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# Dried grape pomace influenced fatty acids composition of Longissimus dorsi muscle and plasma polyphenols spectrum in finishing pigs

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ABSTRACT

The experiment assessed the fatty acids (FA) composition of Longissimus dorsi (LD) muscle and plasma polyphenols spectrumin finishing hybrid Topigs fed diets containing dried grape pomace (DGP). The trial was conducted on 24 hybrids Topigs, 75.54±5.55 kg, randomly assigned for 28-d experimental period, to 2 groups, viz. a control (C diet), and an experimental (E diet) with the additional inclusion of 50 g DGP / kg diet. The FA composition wasanalysed by gas chromatography and all the spectra were recorded by spectrophotometry. The results of the study prove that the DGP, by its high content of polyunsaturated fatty acids (PUFA, over 60% of total FAME) and by the active principles such as polyphenols (lmax =273 nm), is a valuable feed which improvedfatty acids composition of LD muscle. In addition, the polyphenols from GP changed in plasma, maximum changes from 273 nm to 279 nm. These properties were reflectedin a positive influence on muscle n-3 FA composition (>1.27 times in DGP group than control group, p=0.02), particularly alpha-linolenicFA (>1.35 times) and eicosapentaenoic FA (>1.30 times than C diet), known for their health benefits.

**Key words**: Antioxidants, Dried grape pomace, Fatty acids, Muscle, Pigs, Plasma

The dried grape pomace (DGP) is a by-product of vine-making from grapes, which can be used in animal feeding mainly due to its properties of natural antioxidant and also to its valuable content of FA, energy and protein. Studies have been performed generally on the antioxidant effects of grape extracts due to their high content in polyphenols (Brenes *et al.* 2008, Chedea *et al.* 2010), the focus being on the evaluation of the effectiveness of these raw materials on animal products quality (Rojas and Brewer 2008, Carpenter *et al.* 2007).

Presently there are few studies on the use of DGP in pig feeding (Carpenter *et al.* 2007, Sehm *et al.* 2011). Probably due to its rather high content of crude fibre (~25%), the grape pomace (GP) is recommended particularly to ruminants, as a cheap source of feeds which is not in competition with human consumption (Bahrami *et al.* 2010). In general, GP was given in animal feed after ensiling or fermentation or as extract rich in polyphenols. Drying or ensiling the GP are recommended by some authors in order to enhance its feeding value and digestibility, while reducing the costs (Bahrami *et al.* 2010), while other authors (Yan and Kim 2011) recommend fermentation as a means to increase the level of desirable metabolites by the action of microorganisms.The DGP is itself an ingredient with a high level of PUFA (over 60%), of which over 2% n-3 PUFA, or it may complete the other sources of n-3 FA, which are beneficial for the human health, such as the flax (Nuernberg *et al.* 2005, Hãbeanu *et al.* 2014), or camelina (Hãbeanu *et al.* 2011). This aspect is more important as recent investigations focus more on the FA profile and less on composition (Hoehne *et al.* 2012).

The purpose of the study was to quantify the effects of the dietary 5% DGP on the FA composition of LD muscle. The response of the animal organism to high levels of PUFA combined with the antioxidant properties of the DGP reflected in the plasma polyphenol spectrum was also investigated.

## MATERIALS AND METHODS

The by-product used in our study was provided by a Romanian distillery. The wet GP was dried at 90°C in a counter-flow hot air conveyor dryer. Before inclusion into the compound feed, the DGP was ground and screened through 8 mm mash sieves and analysed biochemically.

Animals were treated in accordance with the Romanian Law 305/2006 for handling and protection of animals used for experimental purposes. The experiment was performed on 24 hybrid topigs [(Large White × Pietrain)×(Talent)] (14@& and 10 B&), with an initial weight of 75.54 kg ± 5.55, for 28 days (the finishing period). The animals were assigned randomly to 2 homogenous groups of 12 animals

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Table 1. Chemical composition of control and experimental diets given to the Topigs pigs during the 28d-finishing period

| Items | Control diet | DGP diet |
|---|---|---|
| Dry matter (DM, g/kg diet) | 856.4 | 857.3 |
| Metabolisable energy (Mj/kg) | 12.85 | 12.82 |
| g/kg DM | | |
| Crude protein | 178.54 | 175.55 |
| Digestible crude protein | 140.94 | 139.27 |
| Ether extracts | 50.09 | 51.67 |
| Crude fibre | 66.32 | 74.18 |
| Lysine | 10.27 | 10.27 |
| Digestible lysine | 8.52 | 8.51 |
| Methionine + cystine | 6.88 | 6.88 |
| Calcium | 10.51 | 10.49 |
| Total phosphorus | 7.00 | 6.99 |
| % of total FAME[1] | | |
| Alpha –linolenic fatty acids | 1.50 | 1.64 |
| Linoleic fatty acids | 43.94 | 44.04 |

[1] FAME, fatty acids methyl esters.

