Comparison of texture and biochemical characteristics of three rabbit lines selected for litter size or growth rate

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Abstract

Meat texture and some biochemical characteristics that could influence meat tenderness were studied in rabbit loins. Rabbits from three synthetic lines were compared, lines V and A selected for litter size at weaning and line R selected for growth rate between weaning and slaughter time. The activities of cathepsins, collagen content and textural properties measured by Warner-Bratzler (WB) shear device and by the texture profile analyses (TPA) test were measured. Line R was more tender than line V and line A had an intermediate tenderness. Rabbit meat from line R had higher activity of cathepsins B and B + L, lower total collagen content and lower cohesiveness, springiness and chewiness, shear force and total work (area under the curve obtained with WB device) than line V. Line A had an intermediate texture between lines R and V. Our results show evidence of genetic variation between lines in rabbit meat tenderness.

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Keywords: Bayesian statistics; Cathepsins; Collagen; Texture; Rabbit lines

1. Introduction

Rabbit meat consumption is important in the Mediterranean area, especially in France, Italy and Spain (FAO Stat, 2004). Recently, increasing interest in the sensorial properties of rabbit meat has been shown, and tenderness is probably one of the most important eating quality attributes of rabbit meat (Dalle-Zotte, 2002). Selection programs in rabbit commercial schemes are based in a three way crosses in which two lines are selected for litter size and one line is selected for growth rate (Baselga & Blasco, 1989; Lebas, Coudert, Rochambeau, & Thébault, 1996). Few studies comparing lines selected for different traits have been published. Pla, Hernández, and Blasco (1996), Gomez, Baselga, Rafel, and Ramon (1998) and Pla, Guerrero, Guardia, Oliver, and Blasco (1998) compared lines selected for litter size or growth rate, but they compared the lines at the same weight, thus at different state of maturity, and only Pla et al. (1998) studied textural properties.

Tenderness can be instrumentally measured by Warner-Bratzler (WB) shear device and by the texture profile analysis (TPA) (Honikel, 1998). Meat tenderness depends mainly on the post-mortem changes affecting myofibrillar proteins and on the connective tissue that represents the “background” toughness. Proteolytic enzymes such as cathepsins are involved in structural and biochemical changes taking place during post-mortem storage of meat (Koohmaraie, 1994). However, the contribution of cathepsins to meat tenderization is not clear (see Hopkins & Thompson, 2002, for a review). Several studies in different species have indicated an influence of the genetic type on the activity of cathepsins (Armero, Barbosa, Toldra, Baselga, & Pla, 1999; Hernández, Zomeño, Arino, & Blasco, 2004b, in pork; Schreurs, Van der Heide, Leenstra, & De Wit, 1995, in chicken; and Uytterhaegen et al., 1994 in beef) although no comparisons between rabbit lines are available for the activity of these enzymes. Meat texture
can also be affected by the quantity of collagen as well as its solubility (see Bailey & Light, 1989, for a review). Information about collagen content and its solubility in rabbit meat is scarce (Combes, Lepetit, Darche, & Lébas, 2003). At present, there is no information about the influence of genetic type on collagen content and its solubility in rabbit meat.

Recently, Blasco (2005) has proposed Bayesian statistics and Monte-Carlo Markov Chain (MCMC) as a new and powerful tool for meat quality analyses. In classical statistics, a significant difference does not provide an exact measurement of the evidence provided by the data. A P-value cannot be interpreted as a measure of significance because when repeating the experiment, the P-value changes. An advantage of Bayesian procedures is that we have an exact account of the evidence provided by the experiment. Thus, the probability of differences between lines being higher than 0 can be for example 0.94 or 0.89, and we still can consider that we have evidence enough of these differences for our purposes.

The objective of this study was to compare meat texture and some biochemical characteristics that could influence the tenderness of rabbit meat from lines selected for litter size or growth rate using Bayesian statistical techniques.

2. Materials and methods

2.1. Animals

Rabbits from three synthetic lines were used in the experiment. Line A has a New Zealand origin, line V is a blend of New Zealand and California origins and line R was formed by mixing commercial hybrids used as terminal sires. Line V and A were selected for litter size at weaning (at 4th week of life) and slaughter time (9th week of life) for 24 generations in the farm of the Universidad Politécnica de Valencia.

