Effects of supplementing with red wine solids on the oxidative status in pigs fed diets with different fatty acid profiles

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SUMMARY

Introduction - Nutritionists have suggested the partial replacement of the saturated fatty acids (SFAs) with polyunsaturated fatty acids (PUFAs) in animal diet. However, in the absence of antioxidant protection, PUFAs are subject to lipid peroxidation.
Material and methods - In this investigation, three groups of pigs were fed three different diets, namely an SFA-rich diet (palm oil-based), a PUFA-rich diet (corn oil-based), and a PUFA-rich diet (corn oil-based), supplemented with freeze-dried organic red wine solids (RWS). The objective of this work was to compare the effects of the three diets on serum biochemical parameters (albumin, glucose, triglycerides, cholesterol and alanine aminotransferase) and on serum oxidative status (antioxidant power by anti-ROMs test and reactive oxygen species by d-ROMs test, Diacron s.r.l).
Results and discussion - The results show that pigs fed a diet containing corn oil and RWS have a significantly higher serum antioxidant power, and a lower level of serum reactive oxygen species compared to the other two groups of pigs. Furthermore, in corn oil and RWS-fed pigs, alanine aminotransferase, cholesterol and triglycerides were significantly lower than in the other two groups of pigs.
Conclusions - A diet rich in PUFAs and polyphenols, derived from RWS, may be effective in increasing the body's antioxidant defenses, in decreasing reactive oxygen species in serum, and in improving serum biochemical parameters.

KEY WORDS

Pig, polyunsaturated fatty acid, lipid peroxidation, oxidative status, red wine.

INTRODUCTION

The strong link between diet and health has now been amply demonstrated by numerous scientific studies. Indeed, it seems that some diseases that pose a serious burden on the human health care system, such as cardiovascular disease, neurodegenerative diseases and cancer are also closely related to diet[1]. It follows that the attention of human and veterinary nutritionists has been focused on the research of nutritional strategies that can prevent and overcome this problem. In fact, many studies have shown that diets that are high in saturated fats can cause obvious metabolic imbalances in the body, which can contribute to the pathogenesis of the most common chronic degenerative human diseases[1]. The major cause of these metabolic alterations and tissue damage has always been attributed to the dietary intake of saturated fatty acids (SFAs), typical of food consumed in developed countries. Conversely, lipids containing polyunsaturated fatty acids (PUFAs) are considered beneficial for health due to their lipid-lowering and anti-inflammatory properties[2].
Current strategies for the prevention of these diseases have suggested the replacement of the SFAs content in the diet with PUFAs and the alteration of the relationship between omega-6 and omega-3 PUFAs to favor the latter, which is considered more beneficial for consumer health[2]. However, PUFAs, when administered in high amounts, may undergo lipid peroxidation in the body, as they are more susceptible to the effects of free radicals than SFAs[3]. To overcome this problem, it has therefore been recommended that the use of PUFAs in human and animal diets is accompanied by an adequate antioxidant coverage, also supplied through the diet. In fact, consuming diets rich in antioxidants is often associated with a decreased risk of degenerative diseases related to oxidative stress of the organism[4].
The scientific literature offers a wide range of examples of food and feed with the supplementation of antioxidants, which have resulted in a reduction of lipid peroxidation. Recent studies, for example, have shown that the juice of red grapes contains antioxidant substances, which are considered effective in preventing chronic-degenerative diseases in humans and animals[5]. Indeed, specific investigations carried out into red grapes and their juice have demonstrated that the antioxidant and anti-radical activity directly correlates with the complex matrix of polyphenols within the red grapes[5].
Polyphenols are a large group of organic compounds produced by plants to defend themselves against environmental stress, and are mostly found in the skin and seeds of grapes, along with tannins and resveratrol[6]. From a chemical point

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of view, polyphenols are molecules composed of multiple condensed phenolic cycles[6]. The most studied polyphenols (also called flavonoids) are epicatechin and procyanidins, quercetin and isoflavones. In recent decades, scientific research has correlated polyphenols, contained in plants used in the human diet, with the lower incidence of cardiovascular disease and inhibition of some types of cancer[7].

