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# Influence of feeding dried grape marc to fattening steers on the fatty acids composition of the hepatic tissue

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ABSTRACT

The purpose of this study was to evaluate the effect of feeding dried grape marc to fattening steers on the fatty acids composition of the hepatic tissue (liver). The feeding trial used 20 steers with an average initial body weight of 253 kg assigned uniformly to two groups, control (C diet) and experimental (E diet). The E diet included 20% dried grape marc in the compound feed. The use of this winery by-product improved the feeding value of the liver for the human consumers. The concentration of polyunsaturated fatty acids increased, particularly the concentration of omega 3 and omega 6 fatty acids. Thus, compared to the control group, n-3 fatty acids increased by 18.10%, while n-6 fatty acids increased by 16.14%, in the experimental group.

**Key words**: Dried grape marc, Fatty acids, Liver, Steers

The processing of vegetal materials for human consumption produces large amounts of by-products, or industrial waste. These are not used efficiently in farm animal feeding because of the insufficient amount of available information on their chemical composition, feeding value and possible positive effects of their constitutive elements. For instance, the wine industry produces grape marc which results from crushing the grapes. Presently, this by-product is less known in terms of feeding value and possibilities of using it efficiently in animal nutrition.

The grape marc contains large amounts of resveratrol, one of the strongest antioxidants (Ahn *et al.* 2002), existing mostly in the skin and pulp of red grapes, as well as high levels of polyunsaturated fatty acids (over 60%), more than 2% being omega 3 fatty acids, which are beneficial to human health (Hãbeanu *et al.* 2015). Other research (Chedea *et al.* 2010) also showed that the grape seeds have a variable content of polyphenols (5 to 8%), but the quantification of these compounds is rather laborious, because there are very many categories of such biologically active substances (over 300).

The literature is rather scarce in information on the effect of using winery by-products in ruminant feeding. Some researches evaluated the ruminal parameters of sheep treated with grape marc (Abarghuei *et al.* 2010), dry matter digestibility and growth performance of male lambs (Yadollah *et al.* 2010), performance and plasma biochemical parameters in fattening steers (Voicu *et al.* 2014), dairy cows performances (Toma *et al*. 2015) or blood metabolites and milk composition of dairy cows (Chedea *et al*. 2016). These studies showed that, despite its high content of crude fibre, this by-product is used efficiently by the animals, being consumed with pleasure because of its sourish taste.

We therefore decided to evaluate the effect of using dried grape marc in the compound feeds for growing and fattening steers on the fatty acids content of the liver.

## MATERIALS AND METHODS

All experimental procedures were approved by the Ethical Committee of the National Research-Development Institute for Animal Biology and Nutrition, in accordance with Romanian Law no. 305/2006 regarding handling and protection of animals used for experimental purposes.

*Experimental ingredient.* Wet grape marc exposure to 90°C using a counter-flow hot air conveyor dryer produced dried grape marc containing variable proportions of skin, pulp, seeds and stems. This mixture was milled using 8 mm mash sieves and then incorporated into the experimental compound feed.

*Animals, diets and housing.* The trial was conducted for 91 days, on 20 Romanian Black Spotted fattening steers with an initial average body weight of 253±4.57 kg, kept in collective stalls with slatted concrete flooring and central lane for feed administration. The animals were assigned to 2 homogeneous groups of 10 steers each, divided as follows: a control group fed a classical compound feed formulation

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(C diet) and an experimental group which received 20% dried grape marc in the compound feed formulation (E diet). The compound feeds formulations were isoenergetic and isoproteic and consisted of corn, barley and wheat as energy sources and sunflower meal as protein source, monocalcium phosphate, salt and vitamin-mineral premix. E diet had similar ingredients but barley was entirely replaced by dried grape marc. Alfalfa haylage was the basal dietary ingredient for both groups. The diets were formulated according to Burlacu *et al.* (1991, 2002) and had the same nutritional characteristics (7.94 meat feed units, mFU/day and 676 g intestinally digestible protein, IDP/day). Feed intake and feed leftovers were recorded daily. All animals had *ad lib.* access to the feed and water (constant level drinkers).

*Sample collection, analysis and statistics.* Liver samples were collected from the slaughtered steers in the end of the experiment and analysed for the fatty acid profile. The analytical methods rely on the determination of fatty acid by gas chromatography method (SR CEN ISO/TS 17764-1/2008) with a Perkin Elmer Clarus 500 GC. In order to estimate the susceptibility of the liver PUFA to peroxidation, the fat peroxidation index (PI) was calculated according to the formula proposed by Cosgrove *et al.* (1987):

PI = (% dienoic × 1) + (% trienoic × 2) + (% tetraenoic × 3) + (% pentaenoic × 4) + (% hexaenoic × 5).

All the data were processed statistically using General Linear Model (GLM) procedure with IBM SPSS Statistics (version 20, 2011). One-way multivariate analysis of variance (MANOVA) was used to check the differences between the results obtained from the two groups of animals at 0.05 level of significance. The results are given as means with standard error mean (SEM). The values for fatty acid are expressed as % of total fatty acids ester methyl (FAME).

