Wine by-products feeding on ewe physiological traits, milk quality and the meat quality of their suckling lambs

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SUMMARY

Introduction - Winery by-products, which may constitute undesirable waste, can be used as feeding supplements in ruminants, but they could modify product quality.

Aim - To investigate the effect of wine by-products added to lactating ewes feeding on the ewe's welfare, milk quality and their suckling lamb performance, carcass and meat quality.

Material and methods - A total of 42 Chamarita breed ewes were allocated in one of three experimental diets: a control, based in concentrates without added by-product, and diets supplemented with either 10% grape pomace or 5% grape seed on a dry matter basis, during middle gestation and lactation. Ewe's physiological welfare indicators and milk quality were assessed, and daily gain, carcass and meat quality (pH, cooking and thawing losses, texture and colour) in their suckling lambs were analysed.

Results and discussion - Compared to the control group, cortisol levels was higher in the ewes fed pomace; whereas creatine kinase and non-esterified fatty acids were superior in the ewes fed seed. However, milk composition was minimally affected, showing less lactose concentration the grape pomace and control groups, but the percentage of fat and protein and somatic cell count was similar between treatments. The suckling lambs showed similar performance, but the fattening carcass score of the lambs of the grape seed group was lower than control. The meat of the three treatments reached a similar pH, cooking loss and instrumental texture and only a minor effect in thawing loss and the colour parameter lightness was observed.

Conclusion - The inclusion of grape by-products may be used as a supplementation in lactating ewes without compromising lambs' growths, carcass composition and instrumental meat quality.

KEY WORDS
Grape pomace; grape seeds; animal welfare; performance; meat quality.

INTRODUCTION

Nowadays, Spain is the country with the highest surface area destined for grape production. The regions of Aragón and La Rioja, have a strong wine industry tradition with many designations of origin of international reputation (i.e., Rioja, Cariñena, Campo de Borja, Somontano, Calatayud). The sheep meat industry is also very important in these regions, including the suckling lamb as a delicacy of the Mediterranean cuisine, especially the Chamartín lamb which was recognized as a quality brand in April 2010 and belongs to a rustic breed from La Rioja, included in the Official Catalogue of Spanish Livestock Breeds under Endangered Native Breeds.

During the winemaking process waste by-products are produced in vast amounts, resulting in an environmental problem that could be reduced allocating them for animal feed. Furthermore, not only grapes and wine, but also grape seeds and skin extracts could have favourable effects on human health due to their phenolic content. Therefore, grape by-products could be highly valuable. If they can be obtained from low-cost industrial wastes, production costs of lamb meat can be reduced and profit margins increased, thus reducing the discharge of pollutants into the environment.

Different feeding strategies can affect performance, animal welfare, safety, nutritional value, and eating and technological quality of the milk and the meat. Recent research shows that the use of grape/wine by-products in ruminant feeding could have an immunomodulatory effect in the animal, and could modify the milk composition, the fatty acid composition and the antioxidant activity of the milk, which might affect the quality of their suckling kids. Grape pomace is a major by-product of winery industry, which consists in seed, skin and stem; whereas grape seeds is separated from the pomace and is richer in fibre, ether extract and polyphenols, and both have being tested as feeding supplements in ruminants. However, little is known concerning the effect of feeding ewes wine by-products to
their suckling-lamb meat. Therefore, the aim of this study was to investigate the effect of a feeding regime using wine by-products (grape pomace or seed) on the ewe’s welfare, milk quality and suckling lamb performance, and on carcass and instrumental meat quality of the lambs.

**MATERIALS AND METHODS**

The study was carried out at the Animal Experimentation 75 Service of the University of Zaragoza, Spain (latitude 41°41’N). The area is located in the Ebro Valley, characterized by a dry Mediterranean climate with an average annual temperature of 15°C and an average annual rainfall of 317 mm. The experimental protocol was approved by the Animal Experimentation Ethics Committee of the University of Zaragoza.

**Animals and diets**

All sheep used belonged to the Chamarita breed, a native breed from La Rioja (Spain), included in the Official Catalogue of Spanish Livestock Breeds under Endangered Native Breeds. A total of 42 multiparous adult ewes were oestrus synchronised with intra-vaginal prostaglandin sponges (30 mg Fluorogestone Acetate; Sincropart®, CevaSalud Animal S.A., Barcelona, Spain) and mated (natural controlled). Three experimental groups of 14 ewes each were housed during gestation and lactation in pens at a density of 2 m² per ewe. Each pen was equipped with a metallic water trough (1.5 m x 0.60 m), a metallic feeder (4.5 m x 0.80 m, 27 cm per ewe), and a lick stone for minerals.