Based on this scientific evidence, the aim of our study was to compare the effect on oxidative and metabolic parameters of three groups of pigs fed different diets, containing SFAs, PUFAs and PUFAs supplemented with red wine solids (RWS), respectively. RWS are obtained by freeze-drying organic red wine, and they have a high content of phenolic compounds. These antioxidants are not only beneficial due to their intrinsic properties, but can also safeguard the PUFAs from peroxidation.

MATERIAL AND METHODS

Animals, diets and experimental design

The study was carried out at the Experimental Piggery of the Department of Veterinary Sciences at the University of Turin. A total of 18 pigs belonging to the same genotype, aged 3 months with a mean weight of 74.3±8.3 kg were randomly assigned to three different groups of six (three male and three female pigs each) with equal initial weight variability. Each group of pigs was placed in a box with dimensions 2.3 m x 3 m, under standard conditions at a temperature of 20 °C ± 2 °C, in an environment with artificial light and forced ventilation. Three isoproteic and isoenergetic diets were formulated, namely an SFA-rich diet (palm oil-based, PO), a PUFA-rich diet (corn oil-based, CO) and thirdly a PUFA-rich diet with RWS (3.6 g/kg) supplementation (corn oil-based, CO+RWS). The ingredients of the experimental diets are shown in Table 1.

Table 1 - Ingredients and chemical composition of the experimental diets (from Peiretti et al.[28]).

	PO	CO	CO+RWS
Ingredients (g/kg as-fed basis)			
Barley	800	800	800
Soybean meal	90	90	86.4
Wheat bran	60	60	60
Palm oil	30	–	–
Corn oil	–	30	30
Red wine solids	–	–	3.6
Vitamin mineral premix[#]	17	17	17
Lysine	3	3	3
Chemical composition (g/kg as-fed basis)			
Dry matter	891	911	890
Crude protein	141	140	143
Ether extract	42	46	47
Ash	48	46	50
Gross energy (MJ/kg DM)	18.9	18.3	18.6

PO = diet with palm oil; CO = diet with corn oil; CO+RWS = diet with corn oil supplemented with red wine solids (RWS).
[#] Composition of the vitamin-mineral premix (per kg of diet): vit. A, 312,000 IU; vit. D3, 48,800 IU; α-tocopheryl acetate, 68 mg; vit. B1, 39 mg; vit. B2, 125 mg; vit. B6, 39 mg; vit. B12, 0.75 mg; vit. PP, 623 mg; biotin, 0.75 mg; choline chloride, 12.500 mg; folic acid, 40 mg; D-panthotenic acid, 500 mg; sodium menadione bisulphate, 25 mg; lysine, 18.300 mg; Zn, 275 mg; Fe, 275 mg; Cu, 25 mg; Mn, 17 mg; J, 800 µg; Se, 300 µg.

Diets were administered in flour-form, to avoid possible pelleting, which would result in deterioration of heat-labile nutrients, in particular the RWS. The feed was stored in silos, sheltered from light, to avoid auto-oxidation of the lipid components, and offered *ad libitum* for the duration of the experiment. After 8 weeks, animals (mean live weight 116.8±17.1 kg) were slaughtered according to current standards.

Dosage and analysis of RWS

To determine the optimal dosage of the RWS that would have the greatest effect on pig serum oxidative status, when added in the CO+RWS diet, we consulted the most recent scientific literature.

The content of total polyphenols, anthocyanins and total flavonoids, proanthocyanidins and flavans reactive to vanillin in the RWS were determined as reported by Di Stefano et al.[8]. The antioxidant activity was assessed by spectrophotometry, and expressed as units of equivalent ascorbic acid[8].