Table 2. Fatty acids composition of the *LD* muscle

| Items | Control | DGP | SEM | P-value |
|---|---|---|---|---|
| C18:0 | 11.38 | 11.27 | 0.18 | 0.81 |
| C18:1cis-9 | 43.92 | 42.51 | 0.32 | 0.16 |
| C18:2n-6 | 10.52 | 12.34 | 0.53 | 0.13 |
| C18:3n-3 | 0.34 | 0.46 | 0.02 | 0.004* |
| CLA | 0.09 | 0 .11 | 0.02 | 0.42 |
| C18:4 n-3 | 0.09 | 0.13 | 0.01 | 0.21 |
| C20:2n-6 | 0.50 | 0.49 | 0.01 | 0.70 |
| C20:3n-6 | 0.14 | 0.12 | 0.01 | 0.67 |
| C22:1n-9 | 0 .35 | 0.34 | 0.02 | 0.63 |
| C20:4 n-6 | 0.69 | 0.49 | 0.05 | 0.08[T] |
| C22:2n-6 | 0.12 | 0.16 | 0.01 | 0.14 |
| C20:5n-3 | 0.13 | 0.17 | 0.01 | 0.15 |
| Total SFA | 38.81 | 38.56 | 0.52 | 0.83 |
| Total MUFA | 48.07 | 46.50 | 0.37 | 0.18 |
| Total PUFA | 12.77 | 14.59 | 0.56 | 0.17 |
| Total n-6 PUFA | 12.07 | 13.71 | 0 .56 | 0.19 |
| Total n-3 PUFA | 0.69 | 0,88 | 0.07 | 0.02* |
| n-6/n-3 ratio | 17.49 | 15.57 | 0.88 | 0.07[T] |
| PI[2] | 15.34 | 17.05 | 0.60 | 0.24 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; PI, peroxidability index; Total SFA , C14:0 + C15:0 + C16:0 + C17:0 + C18:0; total MUFA, C14:1 + C15:1+ C16:1 + C17:1 + C18:1cis-9 + C22:1n-9; total PUFA, C18:2n-6 +C18:3n-3 + CLA + C18:4n-3 + C20:2n-6 + C20:3n-6 + C20:3n-3 + C20:4n-6 + C22:2n-6 + C20:5n-3 + C22:6n-3. [2]PI, fat peroxidation index. *Significant differences between groups. T, Tendence to be influenced by treatment.

each: a control group (C diet) fed the classical compound feed formulation and an experimental group (DGP diet) which was treated with 5% DGP. The quality parameters of the feed were in agreement with those mentioned in the rearing guidebook of Topigs hybrid (Table 1).

The compound feeds formulations were isoenergetic and isoprotein and consisted of corn, rice meal, soybean meal, sunflower meal, sunflower oil, choline premix, vitamin-mineral premix. The dried grape pomace diet had similar ingredients but part of the corn and sunflower meal was replaced by the 5% DGP.

Around 200 g LD muscle tissue were collected from 12 animals, from the right half carcass of each animal. The samples were refrigerated 12 days at 4°C. After 12 days the samples were transformed in a homogenous powder using the hammer mill and preserved at -20°C until FA analysis.

Blood samples were collected at the end of the trial. The blood for polyphenol spectra determination was collected 24 hours before slaughtering.

We used standardized methods as per Commission Regulation (EC) no. 152/2009 to determine the gross chemical composition of the DGP and of the muscle.

