-distribution. All data are presented as group mean values \pm standard error of the mean (SEM). Differences were considered to be statistically significant when $P \leq 0.05$. For the Preference Rating evaluation, the Friedman test was applied to identify a significant difference between the samples, comparing the results obtained with the value of chi-square (χ^2), calculated according to the number of samples and consumers used.

3. Results

3.1 Blood Parameters

3.1.1 Blood Antioxidant Status

Table 1 shows the mean values and standard error of the mean (SEM) of the antioxidant status according to dietary treatment and sampling time. No significant differences were observed between the different diets supplemented with aqueous plant extracts or between the two sampling times.

Table 1. Mean Values and Standard Error of the Mean of Blood Antioxidant Parameters

	Sampling Period	Experimental Diets				SEM diet	SEM Time
		CTR	O	R	OR		
AOP (μmol) HClO)	Time 1	321.7	333.2	330.7	326.4	10.54	7.59
	Time 2	348.8	319.7	302.7	307.2		
ROMs (mM) H_2O_2	Time 1	36.8	35.8	35.9	35.7	1.98	1.39
	Time 2	33.9	32.6	34.9	32.1		

CTR: control diet; O: diet supplemented with 0.2% oregano; R: diet supplemented with 0.2% rosemary, and OR: diet supplemented with 0.1% oregano + 0.1% rosemary. SEM: standard error of the mean.

3.1.2 Immune Responses

There were no significant differences in IgG levels at Time 1 among the different dietary groups (data not shown). However, table 2 shows the mean values and standard error of the mean (SEM) of the blood immune parameters evaluated at the end of the experimental period (Time 2). Group R showed the highest value of CD79^+ cells (B^+ cells) and group O the highest IgG level. Therefore, the rosemary water extract administered with the diets was able to increase the number of B cell^+ ; whereas, the oregano supplement increased IgG production.

Table 2. Mean Values and Standard Error of the mean (SEM) of Blood Immune Parameters determined in the different experimental diets at Time 2

Diets	CD4^+ %	CD8^+ %	CD79^+ %	$\text{CD4}^+\text{CD8}^+$ %	IgG mg/ml
CTR	15.2	54.8	14.4 ^a	14.6	81.6 ^{ab}
O	17.2	48.4	15.2 ^{ab}	12.8	97.7 ^a
R	16.6	44.6	19.7 ^b	14.6	74.6 ^{ab}
OR	19.2	45.4	18.2 ^{ab}	17.2	64.5 ^{bc}
SEM	3.2	6.6	1.1	2.4	7.9
P	n.s.	n.s.	0.05	n.s.	0.05

CTR: control diet; O: diet supplemented with 0.2% oregano; R: diet supplemented with 0.2% rosemary, and OR: diet supplemented with 0.1% oregano + 0.1% rosemary. ^{a,b,c}: different letters in the same column indicate significant differences at $P < 0.05$. n.s. indicates not significant.

3.2 Pork Meat Analysis

3.2.1 Glutathione Peroxidase Activity

Figure 1 shows the different levels of GSHPx activity in pork samples according to the dietary treatment. A significant dietary effect was detected ($P < 0.001$). In fact, pigs fed the O diet, combined or not with R, produced pork with the highest GSHPx activity ($P < 0.05$). Although the R diet gave some increase in the GSHPx enzymatic activity, it did not vary significantly with respect to the control diet.

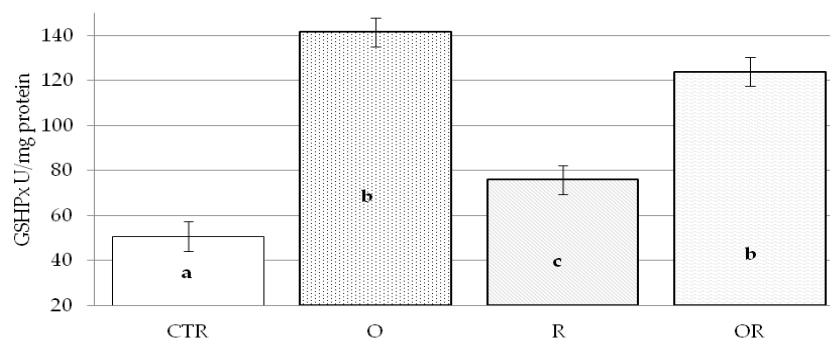


Figure 1. GSHPx Activity in Pork Samples according to Dietary Treatment

CTR: control diet; O: diet supplemented with 0.2% oregano; R: diet supplemented with 0.2% rosemary, and OR: diet supplemented with 0.1% oregano + 0.1% rosemary. ^{a,b,c}: different letters indicate significant differences at $P < 0.05$. Analysis was performed by Dunnett's t test in which the mean of each treatment was compared to the CTR mean.