LC-MS analysis

RWS (25 mg) were diluted in 1 mL of methanol/water 50:50 v/v. The chromatographic separations were run on an Ultimate 3000 HPLC (Dionex, Milan, Italy) coupled to a Surveyor PDA UV detector and a high resolving power mass spectrometer (HRMS) LTQ Orbitrap (Thermo Scientific, Rodano, Italy), equipped with an atmospheric pressure interface and an ESI ion source. Samples were analyzed using an RP C18 column (Phenomenex Luna 150 × 2.1 mm, 3 µm particle size) at a flow rate of 200 µL/min. A gradient mobile phase composition was adopted: 95/5 to 40/60 in 25 min, 0.05% formic acid/acetonitrile. The injection volume was 20 µL. The tuning parameters adopted for the ESI source were: positive ion mode - source voltage 4.5 kV, capillary voltage 17.00 V, and tube lens 45 V; negative ion mode - source voltage 4.5 kV, capillary voltage 17.00 V, and tube lens 45 V. The heated capillary temperature was maintained at 265°C. The mass accuracy of the recorded ions (*vs* the calculated ones) was ± 5 mmu (milli-mass units).

Analyses were run using both full MS (100-1000 m/z range) and MS/MS acquisition in the positive and negative ion modes. The protonated molecular ions were 291.0867 m/z for catechin and isomers. Negative deprotonated ions were 289.0694 m/z for catechin and isomers, 465.1033 m/z for catechin-glucuronides and 479.1190 m/z for methylcatechin-glucuronide (Figures 1, 2 and 3).

Blood collection and biochemical assay

Samples of pig blood were collected via jugular puncture at 150 days, by Veno Jet tube (Terumo, Leuven, Belgium), with a 19-Gauge pin according to Good Veterinary Practices. The blood was allowed to clot and was then centrifuged at 3000 rpm for 10 min at room temperature. The serum was then separated and stored at -70° C until analysis.

Biochemical analyses were carried out in the laboratories of the Department of Veterinary Sciences at the University of Turin. The ILab Aries analyzer (Instrumentation Laboratory, Milan, Italy) was used to analyze serum levels of cholesterol, triglycerides, liver enzyme alanine aminotransferase (ALT), albumin, and glucose.

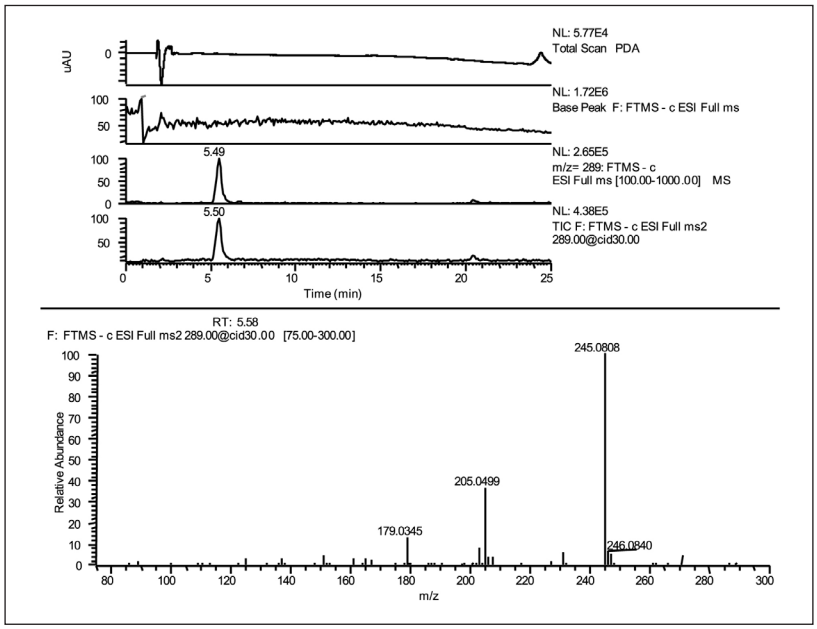

Figure 1
Standard (±)-Catechin: LC-UV and negative ion mode LC-MS chromatogram and spectrum. From the top, the following traces are shown:
1) 240-400 nm UV chromatogram;
2) negative ion mode sum chromatogram;
3) HRMS 289 *m/z* selected mass chromatogram;
4) 289 *m/z* MS/MS chromatogram;
5) 289 *m/z* MS/MS spectrum (75-300 *m/z* range).