An amount of 2.5 g ground DGP was taken for extraction. Twenty millilitres of hot water (almost at boiling point) was added to the weighted meal and left to cool at room temperature (1 h). The mixture was centrifuged at 9,000 rpm for 10 min. The supernatant (the total DGP) was collected, aliquoted and kept at -20° C until further analysis. All the spectra were recorded at room temperature using a spectrophotometer in the UV–Vis range 250–400 nm for DGP and 250-700 nm for the blood plasma. For recording the spectra DGP was diluted 9 × to fit the acceptable absorption range (A < 1). In the blood plasma, the used blank was the plasma of the control variant (fed without GP) so the spectra obtained show the differences given by adding the DGP into the pig feed.

The fatty acids were determined by gas chromatography, which presumes the transformation of the FA from the fat sample into methyl esters followed by the separation of the components in a capillary column and their identification by comparison with the standard chromatograms (reference standard purchased from the Romanian National Office for Standards). We used chromatograph with capillary column with high polarity stationary phase (BPX70, 60m × 0.25 mm inner diameter, 0.25 ìm film); or high polarity cyanopropyl phases, which have similar resolutions for different geometric isomers (THERMO TR-Fame 120m × 0.25 mm ID × 0.25 ìm film). Fat peroxidation index (IP) was calculated starting from the composition of PUFA using the equation reported by Hu *et al.* (1989) namely: IP = (% dienoic × 1) + (% trienoic × 2) + (% tetraenoic × 3) + (% pentaenoic × 4) + (% hexaenoic × 5). This index is used to calculate the susceptibility of the polyunsaturated fatty acids of LD muscle to peroxidation.

*Statistical analysis:* The data were processed by variance analysis using SPSS- general linear model (Statistics version 20, 2011). The results are given as means with standard error of the mean (SEM). The means were considered statistically different at $P<0.05$. The FA were expressed as a percentage of total fatty acids ester methyl (% of total FAME).

## RESULTS AND DISCUSSION

The chemical composition of the DGP, related to 100 g DM, was: 11.77g crude protein, 5.86 g crude fat, 28.54 g crude fibre, 66.18 g NDF, 65.87 g ADF and metabolisable energy 1,912 kcal/kg. The DGP is close as feeding value, to the other by-products used in animal feeding, such as brans, for instance. Its protein level is lower than that of the conventional meals to balance the diet used in this trial, by the replacement of 3% of the sunflower meal, we had to increase by 1% the proportion of soybean meal as protein-rich ingredient (44%) and with a rather high energy content (3,200 kcal ME/kg). The high fibre content is the main inconvenient due to which the inclusion rate of this by-product in monogastric diets is low (below 5%). The DGP had a high level of PUFA (61.98%), of which 58.99% linoleic FA and 2.19% alpha-linolenic FA. The high level of unsaturated FA (79.92%) compared to just 19.71% saturated FA might suggest the predisposition of this by-product to lipoperoxidation, but the spectral analysis of the antioxidant content (Fig. 1) shows that these effects may be diminished.

Significant effects of the supplemental DGP given to the finishing pigs were noticed particularly on the total n-3 PUFA (p=0.02). Thus, the proportion of n-3 PUFA in the muscle from the animals fed the DGP diet was 1.27 times higher compared to the C diet. From the family of the n-3 PUFA, the alpha-linolenic FA was highly significant influenced by the diet (1.35 times higher than C diet, P=0.004). The linoleic FA, predominant among the n-6 PUFA, competes with the alpha-linolenic FA for the enzymatic conversion into long-chain fatty acids (LCPUFA), so that a lower level of this FA into the diet generally leads to the increase of n-3 LCPUFA content into the tissues (Smink *et al.* 2013). In our study, the use of DGP increased the linoleic FA concentration by 1.17 times and n-6 PUFA by 1.14 times compared to group C. As a consequence, the failure to identify FAs derived from alpha-linolenic FA, such as docosapentaenoic FA and docosahexaenoicFA in the LD muscle, was important. The level of arachidonic FA, belonging to the same family, decreased 1.40 times. The diets did not have significant effects on the MUFA.Thereby, the content of MUFA was 3.3%, lower in the DGP pigs than in group C, particularly

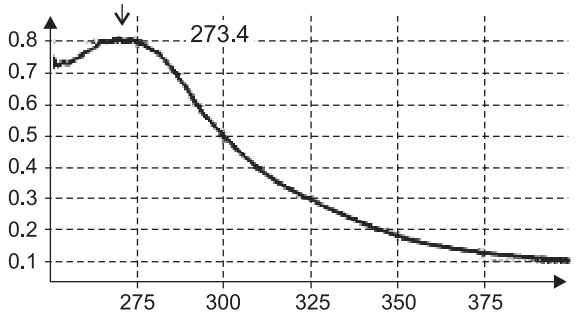


Fig. 1. UV spectrum of the grape pomace aqueous extract (9 × diluted) registered using a spectrophotometer in the UV-VIS range 250-400 nm.