3.2.2 Total Phenolic Content

Figure 2 shows the different levels of total phenolic content in pork samples according to dietary treatment. There was a significant dietary effect ($P < 0.005$). Pigs fed with a combination of O and R in the diet gave the highest total phenolic content in pork samples; whereas, both O and R administered singularly, influenced the total phenolic content to a lesser extent.

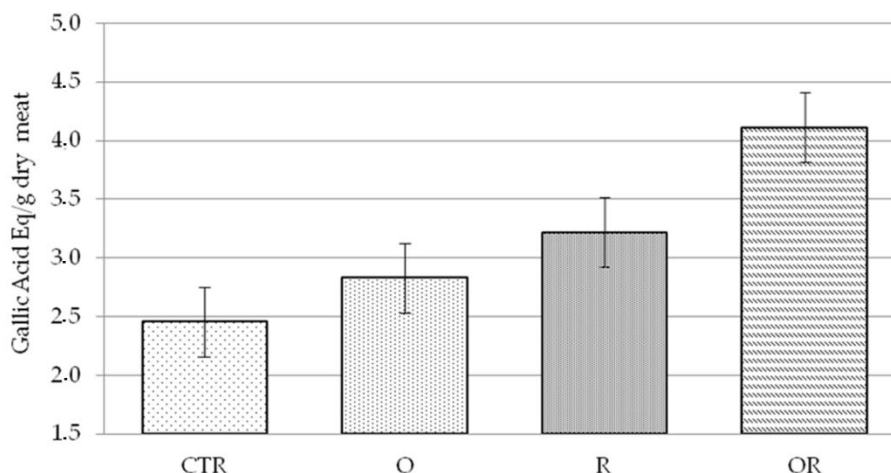


Figure 2. Total Phenolic Content in Pork Samples of Pigs reared with Different Diets

CTR: control diet; O: diet supplemented with 0.2% oregano; R: diet supplemented with 0.2% rosemary, and OR: diet supplemented with 0.1% oregano + 0.1% rosemary. ^{a,b,c}: different letters indicate significant differences at $P < 0.05$. Analysis was performed by Dunnett's t test in which the mean of each treatment was compared to the CTR mean.

3.2.3 Sensory Analysis - Ordering Test on the Basis of Preference

Figure 3 shows the preference rating of “capocollo” burgers evaluated by 60 consumers through the sum of the ranks. Although there were no significant differences between the different burgers, the preference order was: $R > OR > CTR > O$.

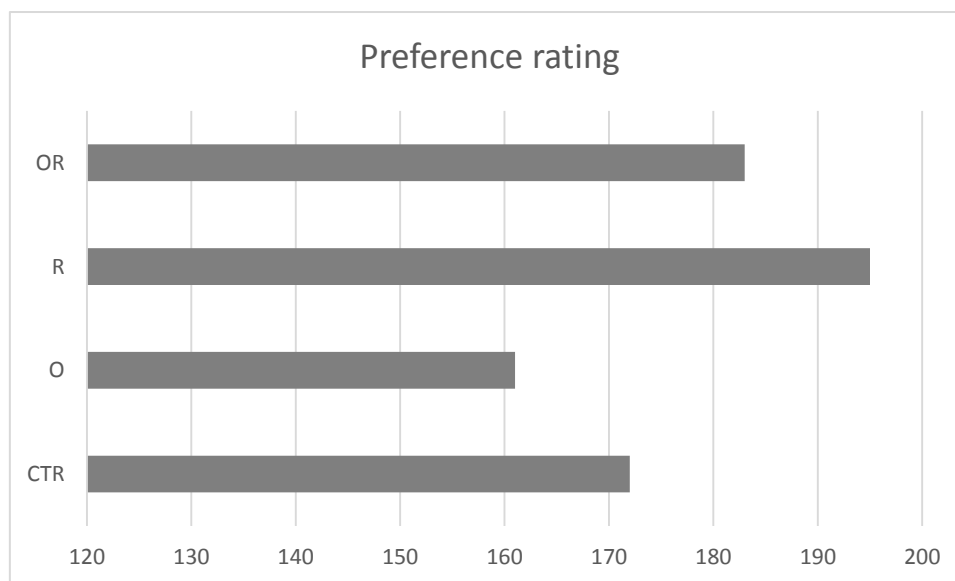


Figure 3. Ordering of Preference of Burgers obtained from Pigs fed Experimental Diets

CTR: control diet; O: diet supplemented with 0.2% oregano; R: diet supplemented with 0.2% rosemary, and OR: diet supplemented with 0.1% oregano + 0.1% rosemary. Data adapted from Beghelli et al. (2014).