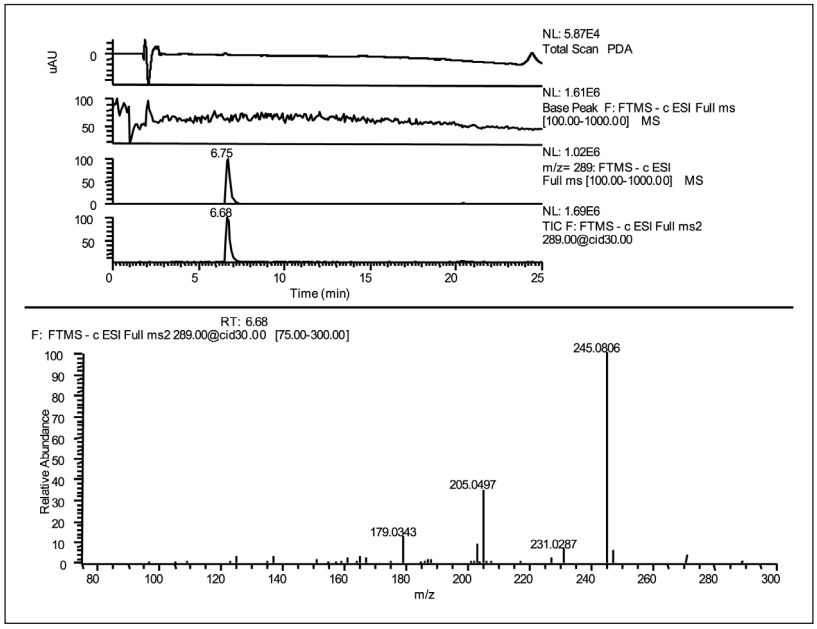

Figure 2
Standard (-)-Epicatechin: LC-UV and negative ion mode LC-MS chromatogram and spectrum (see Figure 1 for details).

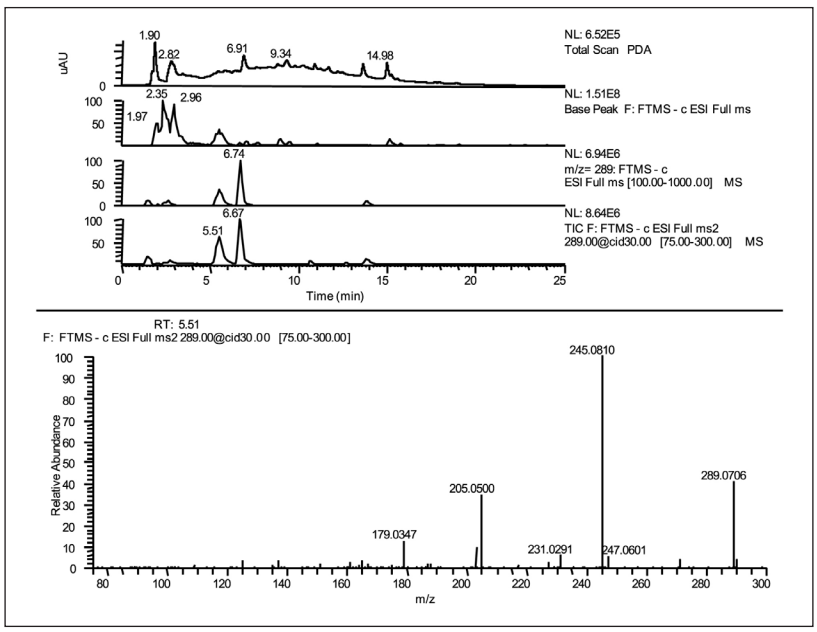

Figure 3
Sample of red wine solids: LC-UV and negative ion mode LC-MS chromatogram and spectrum (see Figure 1 for details).