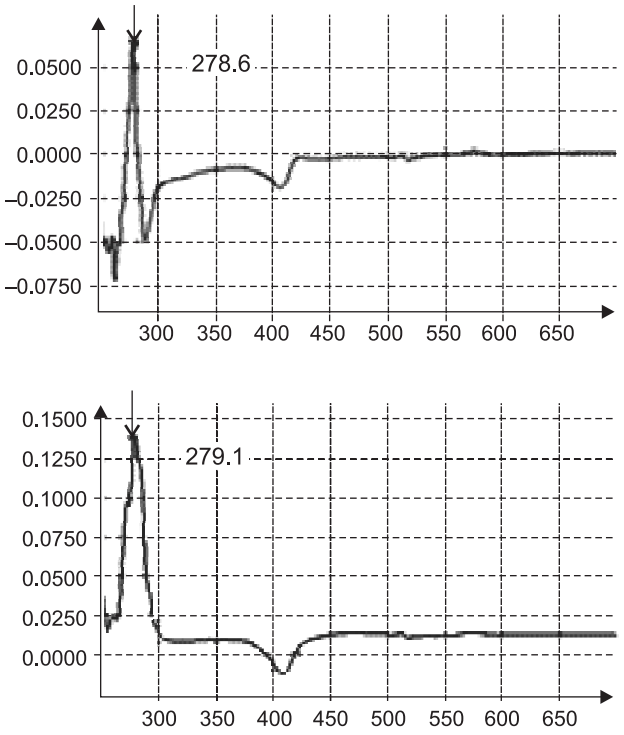


Fig. 2. UV-Vis spectrum of the blood plasma registered using a spectrophotometer. The plasma was separated from the collected blood before slaughtering the pigs fed with GP enriched diet.

the oleic acid (over 40%) which is beneficial to human health because of its effect of reducing LDL-cholesterol. The level of erucic FA, with controversial effects on the human health, was similar whatever the treatment. The values were rather similar in both groups for the saturated FA. The ratio n-6:n-3 tended to decrease by addition of DGP in the diet (1.12 times, p=0.07). Our findings were supported by the generally accepted theory according to which the variation in FA composition is due to the feed but also to the presence of substances with antioxidant effects (Tejerina *et al.* 2012, Rojas and Brewer 2008). In general, it is accepted that the PUFA, particularly those with 4 or more double bonds, are highly prone to peroxidation. The peroxidability index, calculated from the PUFA composition of LD muscle shows the higher predisposition to peroxidation of the meat from DGP pigs (1.11 times higher compared to C group); however, the effects were not significant (p=0.24). As for measuring the spectra, the used blank was the plasma from the pigs fed without DGP, the spectra indicate compounds present in blood as a result of DGP feeding (the blood was collected 24 h before slaughtering, Fig. 2). All spectra obtained from the DGP animals display a trend to produce the same spectrum shape, which denotes the presence of the same type of polyphenol compounds. The data obtained show that $\lambda_{max}$of the blood plasma spectrum ($\lambda_{max}$ =276 to 279 nm) is higher than the maximum wavelength of absorbance for the GP ($\lambda_{max}$ =273 nm).

This bathochromic shift can be explained by a possible

metabolic oxidation of these polyphenolic compounds from the time they are ingested until they reach the blood. The fact that the GP contains antioxidants such as polyphenols and high levels of PUFA potentiates the effects of the DGP on the composition of the n-3 fatty acids muscle tissue composition, such as alpha-linolenic FA (>35.2% than C diet) and its long chain derivatives (eicosapentaenoic FA, >30.7% than C diet).

The addition of 5% DGP as rich source of natural antioxidant and PUFA in the diet for finishing pigs, improved the concentration of total n-3 PUFA especially alpha-linolenic FA in LD muscle. Furthermore, the DGP bio-active compounds potentiated the effects of this valuable by-product by increasing the concentration of C20: 5n-3, known for its health benefits.

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