Study of the influence of genotype and rearing method on muscle fibre characteristics in suckling goat kids

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ABSTRACT

Single born male goat kids (n = 160) from eight breeds, Florida (FL), del Guadarrama (GU), Majorera (MA), Palmera (PA), Payoya (PY), Retinta (RE), Tinereña (TF) and Verata (VE) were involved in the present study. This study aimed to evaluate the effect of genotype and rearing method on muscle fibre populations and morphology. Two different rearing methods were used: natural suckling (NS) and milk replacer (MR). At least 300 fibres from the Semimembranosus muscle of each experimental animal were labelled as Type I or Type II using immunohistochemistry. In this study, the Type II fibres were bigger than Type I. In addition, the VE breed displayed the highest percentage of Type II fibres, and the MA, PA and TF breeds the lowest. On the contrary, the VE breed displayed the lowest percentage of Type II fibres, while the MA breed showed the highest. Goat kids from the VE, RE, GU and PY breeds displayed bigger fibres (i.e. Types I and II) than the other studied breeds. Regarding the rearing method, animals fed MR displayed more presence of Type II fibres than Type I fibres. Natural suckling animals presented bigger fibres (i.e. Types I and II) than MR goat kids.

Introduction

The importance of meat quality for meat industry has been constantly increasing over the last decades. Meat quality is affected by numerous factors such genotype, physiology, health, environment, and management factors (Pophiwa et al. 2020; Prache et al. 2021). Many of these factors have been addressed in goats (Argüello et al. 2005), but the information about how genotype or rearing method (i.e. natural suckling vs milk replacer) may affect muscle fibre characteristics in suckling goat kids is limited.

Muscle fibres can be classified as Type I fibres that are slow-twitch oxidative, and Type II that are fast-twitch glycolytic fibres and show fatigue faster than Type I fibres. The relation between muscle fibre populations and meat quality has been described in cattle (Picard et al. 2020). Thus, some of the variables related to meat quality such as pH decrease after slaughter (Gagaoua et al. 2017), meat colour (Purslow et al. 2020) or meat tenderness (Albrecht et al. 2006) has been correlated with muscle fibre populations.

As previously showed in bulls, muscle fibre populations are affected by genotype (Hocquette et al. 2004), showing that lean animals displayed higher proportions of Type II fibres than those observed in beef animals. Similar studies have proved that there is a strong relationship between muscle fibre composition and growth performance when different breeds compared within a species (Şirin et al. 2017). However, literature on this field is limited and further studies have to be carried out to understand the effect of genotype on muscle fibre populations in goats (Dhanda et al. 2003; Argüello et al. 2005).

Otherwise, nutrition has been reported as a modulator of muscle fibre growth and development (Gagaoua et al. 2017). Dietary restrictions around weaning induce changes in muscle fibre populations in bulls (Brandstetter et al. 1998). Maintenance and nutrition during the postnatal period affect lamb's fibre characteristics as well (Fahey et al. 2005; Hegarty...
et al. 2006). Additionally, a high level of fibre in the diet of cattle has been associated with higher proportions of glycolytic fibres in skeletal muscle, as well fat inclusion increases the oxidative fibres (Kłosowski et al. 1992). Although Argüello et al. (2005) investigated the effects of rearing (i.e. natural suckling vs milk replacer) in muscle fibre populations and morphology, the results in the mentioned research are limited to a single dairy goat breed (i.e. Majorera breed).

Due to the limited literature and the importance of this research area on meat quality, this study aimed to investigate the effect of genotype and rearing method (i.e. natural suckling vs milk replacer) on muscle fibre populations and morphology in suckling goat kids from eight different goat breeds.

**Material and methods**

Procedures involved in the present study follow the guidelines of Directive 2010/63/EU on the protection of animals used for experimental and other scientific purposes. European guidelines for the protection of animals at the time of slaughtering (Regulation (EC) No 1099/2009) were also followed at slaughterhouses.

This study has been performed by the Animal Production and Biotechnology group (Institute of Animal Health and Food Safety, Universidad de Las Palmas de Gran Canaria, Spain) in collaboration with the Faculty of Agronomy (Universidad de La Laguna, Spain), the Experimental Farm of the Pico Research Station (Canary Islands, Spain), the Department of Agricultural and Forestry Science (Universidad de Sevilla, Spain) and the Centro de Investigación y Tecnología Agroalimentaria (Aragón, Spain).