Analysis of the oxidative status of pig serum

Serum antioxidants were evaluated by the anti-ROMs test, carried out using FREE-CARPE DIEM equipment (Diacron s.r.l., Grosseto, Italy). This method exploits the ability of antioxidants to reduce ferric iron to ferrous iron, giving rise to a red-purple coloration, due to the reaction with αα'-dipyridyl. Color intensity increases proportionally according to the quantity of iron reduced by the antioxidants present in the sample. This test enables discrimination between the concentration of the so-called "fast antioxidants", determined at the start by the instrument, i.e. those which are fast-acting, such as Vitamin C or Vitamin E, and the concentration of "slow antioxidants", subsequently determined by the instrument, such as thiol-SH groups, uric acid, polyphenols and anthocyanins. Results are expressed in μEq of reduced iron/liter using ascorbic acid as a standard according to Giongo et al.[9].

Reactive oxygen metabolites (ROMs) were evaluated using the d-ROMs test (Diacron s.r.l., Grosseto, Italy), as described by Benedetti et al.[10]. This test is based on the principle that oxygen free radicals are atoms that possess one or more unpaired electrons in one of their outer orbitals; due to their extreme reactivity, free radicals tend to react with any organic molecule that they come into contact with, generating derivatives or oxygen-reactive metabolites. These latter, equipped with oxidizing power, are more stable free radicals than their predecessors, and can therefore be quantified. In the d-ROMs test, the ROMs (primarily hydroperoxides) contained in the biological sample to be analyzed, generate, in the presence of iron (released from plasma proteins by an acidic buffer), by the Fenton reaction, alkoxyl radicals and peroxyl radicals, which, by reacting with an aromatic amine, oxidize the latter, transforming it into a pink-colored derivative that can be photometrically quantified. The intensity of the color that develops is directly proportional to the ROMs concentration, according to Lambert-Beer's law.

The d-ROMs test results are expressed in arbitrary units known as "Carratelli Units" (CARR U), according to the following formula:

$$\text{CARR U} = F(\Delta\text{Abs} / \text{min})$$

where F is a correction factor with an assigned value (approximately 9000 at 37 °C, according to the results obtained with the standard); (ΔAbs/min) are the mean differences of the absorbances recorded at 1, 2, and 3 min.

Statistical analyses

Statistical analyses were performed using the SPSS software package (version 11.5.1 for Windows, SPSS Inc., USA). Data obtained were analyzed by the General Linear Model using one-way ANOVA, with diet as the main factor. Means were compared by Duncan's test, and P-values<0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

The RWS shows a high antioxidant power, mainly attributed to the high content of total polyphenols and flavonoids (Table 2). The beneficial properties of wine have been acknowledged for centuries, but are flanked by the toxicity and adverse effects of ethanol[5]. RWS have a content of phenolic compounds that is about 20 times higher than that of the corresponding wine, with consequent intensification of the antioxidant power, without the negative presence of alcohol and sulfur dioxide. Therefore, RWS may be a useful dietary supplement for animal studies and human clinical trials. For example, Clifford et al.[11] tested the hypothesis that dehydrated-dealcoholized RWS, when consumed as part of a precisely defined complete diet, can delay tumor onset in transgenic mice that spontaneously develop externally visible tumors, without carcinogen pre-treatment.

Table 2 - Chemical composition, catechin and epicatechin content, antioxidant power (as equivalent ascorbic acid) of organic red wine solids (adapted from Peiretti et al.[28]).

Total polyphenols (mg/g)	49.5
Total anthocyanins (mg/g)	1.03
Total flavonoids (mg/g)	43.3
Proanthocyanidins (mg/g)	58.0
Flavans reactive to vanillin (mg/g)	32.3
Catechin (mg/g)	0.52
Epicatechin (mg/g)	0.18
Antioxidant power	37.0

The polyphenol species present in RWS were characterized by HPLC-UV-HRMS. Figures 1, 2 and 3 show UV and ESI-MS chromatograms, together with MS/MS spectra. Both catechin and epicatechin were recognizable in the RWS sample (Figure 3), whereas no unmodified or metabolized polyphenol species were detected in animal tissues, in agreement with their antioxidant reactivity. Tsang et al.[12] studied the absorption, metabolism and excretion of flavan-3-ols and procyanidins after the ingestion of a grape seed extract by rats. They reported that methylated glucuronide metabolites were detected in liver and kidney. However, these authors gave to rats a concentrate extract of grape seed in experimental conditions, whereas in our work we have gave to pigs the RWS as a dietary supplement under farming conditions.

