Performance and apparent digestibility of growing pigs fed diets with different fat sources and supplemented with organic red wine solids

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Abstract

Nutritional guidelines for consumer health suggest increasing the polyunsaturated fatty acids (PUFA) content of meat, through animal feed. However the replacement of saturated fatty acids (SFA) with PUFA creates problems of conservation of the meat, due to its susceptibility to lipid peroxidation. This problem can however be overcome by supplementing the animal diet with natural substances that have an antioxidant action. In this study we have evaluated the effect of diets containing two vegetable oils with different fatty acid profiles and the supplementation of red wine solids (RWS) by using an alcohol-free phytocomplex (Liofenol^{TM}) obtained from freeze-dried organic red wine on the digestibility of diets and on the performance of growing pigs. Three pig diets were formulated: a diet rich in SFA (palm oil-based); a diet rich in PUFA (corn oil-based); and a diet rich in PUFA (corn oil-based) but supplemented with RWS.

Results indicate that the studied diets can be used for feeding pigs without a decrease in the digestibility of nutrients or the reduction of animal growth performance, thus representing the starting point for further studies on food quality.

Key words: *digestibility, feed intake, growth rate, phytocomplex, red wine solids*

Introduction

The strong link between diet and health has now been widely demonstrated by numerous scientific studies. Many studies have indeed confirmed that a high saturated fat content of the diet contributes to the pathogenesis of the most common chronic degenerative human diseases (Gaudet et al 2012). Saturated fatty acids (SFA), typical of the alimentation of developed countries, have always been considered the major cause of these metabolic alterations and damage to the organism. Current strategies for the prevention of these diseases suggest replacement of the SFA content in the diet with polyunsaturated fatty acids (PUFA) and to improve the relationship of n-6 and n-3 PUFA in favor of the latter, being the most advantageous for consumer health (Tajik et al 2012).

On the basis of these considerations, new strategies are currently being developed for the production of food of animal origin, in the scope of novel prevention of the most common chronic degenerative human diseases. Regarding the improvement of food of animal origin, animal nutrition has an important role because it is a fundamental tool that can provide healthier meat products available to consumers.

By means of the animal diet, it is possible to control the nutritional characteristics of products of animal origin, in order to meet the needs expressed by consumers and current medical nutritional indications. In the case of pork meat, rich in SFA, dietary guidelines for consumer health suggest increasing the PUFA profile of the meat, through the animal feed. However in pig meat, replacement of SFA with PUFA causes problems of preservation due to their susceptibility to peroxidation, in the absence of adequate antioxidant protection (Marnett 2002). Recent studies indicate that it is possible to overcome this problem with the use of natural substances with antioxidant action supplemented in the animal diet. The literature offers a wide range of examples of animal diet formulations with the addition of different antioxidants that have given good results on the protection of fat from phenomena of peroxidation (O'Neil et al 1999; Grau et al 2001; Sáyago-Ayerdi et al 2009).

Many researchers have also described and demonstrated the antioxidant effect of polyphenols derived from wine and its products and their beneficial effects on consumer health (Williamson and Holst 2008; Viveros et al 2011; Suh et al 2011). Recent studies have shown that antioxidants in the juice of red grapes are responsible for the beneficial effects of red wine in the prevention of the most common human and animal chronic degenerative diseases (Geleijnse et al 2002). The inhibitory effects of wine on heart disease, arteriosclerosis, and cancer have been frequently reported (Pace-Asciak et al 1995; Clifford et al 1996; Hayek et al 1997; Miyagi et al 1997; He et al 2008).

Most phenolics, however, are gradually converted during winemaking to other phenolic species. Wine phenolics are divided into flavonoids and non-flavonoids. The family of flavonoids mainly includes flavonols, flavanols, and anthocyanins, whereas non-flavonoids mainly include phenolic acids (benzoic and hydroxycinnamic acids) and stilbenes (Roussou et al 2004). Noteworthy is the antioxidant action of red wine solids (RWS) an alcohol-free phytocomplex obtained from freeze-dried organic red wine (Liofenol^{TM}).

The objective of this study has been to evaluate the effect of diets containing vegetable oils with different fatty acid profiles and the effect of RWS supplementation (added only to the diet rich in PUFA) on the performance and digestibility of diets in growing pigs.