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2.2. Assays of cathepsins activities and cysteine proteinase inhibitors activity

Two grams of *Longissimus* muscle were homogenised in 25 ml of 50 mM sodium citrate buffer, pH 5.0, containing 1 mM EDTA and 0.2% (v/v) Triton X-100. The homogenate was centrifuged at 10,000g for 20 min, and the resulting supernatant filtered through glass wool and used for cathepsin assays.

Cathepsin B, B + L and H were assayed as previously described by Toldrá and Etherington (1988), using N-CBZ-L-arginyl-7-amido-4-methylcoumarin, N-CBZ-L-phenylalanyl-L-arginine-7-amido-4-methylcoumarin, both a pH 6.0, and L-arginine-7-amido-4-methylcoumarin at pH 6.8 as specific fluorimetric substrates of cathepsin B, B + L and H, respectively. The reaction mixture consisted of 50 µl of enzyme extract and 250 µl of reaction buffer, 40 mM sodium phosphate, containing 0.4 mM EDTA, 10 mM cysteine, and 0.05 mM of the specific substrate. The reaction mixtures were incubated at 37 °C and fluorescence was followed continuously at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. One unit of cathepsin activity was defined as the amount of enzyme hydrolysing 1 µmol of substrate in 1 h at 37 °C.

The assay of the lysosomal cysteine proteinase inhibitors was carried out according to Bige, Ouali, and Valin (1985), with the following modifications: to 1 ml of the muscle extract, obtained as previously described for cathepsins, was added 5 N NaOH until the extract reached a pH of 10 and incubated at 37 °C for 60 min in order to destroy any residual proteolytic activity. The pH was readjusted to 6.0 by addition of 5 N HCl, the suspension centrifuged at 14,000g for 10 min at 4 °C, and the pH of supernatant increased to 7.6 with 5 N NaOH. One hundred microliters of the prepared extract was incubated at 37 °C for 30 min with 50 µl of 10 mM papain. Then, 150 µl of reaction buffer (100 mM sodium acetate, pH 6.0, containing 1 mM EDTA, 5 mM dithiothreitol, 0.1% Brij-35 and 20 µM N-CBZ-L-phenylalanyl-L-arginine-AMC) was added and incubated at 37 °C again for 10 min. The fluorescence was measured as previously described for cathepsins. One unit of cysteine proteinase inhibitors was defined as the amount of inhibitor capable of totally blocking one unit of papain activity.

2.3. Collagen content

Total and insoluble collagen contents were measured in the *Longissimus* muscle in twelve animals per line and expressed as mg hydroxyproline/g fresh muscle, as in Bonnet and Kopp (1984). The solubilised samples were used to measure insoluble collagen content following a variation of the method described by Hill (1966). The soluble collagen
was determined as the difference between total collagen and insoluble collagen and the solubility and insolubility percentages were calculated.

2.4. Texture analysis

Muscle samples were thawed at 4°C/24 h in their vacuum-packed plastic bag, cooked at 80°C for 1 h by immersion in a water bath with automatic temperature control (Combes et al., 2003) and then cooled at room temperature (20 ± 2°C) before the analysis. The samples for Warner-Bratzler shear test (WB) were obtained by cutting at least two rectangles of 2 × 1 cm of cross section, parallel to the muscle fibre direction (Guerrero & Guardia, 1999). They were completely cut using a WB shear blade with a triangular slot cutting edge and three parameters were measured: the maximum shear force (Møller, 1980), the shear firmness (Brady & Hunecke, 1985) and the total work performed to cut the sample or the area under the curve obtained. Samples for texture profile analysis (TPA) (Bourne, 1978) were obtained by cutting cubes of 1 cm each side parallel to the muscle fibre direction and then compressing to 75%. In this test the following variables were obtained: hardness, cohesiveness, springiness and chewiness. The Texture Analyser Mod. TA-XT2 (Stable Micro Systems, UK) was used for both tests and all the samples were cut or compressed perpendicular to the muscle fibre direction at a crosshead speed of 5 mm/s. The average value for each Longissimus sample was recorded (mean of two to four replicates).