Furthermore, Donovan et al.[13] measured the levels of a flavonoid catechin and its metabolites in plasma of some volunteers that consumed 120 mL per day of red wine or dealcoholized red wine. These authors showed that catechin was present almost exclusively as metabolites (primarily as a glucuronide conjugate) in human plasma after consumption of red wine, and their levels were independent by ethanol presence.

In the field of animal and human nutritional research, pig plays a key role because it can be used as a model for humans, due to the similarity of the two species in the anatomy and physiology of the digestive system and in metabolic processes[14]. Therefore, it is conceivable that the results of alimentary trials conducted on pigs can be transferable to the human species[15].

The antioxidant activity and the reactive oxygen species content in the serum of pigs fed with the three experimental diets are shown in Table 3.

Regarding the antioxidant power contributed by the fast antioxidants, in particular Vitamins E and C, and the slow antioxidants, such as thiol-SH groups, uric acid, polyphenols and anthocyanins, the values obtained from the three diets

Table 3 - Concentrations (mean ± standard error) of antioxidants and reactive oxygen metabolites in the serum of pigs, at 150 days.

Group code	PO	CO	CO+RWS
n. of Pigs	6	6	6
Fast antioxidants§	16.3±1.8[a]	26.7±1.9[b]	34.2±2.6[c]
Slow antioxidants*	184.5±6.0[a]	209.9±5.7[b]	229.2±5.1[c]
ROMs#	629.5±7.5[b]	630.3±6.9[b]	582.0±7.5[a]

PO = diet with palm oil; CO = diet with corn oil; CO+RWS = diet with corn oil and supplemented with red wine solids (RWS).
§ First concentration of antioxidants (μEq of Fe^{+++}/l) determined by anti-ROMs test.
* Second concentration of antioxidants (μEq of Fe^{+++}/l) determined by anti-ROMs test.
# Reactive oxygen metabolites expressed in arbitrary units known as "Carratelli Units" (CARR U).
[a,b,c] Means with unlike superscripts within a row differ ($P<0.01$).

differ in an highly significant way ($P<0.01$) throughout the experiment. In particular, the antioxidant power of the serum of pigs fed with the CO+RWS diet is significantly higher than that of pigs fed with the diet containing only corn oil. The serum of the CO group, however, has an antioxidant activity that is significantly highly than the serum of the PO group. These results demonstrate that the CO diet induces a greater antioxidant capacity in the serum of pigs compared with the PO diet, and also reveal the additional antioxidant power contributed by the RWS of the CO+RWS diet. The highly significant results that we found when evaluating the antioxidant activity show that RWS intake can be suitable in all cases where it is necessary to increase the body's antioxidant defenses.

Brenes et al.[16] evaluated the effect of grape pomace concentrate on antioxidant activity in chickens**.** These authors showed that the supplementation of grape pomace concentrate had an antioxidant potential as effective as vitamin E. On the basis of these observations, they concluded that grape pomace concentrate could be a new source of antioxidant in animal nutrition.

Choi et al.[17] studied the effects of supplementation with grape seeds extract and grape peels extract on the activity of antioxidant enzymes and on the degree of lipid peroxidation in serum and liver tissue of rabbits fed on high cholesterol diet. In liver tissues, total glutathione contents, glutathione peroxidase and catalase activity resulted significantly higher in rabbit fed diets supplemented with grape seed extract than animal fed diet without grape pomace supplementation. The level of malondialdehyde (MDA) was lower in the serum of rabbits fed with grape seed extract or grape peel powder plus cholesterol than in the serum of rabbits fed with only cholesterol. It is therefore likely that grape seed extract decreased the effects of the oxidative stress induced by cholesterol diet. Therefore, grape pomace (grape seed extract and grape peel powder) supplementation was functional to activate the antioxidant enzyme system and to prevent damage connected to hypercholesterolemia.