The general assessment of diet digestibility and performance of livestock is essential to determine whether the formulated diets can actually be used in animal feed in livestock production. The use of different PUFA and antioxidants in the animal diets is based on the assumption that they do not depress the performance parameters. Therefore, further studies can establish the benefits of dietary supplementation of PUFA in place of SFA on the meat quality and the action of RWS on the prevention of any peroxidative phenomena due to an increased dietary intake of PUFA.

Materials and Methods

Animals and experimental diets, growth trial

The study was carried out at the CISRA (Centro Interdipartimentale Servizio Ricovero Animali) of the University of Turin. A total of 18 pigs belonging to the same genotype, aged 3 months with a mean weight of 74 kg were randomly assigned to three different groups of six (three male and three female pigs each) with equal initial weight variability. Each animal group was placed in a box of 2.3 x 3 m, under standard conditions at a temperature of 20±2°C, in an environment with artificial light and forced ventilation.

Three different diets were formulated: a diet rich in SFA (palm oil-based), a diet rich in PUFA (corn oil-based) and finally a diet rich in PUFA (corn oil-based) but supplemented with 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 117 kg) were slaughtered according to current standards.

Digestibility trial

The apparent digestibility of the three diets was determined in the final week of a growing trial that lasted 56 days. Feces of individual pigs were collected daily during a 4-day period and stored at −20° C for the determination of apparent digestibility, following the recommendations of Moughan et al (1991). Feces were collected at approximately 09:30 A.M. The daily feces of individual pigs were weighed and placed in a two-layer plastic bag to prevent loss of moisture. Frozen samples were individually mixed thoroughly and pooled, ground in a homogenizer (Tecator, Herndon, VA, USA) and representative samples were then weighed on aluminum foil pans, dried in a draft oven at 80 °C to constant weight and stored for chemical analysis.

Analytical procedure

Feed and feces were analyzed for dry matter (DM; cod 930.13), ash by ignition to 550 °C, crude protein (CP; cod. 954.01), and ether extract (EE; cod. 945.16) according to the AOAC method (Association of Official Analytical Chemists 1990). Gross energy (GE) was determined using an adiabatic bomb calorimeter (IKA C7000, Staufen, Germany). The acid insoluble ash (AIA) content was determined according to the method of Van Keulen and Young (1977). All analyses were performed in duplicate.

Determination of digestibility coefficients

Digestibility coefficients were calculated following the indirect digestibility method using AIA as an inert marker. Calculation of the digestibility of DM was as follows:

$$\text{DM digestibility (\%)} = (1 - A/B) \times 100$$

where A and B are the AIA concentrations in the feed and feces, respectively.

Digestibility of the other nutrients (X) was calculated as follows:

$$\text{Digestibility (X in \%)} = (1 - A/B \times XB/XA) \times 100$$

where XA and XB are the concentrations of X in the feed and feces, respectively.

Statistical analysis

Statistical analyses were performed using the SPSS software package (version 11.5.1 for Windows, SPSS Inc., USA). Data obtained in the apparent digestibility determination 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 significant.

Results and Discussion

Three isoproteic and isoenergetic diets were formulated with the following ingredients: barley, soybean meal, wheat bran, vegetable oil (palm oil or corn oil), mineral vitamin supplement and lysine; their percentages in the diets are listed in Table 1. The third diet was supplemented with 3.6 g/kg of RWS.

Table 1. Ingredients (%) of the experimental diets

	PO	CO	CO+RWS
Barley	80	80	80
Soybean meal	9	9	8.64
Wheat bran	6	6	6
Palm oil	3	-	-
Corn oil	-	3	3
Red wine solids (RWS)	-	-	0.36
Vitamin mineral premix[a]	1.7	1.7	1.7
Lysine	0.3	0.3	0.3

PO= *diet with palm oil;* ***CO=*** *diet with corn oil;* ***CO+RWS=*** *diet with corn oil and supplemented with red wine solids (RWS)*

[a] *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, .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*

Table 2 shows the chemical composition of RWS and the corresponding antioxidant power.

Table 2. Chemical composition (mg/g, mean ± S.E.) and antioxidant power (eq. Ascorbic acid) of organic red wine and organic red wine solids (RWS) and their comparison (data were kindly provided by the CRA-ENO, Asti, Italy).