## RESULTS AND DISCUSSIONS

The fatty acids content of the feeds used in this experiment is shown in Table 1. Analysis of the basal dietary ingredient is very important in ruminants because sometimes it may exceed 60% of the total diet. The determination of alfalfa haylage FA content revealed higher concentrations of total UFA (68.80%), of which 50, 20% are PUFA, mainly n-3 FA (25, 52%). As in human diets, a lower ratio of n-6/n-3 in animal feeds is more desirable. The ideal ratio is 1:1, while the optimal one is $\leq$ 5 (AFSSA 2003). Our results highlight a ratio of 0.97. The 20% dried grape marc given to the fattening steers influenced obviously the fatty acids composition of the compound feed for the experimental group. Thus, compared to C diet, the total SFA content from E diet was lower by ~7%, the total MUFA content decreased by ~10%, the total PUFA content increased by 6% and the n-3 FA by 14%. Also, the n-6/n-3 ratio in E diet was lower by 8%.

Table 1. Fatty acids content of the feeds (% of total FAME)

| Item | Alfalfa haylage | C diet | E diet |
|---|---|---|---|
| C16:0 | 20.96 | 18.80 | 16.47 |
| C18:0 | 5.25 | 1.91 | 2.86 |
| C18:1n9 | 15.16 | 23.50 | 21.34 |
| C18:2n6 | 19.92 | 51.46 | 54.67 |
| C18:3n3 | 23.24 | 2.64 | 3.10 |
| C20:2n6 | 3.39 | 0.42 | 0.18 |
| C22:5n3 | 1.71 | 0.19 | 0.14 |
| Σ SFA | 30.42 | 21.42 | 20.11 |
| Σ UFA | 68.80 | 78.59 | 79.89 |
| Σ MUFA | 18.60 | 23.88 | 21.80 |
| Σ PUFA | 50.20 | 54.71 | 58.09 |
| n-3 | 25.52 | 2.83 | 3.24 |
| n-6 | 24.68 | 51.88 | 54.85 |
| n-6/n-3 | 0.97 | 18.33 | 16.93 |

SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Σ SFA, C10:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0; Σ MUFA, C14:1 + C15:1 + C16:1 + C17:1+ C18:1n9 + C24:1n9; Σ PUFA, C18:2n6 + C18:3n6 + C18:3n3 + C18:4n3 + C20:2n6 + C20:3n6 + C20:3n3 + C20:4n6 + C22:2n6 + C22:4n6 + C22:5n3; Σ UFA = Σ MUFA + Σ PUFA.

Generally, in ruminants, unlike in the monogastric animals (pigs, poultry), the higher content of fatty acids *de novo* and the lower proportion of polyunsaturated fatty acids are due to the bacterial biohydrogenation of the dietary fatty acids in the rumen of the herbivores (Hoquette *et al.* 2005), which makes more difficult the manipulation of the fatty acids profile in the tissues. Although no significant effects of the E diet given to the fattening steers were noticed (Table 2), however, the proportion of SFA in the liver of the animals from the experimental group decreased by 9.83% compared to the control group.

World Health Organization places the palmitic acid (C16: 0) in the same group with the trans acids, responsible for the higher risk of cardiovascular diseases (Abeywardena and Patten 2011, Kashani *et al*. 2015). The 20% dried grape marc decreased 1.38 times the proportion of this fatty acid compared to the control group (C diet). The intensity with which the oleic acid (C18: 1) is hydrogenated into stearic acid (C18: 0) depends on the conditions from the rumen. In our case, E diet decreased the C18:1 content by 1.35 times and increased the C18:0 content by 1.15 times. These results were consistent with other research. For instance, the findings of Harfoot and Hazlewood (1997) suggest that the full hydrogenation into C18:0 is inhibited by low amounts of dietary linoleic acid (in our study the C diet contained a lower proportion of C18:2 compared to E diet, 51.46% vs. 54.67%, respectively), while increasing particularly the proportion of C18:1 acid (18.31% for control group vs. 13.60% for experimental group), because the production of stearic acid is low and slow (27.17% for control group vs. 31.28% in the experimental group). It is possible that under specific circumstances (amount of dietary fat, forages to concentrate feeds ratio), the final steps of biohydrogenation can be altered, resulting in the accumulation of C18:1 and reduction of C18:0 (Shingfield *et al.* 2003, Gonthier *et al.* 2004). The diets did not have