2.5. Water holding capacity and cooking losses

To determine water holding capacity, a sample of intact meat (taken at the seventh lumbar vertebra) weighing 300 ± 5 g was placed on a previously desiccated and weighed (0.0001 g accuracy) 7-cm disk of Whatmann No. 1 filter paper. After weighing, the paper with meat was placed between two Plexiglas plates and a load of 2.25 kg was applied for 5 min. The damp paper filter was rapidly placed between two Plexiglas plates and a load of 2.25 kg for 5 min. The damp filter paper weight was recorded after weighing. CL is the ratio (×100) of the difference in weight between the cooked and raw muscle relative to the weight of raw muscle.

2.6. Statistical methods

Data were analysed using a model with line (with three levels, lines A, V and R) and sex effects. A Bayesian analysis was performed. Bounded flat priors were used for all unknowns. Data were assumed to be normally distributed. Marginal posterior distributions of all unknowns were estimated using Gibbs Sampling. After some exploratory analyses we used one chain of 10,000 samples, with a burning period of 2000, thus marginal posterior distributions were estimated with 8000 samples each. Convergence was tested for each chain using the Z criterion of Geweke (Geweke, 1992). Monte Carlo standard errors were calculated. Details of the procedure can be found in Blasco (2001) and in Sorensen and Gianola (2002).

3. Results and discussion

Inferences were made from features of marginal posterior distributions of the differences between lines. Posterior distributions were symmetrical, thus median mean and mode were almost same. We offer the median because it has good statistical properties (see Blasco, 2005, for a review). Monte Carlo standard errors were very small and they are not offered in the tables (the highest MCse was lower than 0.001). The Geweke test did not detect lack of convergence in any case. In order to answer the question of whether two of the lines are different or not, we calculated the probability of this difference being higher than 0 (P > 0). An advantage of Bayesian procedures is that we have an exact account of the evidence provided by the experiment. Thus, the probability of differences between lines being higher than 0 can be for example 0.94 or 0.89, and we still can consider that we have evidence enough of these differences for our purposes. When differences are lower than zero, high evidence of these differences is provided by probabilities near 0, for example, (P > 0) = 0.02 or (P > 0) = 0.09. A comparison between growth curves reveals that at 9 week of age, animals of the three lines have almost the same percentage of adult weight (Blasco, Piles, & Varona, 2003; Pla, Piles, & Valde- vira, 1997), therefore differences between lines should not be attributed to differences in maturity since lines were measured at the same age, thus they are close to the same stage of maturity.

3.1. Cathepsin activities and their inhibitors

Table 1 shows the features of the estimated marginal posterior distributions of the differences between lines for cathepsin activities and their inhibitors. Several studies have indicated differences in the genetic type for the activity of cathepsins in pork (Armero et al., 1999; Hernández et al., 2004b), in chicken (Schreurs et al., 1995) and in beef (Uytterhaegen et al., 1994). However, at present there are no studies about the influence of genetic type in the activity of these enzymes in rabbit meat. Line R showed higher activity of cathepsin B and B + L than lines V and A, and line A showed a higher activity of cathepsin B than line V. However, the rate cat (B + L)/cat B, which is an indicator of the activity of cat L, showed differences between lines A and V with a higher value in line V, but no differences were found when compared with line R. The activity of cathepsin H was higher in line A than in lines V and R.
No differences between lines were found for the cysteine protease inhibitors. A sex effect was found for the activity of cathepsin B and B + L (data not shown) with higher values for females (differences between males and females of −0.06 and −0.16 for cathepsin B and B + L, respectively).

In chicken, Schreurs et al. (1995) found differences in the activity of cathepsins and their inhibitors, cystatins, among strains with different growth rates. In this experiment, the experimental broiler line selected by high body weight, showed lower cathepsin B, D and H activities. However, Gil et al. (2006) found that selection for growth rate did not affect the activities of proteolytic enzymes and of their inhibitors in rabbit meat. In our experiment the line R, selected for growth rate, had a higher activity of cathepsins B and B + L than the lines selected for litter size at weaning. Differences between lines are not only due to growth rate, but to other traits derived from their different genetic background. Russo et al. (2000) found a moderate heritability (0.23–0.28, although with high standard errors) in pigs, for the activity of cathepsin B, suggesting the possible use of cathepsin B as a selection criteria for genetic improvement of meat tenderness.