	Red wine	**RWS**	**RWS/Red wine**
Total polyphenols	2.23±0.01	49.5±0.11	22.2
Total anthocyanins	0.06±0.001	1.03±0.05	17.9
Total flavonoids	2.09±0.01	43.3±4.42	20.7
Proanthocyanidins	2.61±0.02	58.0±3.53	22.3
Flavans reactive to vanillin	1.58±0.01	32.3±0.17	20.4
Antioxidant power	1.62±0.01	37.0±2.40	22.8

The content of total polyphenols, anthocyanins and total flavonoids, proanthocyanidins and flavans reactive to vanillin were determined as reported by Di Stefano et al (1989).The antioxidant activity was determined by spectrophotometry and expressed in equivalent ascorbic acid, as reported by Kim et al (2002).

The beneficial properties of wine have been acknowledged for centuries; the benefits of wine, however, are flanked by the toxicity and adverse effects of ethanol (Lamont et al 2012). RWS have a content of phenolic compounds of 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 (1996) tested the hypothesis that dehydrated-dealcoholized RWS, when consumed as part of a precisely defined complete diet, would delay tumor onset in transgenic mice that spontaneously develop externally visible tumors without carcinogen pretreatment.

Table 3 shows the proximate analysis of the three diets.

Table 3. Chemical composition (%) of the experimental diets

	PO	**CO**	**CO+RWS**
Dry matter	89.1	91.1	89.0
Organic matter	95.2	95.4	95.0
Ash	4.8	4.6	5.0
AIA	0.55	0.57	0.60
Crude protein	14.1	14.0	14.3
Ether extract	4.2	4.6	4.7
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 organic red wine solids (RWS)*

The apparent digestibility coefficients obtained from the digestibility trials are reported in Table 4.

Table 4. Percent of the apparent digestibility coefficients obtained using AIA (acid insoluble ash) as the internal marker

	PO	**CO**	**CO+RWS**	**SEM**	**Prob.**
Number of observations	6	6	6		
Dry matter	74.2	75.5	73.8	0.90	0.78
Organic matter	76.1	77.1	75.5	0.88	0.78
Crude protein	63.0	65.9	66.1	1.65	0.74
Ether extract	41.1	48.5	43.4	2.78	0.58
Gross energy	73.5	75.0	73.5	0.95	0.79

PO*= diet with palm oil;* ***CO****= diet with corn oil;* ***CO+RWS****= diet with corn oil supplemented with organic red wine solids (RWS)*

The statistical analysis of data shows that there are no significant differences between the groups of pigs fed the three different diets. This indicates that, the use of vegetable oil and the supplementation with RWS did not result in adverse changes in diet digestibility in pigs.

The digestion coefficient of animal feeds and diets can be determined using different *in vivo* or *in vitro* methods. The *in vivo* digestibility methods are most commonly determined by total collection of feces or by markers. Total collection method is time consuming and costly and it involves some practical problems. Measuring the concentration of markers found in both the diet and in the feces, eliminates the need for the compulsory use of the total collection of feces, and thereby facilitates digestibility studies in the field (McCarthy et al 1974). Ly et al (2002) studied, in Mong Cai pigs, the interrelationship between the direct method of digestibility estimation, which implies the total collection of feces and feed, and the indirect method, using the technique recommended by Van Keulen and Young (1977) for the estimation of nutrient digestibility in ruminants. These authors suggested that the indirect method of digestibility determination by using AIA as a reference substance can be used for the nutritional evaluation of diets given to young pigs. The method suggested by Van Keulen and Young (1977) has also been used in the evaluation of Cambodian feed for pigs (Ly et al 2002; Nguyen Thi Thuy and Ly 2002). Several studies have also reported comparisons of apparent fecal digestibility, determined using the digestibility markers Cr_2O_3 and AIA (Moughan et al 1991; Bakker and Jongbloed, 1994). However, problems determining digestibility using Cr_2O_3 have been reported, due to interference from other minerals in the diet; therefore, AIA, as a natural marker, shows greater promise for use in digestibility studies with young pigs (Van Leeuwen et al 1996 ; Moughan et al 1991).