4. Discussion

The dietary recommendations for humans discourage the consumption of meat with high concentrations of saturated FA (SFA) in favor of meat with more PUFA or mono-saturated FA. It is known that lipid metabolism in nonruminants, and thus also the carcass lipid quality, could be somewhat managed depending on the composition of the FA that are supplied through the diet as well as by *de novo* lipogenesis and environmental stressors (White, Richert, & Latour, 2013). However, as more unsaturated fats are supplied with food, and as the degree of unsaturation increases, the more does the consistency of the fat tissue change, gradually becoming soft (Cameron et al., 2000), which affects the technological properties of the meat. Furthermore, meat containing a high quantity of unsaturated fat tends to become rancid due to rapid oxidation, with the consequent degradation of sensory properties and reduction of its shelf life. Therefore, the nutritional and technological qualities of the meat are inversely related, but the combined use of aromatic plant extracts and CLA for their antioxidant activity or supply of polyunsaturated FA, respectively, could represent a good compromise for obtaining added nutritional value in the absence of early rancidity of animal meat.

Oregano (*Origanum vulgare* L.) and rosemary (*Rosmarinus officinalis* L.) contain important active compounds such as carvacrol and thymol (Adam, Sivropoulou, Kokkini, Lanaras, & Arsenakis, 1998; Calsamiglia, Busquet, Cardozo, Castillejos, & Ferret, 2007) or phenolic diterpenes, such as carnosol, carnosic acid, rosmanol, epirosmanol and isorosmanol (Cuppett & Hall, 1998), respectively, that have been particularly studied for their significant antioxidant properties (Botsoglou, 2004). Likewise, CLA isomers are known for their health promoting effects (Beluroy, 2002; Raes, De Smet, & Demeyer, 2004).

The aim of the present study was to verify whether the addition of these aromatic herbs is able to modify the trends of some redox parameters, not only in the blood but primarily in the meat. These herbs are very common in the Mediterranean diet and their traditional use has always provided a natural source of antioxidants and phenolic compounds (Ogce, Ceber, Ekti, & Oran, 2008).

Previously it was reported, as shown in Table 1, that the use of conjugated linoleic acid in the diet during the finishing phase combined or not with oregano and/or rosemary water extracts did not affect the blood redox status or the blood lipid parameters investigated (Beghelli et al., 2014).

Other authors (Forte et al., 2017; Ranucci et al., 2015), however, reported an influence of a diet supplemented with oregano and sweet chestnut wood extracts or oregano alone on pig blood AOP values. However, in those studies, animals were reared outdoors, so they probably had to cope with greater environmental stress (physical exercise, higher lipid oxidation for the energetic scope and antigenic pressure) that stimulated greater involvement of antioxidant mechanisms in the supplemented animals. However, when animals were reared indoors this 'plant extract effect' on the blood redox status did not occur (Forte et al., 2017).

However, in the present study, even if there was not an evident effect of supplemented diets on the blood antioxidant status, some variations were obtained in the meat.

Indeed, in meat samples the dietary administration of oregano and/or rosemary, together with a further 0.5% CLA addition in the finishing period, showed a significant effect on GSHPx activity and total phenolic content (Figures 1 and 2). This finding is in accordance with data previously reported (Forte et al., 2017; Ranucci et al., 2015), but given the design of the present study, it is not possible to determine whether the increased GSHPx enzymatic activity and the higher total polyphenolic content could be directly correlated to the effect of oregano and/or oregano extracts and CLA on transcriptional regulators that drive the increased expression of the gene of this antioxidant enzyme.

However, in a recent study Vitali et al. (2018) evaluated the effect of a diet enriched with both n-3 PUFA and antioxidants or polyphenols (source plant extracts) on the expression of genes involved in the lipid metabolism of pig muscle. They demonstrated that the addition of n-3 PUFA alone or n-3 PUFA and plant extract led to significant differences in the expression of the genes involved in lipid and energy metabolism in swine, leading to a mutual interaction between lipogenesis and oxidative processes in the *Longissimus thoracis* muscle of pigs.