Goat kids from eight Spanish goat breeds (20 kids per breed) were involved in the study: Florida (FL), del Guadarrama (GU), Majorera (MA), Palmera (PA), Payoya (PY), Retinta (RE), Tinerfeña (TF) and Verata (VE). Goat kids were reared in two or three different farms per breed. All goat kids were males from a single partum of multiparous goats. Two different rearing systems were used: natural suckling (NS) system where goat kids had free access to the dams; and milk replacer (MR) system where goat kids were immediately removed from dams after birth and were fed colostrum for two days according to the management suggested by Castro et al. (2005). After that, animals were ad libitum fed warm (40°C) reconstituted (17% w/v) milk replacer (total fat 25 ± 0.6%, crude protein 24 ± 0.5%, crude cellulose 0.1 ± 0.0%, ash 7 ± 0.6%, Ca 0.8 ± 0.1%, Na 0.5 ± 0.2%, P 0.7 ± 0.0%, Fe 36 ± 4.0 mg/kg, Cu 3 ± 1.7 mg/kg, Zn 52 ± 18.8 mg/kg, Mn 42 ± 14.4 mg/kg, I 0.22 ± 0.06 mg/kg, Se 0.1 ± 0.06 mg/kg, and butylhydroxytoluene (BHT) 65 ± 30 ppm). Goat kids from both experimental groups had no access to concentrates, hay or forages during the entire study.

Goat kids were slaughtered at 8.47 ± 0.077 kg BW (body weight) in commercial slaughterhouses after approximately 30 days of rearing. The mean time between farm and slaughterhouse was less than 3 h. Electrical head stunning was applied (1.00 A) to the goat kids. Subsequently, animals were exsanguinated and dressed. Thereafter, hot carcasses, including the head and kidneys, were weighed, achieving a hot carcass weight of 4.97 ± 0.061 kg. Afterwards, carcasses were hung by the common calcaneal tendon and chilled for 24 h at 4°C. After that, carcasses were split into half and each left half carcass was divided into five primary cuts as described by Argüello et al. (2001). The left long leg was vacuum-packed and frozen at −20°C until analyses were performed later. The longissimus thoracis muscle (LT) of the right half of the carcasses was extracted, vacuum-packed, and aged for 3 days. A layer of muscle was retired, and the colour was measured. Then, pH was measured with a pH metre equipped with a Crison 507 penetrating electrode (Crison Instruments S.A., Barcelona, Spain).

To determine muscle fibres, the left Semimembranosus muscle was dissected, and all connective tissue was removed. This muscle is often used while determining goat and other species meat quality studies (Bhuiyan et al. 2017). Due to its propulsive nature, a high number and full diversity of fast-twitch fibres in this muscle can be expected (Adeyemi et al. 2015). Muscle samples (3 × 1 × 1 cm) were removed from the mid-belly region of the muscle. Samples were fixed in 4% neutral-buffered formalin for 24 h. Afterwards, they were sculpted (two 2 × 1 cm pieces/sample) and embedded in a paraffin bath to be able to slice and stain them. The samples were then stained using haematoxylin-eosin to assure the right transversal orientation of the muscle fibres. After that, transverse serial sections at 5 μm thicknesses were performed using a Leica RM2135 microtome (Leica Microsystems S.L.U., Barcelona, Spain) and mounted on poly-L-lysine-coated glass slides for immunohistochemistry analyses. The mounted sections were heated at 37°C overnight.

The muscle colours were measured using a Minolta CM-2006d Spectrophotometer (Konica Minolta Holdings, Inc., Osaka, Japan) in CIEL*ab* space (CIE 1986) with the specular component included, 0% UV, an observer angle of 10° and zero, and white calibration. The integrating sphere had a diameter of 8 mm was covered with a CMA149 dust cover (Konica Minolta Holdings, Inc.). The illuminant used was D65. The spectrophotometer was rotated 90° on the horizontal plane before each reading, and the mean of three readings was used for analysis. The lightness (L*) index was recorded using the SpectraMagic NX software (Minolta Co. Ltd., Osaka, Japan).