All of the ewes were fed pelleted concentrate (11.5 MJ ME/kg DM and 15.5% crude protein; approximately 0.750 kg per day) and straw ad libitum until the middle of gestation. Then they were allocated in one of three experimental diets, balanced for body weight and number of lactation (2-3 lactations), as follows:

1. Concentrate without added wine by-products (control);
2. Concentrate supplemented with 10% of grape pomace, on a dry matter (DM) basis (grape pomace);
3. Concentrate with 5% of grape seed, on a DM basis (grape seed).

Grape pomace was supplied by a family winery from La Rioja (Spain), whereas grape seeds were obtained from Agralco S. Coop. Ltda. (Estela, Navarra, Spain). All the ingredients of each concentrate were mixed and ground in a mill and fed as dry meal. The ewes were fed twice a day (1 kg/day per ewe), in the morning between 08.00 and 08.30 h and in the afternoon between 15.00 and 15.30 h, apart from ad libitum (5.02 MJ ME/kg DM and 3.5% crude protein) until the middle of gestation. Then they were allocated in one of three experimental diets, balanced for body weight and number of lactation (2-3 lactations), as follows:

1. Concentrate without added wine by-products (control);
2. Concentrate supplemented with 10% of grape pomace, on a dry matter (DM) basis (grape pomace);
3. Concentrate with 5% of grape seed, on a DM basis (grape seed).

**Physiological welfare indicators**

Blood samples were taken from all ewes by jugular venepuncture with vacuum tubes in the middle of the milking period (15 days of lactation, before weighing) to evaluate their physiological responses to stress (two 10 ml tubes per animal, with and without anticoagulant, EDTA-K3). Blood was sampled using the necessary precautions to avoid sampling error on stress indicators. Samples were kept on ice for a maximum of 2 h and taken to the laboratory for routine haematological measurements. EDTA plasma and serum were centrifuged at 3000 rpm for 10 min, and aliquots were frozen and kept at -30°C until analysed as described in Miranda de la Lama et al.

**Milk quality**

Individual milk samples were collected at the beginning (4th to 5th day after lambing) and middle (15 days of lactation) of the milking period in 50 ml sterile plastic containers after cleaning and disinfecting teats (70% ethyl alcohol) and discharging foremilk. Samples were sent to the laboratory for analysis 136 of milk gross composition (i.e., fat, protein, lactose and dry matter content) with the MilkoScan 4000 (Foss Electric, Hillerød, Denmark), and for measurement of the somatic cell count with a Fossomatic 5000 (Foss Electric, Hillerød, Denmark).

**Lamb’s productive, carcass and meat quality variables**

Lambs were weighed at birth (BW) and at weaning (WW). Pre-weaning average daily gain (ADG) was estimated by the difference between WW-BW divided by the total milking period. A total of 10 lambs from each group were selected to study the meat quality. All of them were slaughtered within the weight range of suckling lamb-type category at an EU-approved abattoir located in the city of Zaragoza. After overnight lairage, lambs were electrically stunned and dressed using standard commercial procedures. Cold carcass weights were taken after 24 h in the cold room at 2°C. The chilling system complied with the quality certification GS1 standard AECOC (http://www.aecoc.es). Carcass conformation score and carcass fatness were graded according to the European classification system (European Union, 1993), the EUROP conformation scale (converted to a 15 points scale) and the carcass fatness scale (converted to a 15 points scale). To determine pH₇₅₀ of the M. longissimus, a portable pH meter (fitted with a penetration electrode 52.00 from Crison Instruments®) was used, which was inserted into a small in-
cision in the right loin (L2-L3 vertebrae). The pH meter was re-calibrated at the same temperature of the operation room (5°C) after every five samples, using two standard buffer solutions at pH 7.0 and 4.0. After chilling for 24 h, the left rack was removed from T1 to L6 vertebrae and transferred to the Meat Laboratory at the Faculty of Veterinary Medicine of the University of Zaragoza without disrupting the cold chain. The M. longissimus was removed and sampled for the meat quality analyses. At 72 h post-mortem and after 1 h of blooming, instrumental colour was evaluated using a Minolta CM 202 calibrated chromameter with a standard illuminant D65 and a 10° observer with an aperture size of 2.54 cm, following the CIE L*a*b* system, measured the colour of fresh meat on the cut surface of the 13th thoracic vertebra of the Longissimus thoracis. The colorimetric indices of chromaticity \(C^* = (a^* + b^*)^{1/2}\), quantity of colour) and hue \(h^* = \tan^{-1}(b^*/a^*)\), real colour) were calculated. Final values were the average of three measurements. Samples for further analyses were vacuum-packed, frozen, and stored at -20°C after 72 h of ageing. Samples from T10 to T13 were thawed for 24 h in a refrigerator (2-4°C) in their vacuum-sealed plastic bags prior to testing and weighted to calculate thawing losses. To perform texture, the same samples were cooked in their vacuum-sealed plastic bags in a 75°C water bath (GLF-D3006), until the internal temperature of the meat (measured with a penetration thermometer) reached 70°C, then cooled for 30 min under flowing cold water and weighted to calculate cooking losses. The samples were randomly allocated in two batches. The texture of the cooked meat was measured using an Instron 4301 equipped with a Warner-Bratzler shear. To cut 1 cm² pieces (in the direction of the muscle fibres), we used a digital calibre MITUTOYO® Series 500 (Mitutoyo Corporation USA). For each animal, at least three measurements were taken. The sample gauge was 10 mm, gauge length was 30 mm, load cell was 100 kg (minimum load level 0.001 kg), crosshead speed was 150 mm/min (high extension limit = 30 mm), and the sampling rate was 20 points/s.