### 3.3. Collagen content

The features of the estimated marginal posterior distributions of the differences between lines for whole collagen content and the percentages of solubility and insolubility are summarized in Table 2. Collagen content is influenced by rabbit line, line R having a lower content than lines A and V; while the probability of the differences between lines A and V being higher than zero is low ($P > 0$) = 0.55. Collagen content varies between breeds in different species (Sanudo et al., 2004, in beef; Martínez-Cerezo et al., 2005, in lamb) but at present there is no comparison between different rabbit genetic types. Regarding the percentages of soluble and insoluble collagen, no differences were found between lines A and V but there was some evidence of differences between line R and the other lines. The percentage of soluble collagen is remarkable high in rabbit meat (around 60%) compared with other species (Combes et al., 2003). Regarding the sex effect, differences between males and females for collagen content were 0.49 with a ($P > 0$) = 0.97 and no differences between sex were found for the percentage of collagen solubility and insolubility.

### 3.4. Textural properties

Table 3 shows the features of the marginal posterior distributions of the differences between lines for instrumental texture variables measured by the WB and TPA methods.

The largest differences appeared between line R selected for growth rate and the other lines. The results from the WB test showed significant differences for shear force and total work (defined as area) and no differences among lines (low probability) were found for shear firmness. Line V had higher values of shear force and area than line R and line A had a higher value of area than line R, while no differences were found between lines A and V. The results from the TPA (Table 3) showed differences between groups in cohesiveness, springiness and chewiness whereas the evidence for differences in hardness was low. According to these results, line V had higher cohesiveness, springiness and chewiness than line R and higher cohesiveness and chewiness than line A, and no differences were found between males and females for collagen content were 0.49 with a ($P > 0$) = 0.97 and no differences between sex were found for the percentage of collagen solubility and insolubility.

### Table 1
Activity of cathepsins and their cysteine proteinase inhibitors (U/g) of *Longissimus* muscle of three lines of rabbits

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>CV</th>
<th>A-V</th>
<th>Median</th>
<th>HPD</th>
<th>$P &gt; 0$</th>
<th>HPD</th>
<th>$P &gt; 0$</th>
<th>HPD</th>
<th>$P &gt; 0$</th>
<th>HPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>0.710</td>
<td>21.0</td>
<td>0.070</td>
<td>0.95</td>
<td>−0.018; 0.167</td>
<td>−0.098</td>
<td>0.02</td>
<td>−0.196; 0.007</td>
<td>−0.176</td>
<td>0.00</td>
<td>−0.273; 0.079</td>
</tr>
<tr>
<td>B + L</td>
<td>2.28</td>
<td>15.8</td>
<td>0.085</td>
<td>0.79</td>
<td>−0.137; 0.315</td>
<td>−0.379</td>
<td>0.00</td>
<td>−0.615; 0.163</td>
<td>−0.471</td>
<td>0.00</td>
<td>−0.704; 0.240</td>
</tr>
<tr>
<td>(B + L)/B</td>
<td>3.27</td>
<td>11.7</td>
<td>−0.203</td>
<td>0.05</td>
<td>−0.458; 0.010</td>
<td>−0.050</td>
<td>0.34</td>
<td>−0.288; 0.161</td>
<td>0.154</td>
<td>0.89</td>
<td>−0.085; 0.370</td>
</tr>
<tr>
<td>H</td>
<td>0.642</td>
<td>20.5</td>
<td>0.065</td>
<td>0.94</td>
<td>−0.017; 0.149</td>
<td>0.089</td>
<td>0.98</td>
<td>0.004; 0.170</td>
<td>0.022</td>
<td>0.69</td>
<td>−0.063; 0.107</td>
</tr>
<tr>
<td>CPI</td>
<td>6.52</td>
<td>12.6</td>
<td>0.190</td>
<td>0.77</td>
<td>−0.355; 0.648</td>
<td>−0.051</td>
<td>0.41</td>
<td>−0.568; 0.463</td>
<td>−0.240</td>
<td>0.16</td>
<td>−0.754; 0.224</td>
</tr>
</tbody>
</table>

Features of the marginal posterior distributions of the differences between rabbit lines, differences between lines A and V (A–V), lines A and R (A–R) and lines V and R (V–R). CV, coefficient of variation

### Table 2
Collagen content (mg/g of fresh muscle), soluble collagen % (CS) and insoluble collagen % (CI) of *Longissimus* muscle of three lines of rabbits