Table 2. Fatty acids content of the liver (% of total FAME)

| Item | C group | E group | SEM | p-value |
|---|---|---|---|---|
| C14:0 | 0.75 | 0.39 | 0.19 | 0.446 |
| C14:1 | 0.18 | 0.17 | 0.03 | 0.892 |
| C15:0 | 0.29 | 0.19 | 0.06 | 0.479 |
| C15:1 | 0.34 | 0.42 | 0.02 | 0.245 |
| C16:0 | 14.68 | 10.62 | 2.02 | 0.421 |
| C16:1 | 1.25 | 0.87 | 0.34 | 0.636 |
| C17:0 | 0.69 | 0.35 | 0.12 | 0.300 |
| C17:1 | 1.13 | 1.93 | 0.38 | 0.403 |
| C18:0 | 27.17 | 31.28 | 2.39 | 0.480 |
| C18:1n9 | 18.31 | 13.60 | 2.08 | 0.374 |
| C18:2n6 | 13.52 | 15.28 | 0.54 | 0.243 |
| C18:3n3 | 2.19 | 2.22 | 0.11 | 0.906 |
| CLA | 0.29 | 0.34 | 0.02 | 0.365 |
| C20:2n6 | 0.09 | 0.0 | 0.05 | 0.423 |
| C20:3n6 | 0.39 | 0.43 | 0.06 | 0.752 |
| C20:3n3 | 2.60 | 3.29 | 0.56 | 0.599 |
| C20:4n6 | 7.38 | 8.82 | 0.38 | 0.201 |
| C20:5n3 | 0.59 | 1.11 | 0.27 | 0.434 |
| C22:2n6 | 1.05 | 1.53 | 0.27 | 0.467 |
| C24:1n9 | 0.68 | 0.85 | 0.02 | 0.070 |
| C22:5n3 | 4.71 | 5.14 | 0.19 | 0.367 |
| C22:6n3 | 0.91 | 1.22 | 0.19 | 0.484 |
| Σ SFA | 43.57 | 42.82 | 0.26 | 0.291 |
| Σ UFA | 55.58 | 57.18 | 0.27 | 0.098 |
| Σ MUFA | 21.88 | 17.82 | 2.08 | 0.432 |
| Σ PUFA | 33.7 | 39.36 | 2.19 | 0.326 |
| Σ n-6 | 22.43 | 26.05 | 1.15 | 0.256 |
| Σ n-3 | 10.99 | 12.98 | 1.06 | 0.445 |
| n-6/n-3 | 2.07 | 2.01 | 0.09 | 0.763 |
| PI | 72.86 | 86.23 | 5.36 | 0.338 |

SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Σ SFA, C14:0 + C15:0 + C16:0 + C17:0 + C18:0; Σ MUFA, C14:1 + C15:1 + C16:1 + C17:1+ C18:1n9 + C24:1n9; Σ PUFA, C18:2n6 + C18:3n3 + C20:2n6 + C20:3n6 + C20:3n3 + C20:4n6 + C20:5n3 + C22:2n6 + C22:5n3 + C22:6n3; Σ UFA = Σ MUFA + Σ PUFA; PI, fat peroxidation index.

significant effects on the monounsaturated fatty acids levels from the hepatic tissue, thereby the total MUFA content was with 19% lower in the fattening steers' experimental group than in control group. The proportion of the PUFA in the liver of the animals fed E diet was 1.17 times higher compared to C diet. Among n-6 PUFA, the E diet increased the linoleic FA concentration by 1.13 times compared to C diet, as also noticed by other studies (Voicu *et al.* 2006, 2008), which is good for human health. The level of arachidonic FA (C20:4n6) also increased by 1.20 times. From the n-3 PUFA family, the alpha-linolenic FA was not influenced by the diet (P=0.906), but it was noticed that the use of dried marc increased the proportion of long chain FA, derivatives of linolenic FA, such as C20:5n-3, C22: 5n-3 and C22:6n-3. Compared to C diet the level of these important three fatty acids was higher in the liver of the animals from the experimental group, by 47% for eicosapentaenoic acid, 8% for docosapentaenoic acid and 25% for docosahexaenoic acid respectively. Also, the eicosatrienoic acid (C20:3n3) was 1.27 times higher in the liver of the animals fed E diet. The n-6:n-3 ratio was slightly lower by addition of the dried grape marc in the diet (2.01 vs. 2.07), which is a desirable value of animals products for human consumption. Because this ratio is a risk factor for some diseases, Enser (2001) recommends an n-6:n-3 ratio lower than 4. The use of plant materials with antioxidant effects have the potential to reduce and provide limited protection against lipid oxidation. Polyunsaturated fatty acids in tissue lipids, especially those with four or more double bonds, are particularly susceptible to oxidation (Rojas and Brewer 2008). The fat peroxidation index (PI), calculated from the PUFA content of the liver shows higher predisposition to peroxidation of the liver from steers belonging to E group (1.18 times higher compared to control group). However, the differences were not significant (P=0.338). Generally, higher antioxidative effects depend on the type and concentration of the polyphenols contained by the ingredient used (Lau and King 2003, Sánchez-Escalante *et al.* 2003).

Although the addition of 20% dried grape marc in the fattening steers diet did not lead to significant differences regarding the liver fatty acid composition when compared to a control diet, however, the use of this ingredient improved the liver concentration of total PUFA, especially the long chain fatty acids, such as C20:5n-3, C22:5n-3 and C22: 6n-3 respectively, known for their beneficial effects on human health.

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