### Statistical analysis

Data were analysed using the General Lineal Model (GLM) procedure of SAS (2001). The diet treatment was considered as the fixed effect (three levels) whilst residuals were considered as random effects. The general representation of the model used was: \(y = Xb + e\), where \(y\) was an \(N \times 1\) vector of records, \(b\) denoted the fixed effect in the model with the association matrix \(X\), and \(e\) was the vector of residual effects. Meat and carcass quality variables were co-varied with cold carcass weight. A probability value of \(p<0.05\) was considered statistically significant.

### Table 1 - Chemical composition of grape by-products and concentrates used in the diets. Fatty acid composition (g/100 g FAME) of the concentrates*.

<table>
<thead>
<tr>
<th></th>
<th>Grape by-products</th>
<th>Concentrates*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grape pomace</td>
<td>Control</td>
</tr>
<tr>
<td>Dry matter (DM, %)</td>
<td>88.18</td>
<td>88.46</td>
</tr>
<tr>
<td>Organic matter (OM, % DM)</td>
<td>92.14</td>
<td>96.41</td>
</tr>
<tr>
<td>Ash (% DM)</td>
<td>7.86</td>
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<tr>
<td>Ether extract (%)</td>
<td>8.94</td>
<td>2.51</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>13.26</td>
<td>19.28</td>
</tr>
<tr>
<td>Neutral detergent fibre (% DM)</td>
<td>36.26</td>
<td>14.03</td>
</tr>
<tr>
<td>Acid detergent fibre (% DM)</td>
<td>29.19</td>
<td>4.55</td>
</tr>
<tr>
<td>Acid detergent lignin (% DM)</td>
<td>17.65</td>
<td>0.02</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td></td>
<td>39.80</td>
</tr>
<tr>
<td>C16:0</td>
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<td>0.29</td>
</tr>
<tr>
<td>C16:0</td>
<td></td>
<td>18.10</td>
</tr>
<tr>
<td>C18:0</td>
<td></td>
<td>2.44</td>
</tr>
<tr>
<td>C20:0</td>
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<tr>
<td>Monounsaturated fatty acids</td>
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<td>19.90</td>
</tr>
<tr>
<td>C18:1 n-9</td>
<td></td>
<td>18.41</td>
</tr>
<tr>
<td>C18:1 n-11</td>
<td></td>
<td>0.99</td>
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<td>C20:1</td>
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<td>Polyunsaturated fatty acids</td>
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<td>54.13</td>
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<tr>
<td>C18:3 n-3</td>
<td></td>
<td>4.50</td>
</tr>
</tbody>
</table>

FAME: total fatty acids methyl ester.

*Data presented in Resconi et al. (2018)².

²: Common ingredients in the concentrates: soya (18%), sunflower oil (0.5%), lysine (0.23%), methionine (0.03%), sodium chloride (0.4%), calcium carbonate (1.12%), dicalcium phosphate (0.65%), vitamin supplement corrector (0.4%), barley (78.67% for the control treatment, 68.67% for the grape pomace group and 73.67% for the grape seed group). The roughage source was Lucerne hay offered ad libitum.
RESULTS AND DISCUSSION

Ewe physiological welfare indicators

Physiological indicators provide evidence about the welfare status of farm animals²⁶. Table 2 shows the live weight and physiological indicators of stress response in lactating ewes fed with three types of concentrate. The stress levels observed in our study fell within normal ranges for sheep and indicate that the welfare of the ewes was not compromised in any of the experimental groups²⁶. However, results indicate significant differences in some stress indicators between the groups studied (Table 2).