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>CV</th>
<th>A-V</th>
<th>Median</th>
<th>HPD</th>
<th>$P &gt; 0$</th>
<th>HPD</th>
<th>$P &gt; 0$</th>
<th>HPD</th>
<th>$P &gt; 0$</th>
<th>HPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>6.80</td>
<td>12.6</td>
<td>0.006</td>
<td>0.55</td>
<td>−0.521; 0.813</td>
<td>0.947</td>
<td>1.00</td>
<td>0.376; 1.65</td>
<td>0.971</td>
<td>0.99</td>
<td>0.390; 1.65</td>
</tr>
<tr>
<td>CS, %</td>
<td>60</td>
<td>9.84</td>
<td>−0.522</td>
<td>0.37</td>
<td>−4.35; 4.85</td>
<td>2.21</td>
<td>0.85</td>
<td>−1.72; 7.08</td>
<td>3.13</td>
<td>0.92</td>
<td>−0.872; 7.84</td>
</tr>
<tr>
<td>CI, %</td>
<td>40</td>
<td>15.7</td>
<td>1.34</td>
<td>0.71</td>
<td>−2.78; 7.12</td>
<td>−2.01</td>
<td>0.23</td>
<td>−6.34; 3.13</td>
<td>−3.01</td>
<td>0.10</td>
<td>−7.32; 2.05</td>
</tr>
</tbody>
</table>

Features of the marginal posterior distributions of the differences between rabbit lines, differences between lines A and V (A–V), lines A and R (A–R) and lines V and R (V–R). CV, coefficient of variation × 100. $P > 0$, probability of the difference of being higher than 0. HPD, high posterior density interval at 95% of probability.
lines A and R. No differences between sexes were found for instrumental texture characteristics measured by the WB and TPA methods.

Most of the texture variables measured indicate that the meat from rabbits of line R is the most tender and the meat from rabbits of line V is the least tender, line A having an intermediate tenderness. Results from sensory analysis of the same samples (Hernández, Arín, Pla, & Blasco, 2005) showed line V was harder, more fibrous and less juicy than lines A and R. No differences between lines A and R were found for these traits. Pla et al. (1998) studied texture properties measured by the WB method comparing lines selected for litter size or growth rate, but they compared the lines at the same weight, thus at different stage of maturity. In our experiment the differences between lines are not due to different stage of maturity because all lines were measured at the same age and not at the same weight, i.e., close to the same stage of maturity.

### 3.4. Water holding capacity and cooking losses

Low evidence of differences between lines were found for water holding capacity and cooking losses (Table 4). In rabbits, our group have reported a negative effect of selection for growth rate on water holding capacity (Hernández, Aliaga, Pla, & Blasco, 2004a; Piles, Blasco, & Pla, 2000). However, the line R selected for growth rate had similar values for PRW to lines V and A, selected for litter size. Similarly, Pla et al. (1998) did not find differences in water holding capacity between rabbit lines but compared at the same weight. Comparable cooking losses were found by Combes et al. (2003) in rabbit meat cooked as in our experiment. These authors found that cooking losses increase with the increase of temperature to reach a maximum at 80 °C and then remain stable between 80 and 90 °C. Nevertheless, the largest part of cooking losses are achieved after 20 min heating. No sex effect was found for percentage or released water and cooking losses.

In conclusion, our results show evidence of genetic variation between lines in rabbit meat tenderness. Meat from the high size line selected for growth rate (line R) was more tender than meat from rabbits of line V, and line A had an intermediate tenderness. Rabbit meat from line R had higher activity of cathepsins B and B + L, lower total collagen content and lower cohesiveness, springiness and chewiness than line V. Also, line R had a lower shear force than line V, and a lower total work (area under the curve obtained with WB device) than in line V was necessary to cut the sample. Line A presented an intermediate texture characteristic between lines R and V. Although meat tenderness is one of the main attributed that determines meat quality, at present this characteristic is not taken into account in the price of rabbit meat. However, these better texture characteristics could be an added value in the use of lines with better meat quality properties measured by the WB method comparing lines selected for litter size or growth rate, but they compared the lines at the same weight, thus at different stage of maturity. In our experiment the differences between lines are not due to different stage of maturity because all lines were measured at the same age and not at the same weight, i.e., close to the same stage of maturity.

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References