Regarding the influence of the lipid component of the diet upon fat digestibility, Cera et al (1990) have found that fat digestibility increases with post-weaning time, and animal fats are less digestible than those of vegetable origin. Responses have been attributed to the high degree of saturation and long chain length of fatty acids in animal fats, factors that decrease micelle formation (Freeman, 1969). Freeman et al (1968) reported that, in young pigs, the capacity of the small intestine to absorb micellar lipids exceeds normal influx into the gut. Therefore, entry of fatty acids in the micellar phase probably limits fatty acid digestibility (Bayley and Lewis 1983). Jones et al (1992) carried out three experiments to determine the utilization of fat from diets for early weaned pigs; in this work soybean oil and coconut oil were found to be more digestible than tallow and lard. Differences observed in fatty acid digestibility between the soybean oil and tallow treatments could be related to differences in their unsaturated fatty acid/saturated fatty acid ratio.

We did not, however, find any differences in fat digestibility between the three diets. This is probably due to the fact that, in our study, animals were in the growth phase and not the post-weaning phase as in the previous studies described in the literature.

The data obtained from livestock performance evaluation are shown in Table 5. Performance parameters show similar value between the groups of pigs fed the three experimental diets. The study by Jones et al (1992) demonstrates that the fat source does not improve the growth performance for the first 7 to 14 days post-weaning compared with a control diet with no added fat.

Different studies have been carried out regarding the effect of phyto-complexes on livestock diets. Several studies have provided evidence for improved digestion by herbs and spices (Platel and Srinivasan 2000; Rao et al 2003). Herbs and botanicals are supposed to enhance feed intake, digestive secretions and to stimulate immune functions. Due to several assumptions for the mode of action, phytogenic substances may contribute to resolving post-weaning problems in piglets (Rao et al 2003; Wenk 2003; Verdonk et al 2005; Watzl et al 2005).

Table 5. Mean growth performance of pigs fed experimental diets

	PO	CO	CO+RWS	SEM	Prob.
Pigs, n	6	6	6		
Initial live weight, kg	73.9	73.2	75.8	1.96	0.87
Final live weight, kg	117	114	119	4.03	0.86
Weight gain, kg	43.3	40.7	43.8	2.36	0.86
Average daily gain, g	772	726	781	42	0.86
Feed intake[a], kg	167	167	168	-	-
Average daily feed intake[a], g	2987	2988	2991	-	-
Feed conversion rate, g/g	3.87	4.11	3.83	0.23	0.99

***PO**= diet with palm oil; **CO**= diet with corn oil; C**O+RWS**= diet with corn oil supplemented with red wine solids (RWS)*

[a] determined for each group

However Muhl and Liebert (2007a) conducted two experiments to investigate the effects of a commercial phytogenic feed additive containing thymol, carvacrol (from oregano oil) and inulin, on growth and microbial parameters in weaned piglets (Muhl and Liebert 2007b).These authors showed that there was no evidence of any influence of the studied additive supplementation on enzyme activities.

Conclusions

- Our results showed that the different composition in vegetable oil (palm oil and corn oil) added to the diets and the RWS supplementation in the corn-oil diet, had no adverse effects on the diet digestibility and on the performance parameters

- Further research is needed to clarify the metabolic mechanism of flavonoids and non-flavonoids contained in RWS. In addition, more specific studies on the effects of these antioxidants on pork meat quality should be carried out to evaluate the potential of RWS in animal feeds.

Acknowledgements

Research was partially financed by the Ministero dell'Università e della Ricerca (Fondo ex-60%). Thanks are due to: Mr. G. Cerato (CISRA) for the preparation of the diets, Mr. R. Maritano (CISRA) for the care of the pigs; Dr. G. Perona (CISRA) and Mrs. C. Bianchi (Department of Veterinary Sciences, University of Torino) for their technical support, Azienda Agricola Ca'Novella (organic wine "Gocciorosso") who kindly provided Liofenol^{TM}, Dr. D. Borsa and Dr. F. Piano (Consiglio per la Ricerca e Sperimentazione in Agricoltura - Centro di Ricerca per l'Enologia of Asti) for the organic red wine and organic RWS analysis, and Mrs. Srinivasan for the English revision of the manuscript.

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Received 24 July 2013; Accepted 11 September 2013; Published 1 October 2013