Cortisol levels are recognised as a good indirect measure of the stress experienced by an animal²⁶, and could increase when animals are exposed to adverse conditions, such as restraint or a novel environment. Higher white blood cells (WBC) may also indicate stress²³. Ewes fed on grape pomace had higher levels of NEFA and WBC, which may be due to an increase in energy demand²⁹. In our study, the energy demand on the ewes was high since they were lactating, so it is possible that ewes feeding on grape seed needed to mobilise their reserves more than did the controls, probably due to the reduction of the concentrate supplemented with that by-product (Table 1²⁸). However, the live weight of the sheep was not compromised (Table 2).

Milk composition and somatic cell count

Sheep milk composition is influenced by ewe nutrition, especially in highly productive animals. In our study, the changes in diet affected the percentage of lactose and total dry matter (Table 3). Results of the milk fatty acid composition are published in Resconi et al.²². Grape pomace reduced while grape seed increased lactose percentages, with respect to the control diet, but milk yield was not directly assessed.

In another study, grape pomace at 5 and 10 g/100 g of DM in Churra ewes also decreased lactose concentration with respect to control diets²⁴, but 300 g/230 d per head of grape seeds in Sarda ewes did not affect milk composition²⁵. We did not find differences in milk fat and protein concentrations between treatments, in agreement with previous studies including winery/grape residues in ruminant diets, where milk yield was neither affected²¹,²²,³¹. However, Moate et al.²⁶ found higher concentration of milk fat from cows with diets including pellet and ensiled grape marc.

Lamb production, carcass characteristics and meat quality

Least square means for productive and carcass traits of the lambs born from ewes of the three experimental groups are shown in Table 4. The results are similar between groups for the initial and final live weights, the average daily gain and for all the parameters measured in the carcass except for fat-tension score. Regarding instrumental meat quality, we found significant differences in thawing losses and meat colour in terms of L* (lightness) between the grape pomace and the grape seed groups (Table 5).
In our study, milk composition was slightly modified by the diets and therefore, the lack of effect in the performance of the lactating lambs was expected. In fact, studies with lambs fed diets supplemented with grape residues did not find an effect in the growth rate of the animals neither on carcass weight\textsuperscript{32,33}. Furthermore, Bahrami and Chekani-Azar\textsuperscript{34}, found that lambs fed rations containing 5% and 10% dried grape seed even improved their growth and feed conversion compared to controls. We found a higher fattening score in the control group compared to the grape seed group. The energy content of the grape seed could have been reduced compared to that of control, which could lead to a lower milk production and thus to a lower energy deposition of fat. By the other hand, phenolic compounds maybe transferred to milk could have also reduced obesity in lambs, as it was observed in hamsters supplemented with grape seed procyanidin extract\textsuperscript{35}.

The pH values were below 6.0, within the range of optimum commercial quality, and the ewes’ diet had no effect on the ultimate pH of their lambs’ meat. However, we found signifi-
**CONCLUSIONS**

The results suggest that the inclusion of grape by-products could be effectively used to supplement ewe diets without affecting negatively through lambs’ growth. Although some indicators of stress were lower in lactating ewes fed with the control concentrates, the welfare of the ewes in the three treatments evaluated was not compromised. Finally, grape seeds in the ewes diets reduced the fatness score in the carcass and lightness in the meat of their suckling lambs, whereas thawing losses were increased; but all the differences were minor and therefore the relevance at commercial level might negligible.

**ACKNOWLEDGEMENTS**

This study was supported by the Spanish Ministry of Science (project AGL-2008-02088/GAN). The Government of the Autonomous Community La Rioja, funded the PhD Scholarship of Mrs. M. Pascual-Alonso (Programa de Ayudas Predoctorales del Gobierno de la Rioja). V.C. Resconi was supported by the Juan de la Cierva-Incorporación Program (MINECO, Spain). Thank you very much to the staff of the Animal Experimentation Service (SEA) of the University of Zaragoza. Special thanks to Dr. A. De Vega for his assessment and analyses of the diet compositions, and to the Milk Laboratory Service of Aragón (Laboratorio Interprofesional Lechero de Aragón) for their assessment and help with the milk analysis.