Tous et al. (2012) reported that dietary conjugated linoleic acid (CLA) can affect the expression of both porcine lipogenic and regulatory genes in a tissue-specific manner. Moreover, some studies have reported that supplementing the diet with antioxidants or polyphenols can also influence nutrient digestibility, gut microbiota, expression of pro-inflammatory genes and meat quality traits in pigs (Fiesel, Gessner, Most, & Eder, 2014; Lipiński, Mazur, Antoszkiewicz, & Purwin, 2017; Zhang et al., 2015).

Therefore, it is possible to hypothesize that the combined administration of CLA and aromatic plant extracts could give rise to both a higher expression of the GSHPx gene (O and OR groups vs R and CTR) and the

distribution of plant polyphenols in the skeletal muscle (group R, in particular).

The immune profile evaluated in the present work revealed higher percentages of CD79⁺ cells in pigs supplemented with plant extracts, with significantly higher values in R vs CTR ($P < 0.05$). Furthermore, the IgG concentrations were significantly higher in O vs OR treated animals ($P < 0.05$).

The higher percentages of blood CD79⁺ cells in plant extract supplemented pigs vs CTR (significant difference only for group R) is difficult to interpret when evaluated in parallel with the IgG results. The B cell receptor complex (BCR) is composed of immunoglobulin (Ig) heavy and light chain on membrane with heterodimers of CD79a (Iga) and CD79b (Igb) responsible for mediating a signal downstream of the cell. Therefore, CD79⁺ supports B cell development, the BCR assembly and trafficking to the cell surface (Lee et al., 2008). There is no other pan-B cell marker for swine than intracellular CD79a detected by cross-reactive anti-human monoclonal antibodies (Sinkora & Butler, 2009). However, this marker is not able to differentiate between naïve, memory B cells, unactivated immature B cells and pro-B cells; furthermore, the expression of CD79a has also been observed in non-B-cell lineage including thymocytes and T cell lineage neoplasm (Lee et al., 2008). Maybe it is because of this wider distribution of CD79 that a clear correspondence between percentages of CD79⁺ cells and IgG concentrations has not been able to be determined. Furthermore, in the present study the percentages of CD4⁺, CD8⁺ and CD4⁺CD8⁺ cells did not vary between experimental and/or CTR groups. These data differ somewhat from what has been reported by other authors who observed a non-specific immune-stimulatory effect on T lymphocytes (CD4⁺, CD8⁺, CD4⁺CD8⁺), but not on immune B cells in pigs fed an oregano supplemented diet (Walter & Bilkei, 2004). However, in the cited work, pigs were growth-retarded, low-weight growing finishing animals, which may have exerted a different immune modulatory influence.

The increased total IgG values obtained in group O at Time 2 may indicate a more intense immune response to vaccine antigen or environmental antigenic pressure by these subjects. The total IgG values at the end of the trial were greater than those reported in the literature (Forte et al., 2017) although they could be related to the different procedures followed. However, dietary immune-modulators can amplify or decrease the magnitude of the reaction to a challenge through their effects on other immune cells (Dietert, Golemboski, Bloom, & Qureshi, 1991). Hashemipour, Kermanshahi, Golian, Veldkamp, & Hashemipour (2016) found an improved immune response in broilers fed a diet supplemented with thymol and carvacrol, characterized by an enhancement in hypersensitivity and an increase in total IgG and IgG anti-sheep red blood cells with a decreased heterophil to lymphocyte ratio. However, higher antibody titres in animals supplemented with plant extracts were also reported by other authors both in chickens (Franciosini et al., 2016; Hashemipour et al., 2013; Varshney, Dash, Goe, & Bhatia, 2013) and in ruminants (Ozkaya, Erbas, Ozkan, Baydar, & Aksu, 2017).

Finally, in the present study the sensory analysis revealed a greater appreciation of meat obtained from animals fed a diet supplemented with rosemary (lower when combined with O) and CLA; these findings disagree with data reported by Cullen et al. (2005) and with those of Janz, Morel, Wilkinson, & Purchas (2007) with regard to oregano.

5. Conclusions

In the present work dietary supplementation with oregano and/or rosemary (*Origanum vulgare* L. and/or *Rosmarinus officinalis* L.) in growing pigs receiving a CLA supplement in the finishing period was shown to modulate immune responses and to influence the antioxidant defense and polyphenol content of the meat. Plant extracts have a number of recognized biological properties (antioxidant, antibacterial, anti-fungal, immune modulatory activities) and active ingredients able to promote animal growth and improve feed efficiency, and are thus very promising. Greater understanding of the effects and mechanisms of the action of plant extracts and their possible ability to modulate genes involved in different metabolisms and modes of action is an important challenge which will aid in the design and application of the more effective use of essential oils in swine production.

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