In our work the reactive oxygen metabolites (ROMs) values can be seen in Table 3. The values obtained from the d-ROMs test in pigs fed the CO diet (rich in PUFA) were similar to those found in pigs fed the PO diet, which may be due to the action of the antioxidants present in the corn oil, some of which (for example, Vitamin E) are able to preserve the oxidation. The lowest ROMs values of the three different groups were found in pigs fed the CO+RWS diet, which means that the RWS supplement further protects, PUFA of CO diet and the body, from the formation of reactive oxygen species. By analyzing the total oxidative status (antioxidant activity and reactive oxygen metabolites), it can be concluded that the RWS additive therefore plays a protective role, and prevents the generation of reactive oxygen species in diets rich in PUFA. Expressing our data in mg H_2O_2/dL by the following relation: 1 CARR U = 0.08 mg H_2O_2/dL, we found d-ROMs values similar to those reported in crossbred pigs by Brambilla et al.[18].

Koga et al.[19] determined the antioxidative potential of rat plasma by oral administration of proanthocyanidin-rich extract from grape seed. These authors suggested that the intake of proanthocyanidins, the major polyphenols in red wine, increased the resistance of blood plasma against oxidative stress. This shows the physiological functions of plant food, including wine, through *in vivo* antioxidant capacity.

Simonetti et al.[20] evaluated the effect of supplementation with procyanidins from *Vitis vinifera* on human markers of oxidative stress and suggested that dietary procyanidins exerted their antioxidant protection *in vivo* by sparing liposoluble vitamin E and reducing DNA oxidative damage.

The biochemical profile of pigs at 150 days is shown in Table 4. Regarding the serum albumin levels, there were no differences between the three groups of pigs in the period evaluated. Serum albumin levels were observed in the standard, which shows that the pigs had a normal nutrition state, in relation to the protein fraction.

In addition, there were no significant differences in blood glucose levels between the three groups of pigs. Moreover, blood glucose levels were in the normal range, confirming that the pigs showed no signs of predisposition to diabetes or hyper-insulinemia.

The levels of serum triglycerides were significantly different between the three groups ($P<0.05$). The triglyceride level in the serum of pigs fed with the CO+RWS diet was significantly lower than the triglyceride level found in the CO group. In addition, the CO group demonstrated a significantly lower serum triglyceride level than in the PO group.

The enhanced removal of serum triglycerides, due to an elevated consumption of PUFAs (present in high quantities in corn oil), is related to an increase in the activity of the lipoprotein lipase, an enzyme localized in the vascular endothe-

Table 4 - Biochemical parameters (mean ± standard error) in the serum of pigs, at 150 days.

Group code	PO	CO	CO+RWS
n. of Pigs	6	6	6
Albumin (g/dL)	3.25 ± 0.13	3.48 ± 0.12	3.60 ± 0.19
Glucose (mg/dL)	86.5 ± 1.4	87.5 ± 3.2	87.7 ± 2.3
Triglycerides (mg/dL)	55.5 ± 3.6[a]	41.7 ± 3.2[b]	31.3 ± 2.6[c]
Cholesterol (mg/dL)	107.8 ± 3.4[a]	94.7 ± 3.0[b]	85.5 ± 2.8[b]
ALT (U/L)	56.8 ± 2.8[a]	44.8 ± 2.7[b]	39.5 ± 2.1[b]

PO = diet with palm oil; CO = diet with corn oil; CO+RWS = diet with corn oil and supplemented with red wine solids (RWS); ALT = alanine amino transferase.
[a,b,c] Means with unlike superscripts within a row differ ($P<0.05$).

lium, which appears to be the main determinant of triglyceride removal of the peripheral circulation. PUFAs are therefore able, by influencing secretion and triglyceride metabolism, to reduce the level of triglycerides[2].

Experimental and clinical studies have documented that high amounts of PUFAs suppressed the hepatic production of triglyceride-rich lipoproteins, and tended to accelerate the removal of serum triglycerides. This function occurs especially at hepatocyte level, and it is linked to a reduced production of apolipoprotein B, the protein component of low-density lipoprotein (LDL). This leads to a reduced entry of circulating triglycerides[21].

The recent study by Wang et al.[22] has shown that polyphenols are able to reduce serum levels of triglycerides and MDA (a terminal product of lipid peroxidation) in hyper-cholesterolemic animals, and to increase the antioxidant capacity. Similar results were obtained with the polyphenolic extract of the outer part of the peanut[23].

Serum cholesterol is significantly higher in the PO group compared to the CO and CO+RWS groups, while there is no significant difference in cholesterol levels evident between the CO and CO+RWS groups.

Many researches indicate that dietary PUFAs decrease serum cholesterol in animals and humans. According to Stewart et al.[24] the manipulations of diets that decreased the total fat content or substitute unsaturated vegetable oils for saturated fats resulted, in humans, in a reductions of total plasma and LDL cholesterol concentrations. Monounsaturated fatty acids and PUFAs are reported to decrease total cholesterol, LDL cholesterol, and high-density lipoprotein (HDL) cholesterol by several mechanisms. These mechanisms included the decrease of LDL apolipoprotein B production rates and increased HDL apolipoprotein catabolism. Many studies reported that PUFAs increase LDL apolipoprotein B catabolism and may also decrease total and LDL cholesterol modifying the oxidation-reduction or phosphorylation state of a nuclear transcription protein that cause the synthesis of fatty acid synthase, acetyl-CoA carboxylase, or stearoyl-CoA desaturase[24].

The hypo-cholesterolemic and antioxidant effects of wines has been reported in the study by Suh et al.[7], who investigated rats fed on a hyper-cholesterolemic diet for twelve weeks. These authors found a positive effect on animals, and concluded that the antioxidant and antiatherogenic effects of dealcoholized wines can be attributable to their polyphenolic fraction.

Red wine and its polyphenolic constituents have some lipid- and lipoprotein-lowering effects. Agouni et al.[25] showed that red wine reduces circulating triglycerides and total cholesterol levels, as well as the ratio between LDL and HDL cholesterol in obese rats compared to lean rats of control. It may be explained because polyphenols may reduce cholesterol absorption due to the interaction of these compounds with cholesterol carriers and transporters present across the brush border membrane.

In this current study, we have shown a similar trend for cholesterol as for the liver enzyme ALT. In a recent study, Saligram et al.[26] found that elevated ALT levels were significantly correlated with abnormal hepatic functions, along with high levels of triglycerides and LDL cholesterol. Given that high levels of ALT are associated with an abnormal liver function, it can be concluded that, in our study, the CO diet and particularly the CO+RWS diet, can induce a protective effect on the animal liver.

Thus, the increase in antioxidant activity in the serum of pigs fed with the CO+RWS diet suggests that consumption of the studied RWS is suitable in all cases where it is necessary to increase the body's antioxidant defences. Moreover, in our study, the CO and CO+RWS diets have hypocholesterolemic and hypotriglyceridemic effects. Although medical research has resulted in the production of highly efficient and readily-available drugs to counteract hyper-triglyceridemia and hyper-cholesterolemia, these cannot be administered to all patients due to their potential side effects[27]. Compared with these medications, herbal products have been the focus of great attention in recent years as they are considered non-toxic and free from side effects[27].

CONCLUSIONS

The PUFAs are considered healthy for their lipid-lowering and anti-inflammatory properties; but, when they were administered in high amounts, may undergo lipid peroxidation. To overcome this problem it is recommended that the use of PUFAs in human and animal diets is joined with an adequate antioxidant coverage, also supplied through the diet.

In this paper, we have shown, using the pig as an animal model, that the adoption of a diet rich in PUFAs and polyphenol derived from RWS can be effective in increasing the body's antioxidant defenses, in decreasing the levels of serum ROS, triglycerides and cholesterol, and in better preserving liver function. Although the animals we used had no pathological conditions, the results have shown that these diets can be recommended for the prevention of dyslipidemia, hypercholesterolemia, oxidative stress and the most common related diseases.

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