**Immunohistochemistry**

Muscle samples were immunostained to detect slow (Type I) and fast (Type II) heavy-chain myosin isoforms using monoclonal antibodies: anti-myosin skeletal slow M8421 (Sigma Co., St. Louis, MO, USA) and skeletal fast M4276 (Sigma Co., St. Louis, MO, USA) were used at 1:500 and 1:750 dilution factors, respectively. A modified version of the immunohistochemistry technique according to Rivero et al. (1996) was carried out. Before the immunohistochemistry procedure, the muscle slides were dewaxed, dehydrated and rehydrated. The antigen retrieval was performed using 1% protease solution (PS147-5G, Sigma-Aldrich). Samples were first incubated in rabbit serum X090210-8 (Agilent Technologies, Dako Denmark A/S, Denmark) and then with the primary antibody for 18 h, both procedures in a humidity chamber. Sections were washed, and polyclonal biotinylated mouse anti-rabbit secondary antibody E035401-2 (Agilent Technologies, Dako Denmark
Area: Reports the area of each object. Measured in μm².
Area polygon (μm²): Reports the area of the polygon that defines the object’s outline. Perimeter: the length of the outline of each object. Measured in μm.
Maximum diameter (μm): Reports the length of the longest line joining two outline points and passing through the centroid.
Minimum diameter (μm): Reports the length of the shortest line joining two outline points and passing through the centroid.
Mean diameter (μm): Reports the average length of the diameters measured at two-degree intervals joining two outline points and passing through the centroid.
Maximum radius (μm): Reports the maximum distance between each object’s centroid pixel position and its perimeter.
Minimum radius (μm): Reports the minimum distance between each object’s centroid pixel position and its perimeter.
Maximum: minimum radius: Reports the ratio between maximum and minimum radius.

Statistical analysis
Statistical analysis was performed with the R studio package. Normality was tested with the Shapiro test. All variables were non-normal distributed. The Wilcoxon test was used to evaluate differences in morphometry of Type I and Type II fibres. A Kruskal test with pairwise Wilcoxon test (P adjust method = ‘bonferroni’) was used to analyse genotype and rearing method effects. The model included genotype, rearing method, and the interaction between both as fixed effects. The interaction was non-significant and the significance was set as P < 0.05.

Results
In Table 1 are shown the percentages values of type of fibre in Semimembranosus muscle in goat kids from eight different breeds. Values ranged from 17.9% to 36.7% for Type I fibre and from 63.3% to 82.1% for Type II fibre. No interactions between genotype and rearing method were observed for any of the variables measured in this study (P > 0.05).

As can be observed in Table 1, genotype affected muscle fibre populations (P < 0.05). The VE breed showed the highest Type I percentage (P < 0.05) and the MA, PA and TF breeds the lowest (P < 0.05). On the contrary, the VE breed showed the lowest percentage of Type II fibres (P < 0.05), while the MA breed showed the highest (P < 0.05). Similarly, the rearing method also affected the muscle fibre populations (P < 0.05). Thus, goat kids reared under the MR method showed a lower percentage of Type I fibres and a higher percentage of Type II fibres than those animals reared under the NS method (P < 0.05).

Regarding differences between fibre types (i.e. Type I vs Type II; Table 2), Type II fibres showed higher values for all morphometric variables (i.e. area, maximum diameter, minimum diameter, medium diameter, maximum radius, minimum radius, perimeter, radius ratio and area polygon) than those obtained in Type I fibres (P < 0.05).

The morphology of Type I fibres was affected by genotype (Table 3; P < 0.05). Indeed, the VE and the PY breeds had the biggest Type I fibres (P < 0.05), while FL had the smallest (P < 0.05). The morphology of Type I fibres was also affected by the rearing method (P < 0.05), showing higher values for all measured variables in goat kids from the NS group than the ones obtained in the MR group (P < 0.05).

The morphology of Type II fibres was affected by genotype (Table 4; P < 0.05). The VE and the PY breeds showed the biggest Type II fibres while the FL and the MA breeds displayed the smallest ones. The rearing method (NS vs MR) also affected the morphology of Type II fibres (P > 0.05), showing higher values for all measured variables in goat kids from the NS group than the ones obtained in the MR group (P < 0.05).

In Table 5 are shown pH values which ranged from 5.49 to 6.38. There was a significant interaction between the breed and rearing method (p < 0.001). The rearing method did not modify the pH in most of the breeds (P > 0.05). However, Palmera, Payoya, and Tinerfaña kids reared with milk replacers had a higher pH than natural suckled kids (P < 0.05). Colour parameters of LT are also shown in Table 5, lightness (L*), and chroma (C*) variables were affected by the interaction between the breed and rearing system (P < 0.001). The use of milk replacers increased the L* of Payoya (p < 0.05) and decreased L* of Tinerfaña (P < 0.05), but the other breeds were not affected (p > 0.05).

Discussion
Muscle fibre Type I and Type II percentages are similar to the percentages recently reported by Castro et al. (2005) in the
Table 1. Genotype and rearing method effects on Type I and II fibres percentages.

| Type I, % | Genotype | Rearing method |
|----------|----------|----------------|
| FL GU MA PA | Type I | NS MR SD G R |
| 24.8 27.4 | FL | 28.1 24.7 9.6 | <0.001 0.007 |
| 17.9 20.1 | GU | 71.9 75.3 9.6 | <0.001 0.002 |
| 29.5 31.9 | MA | | |
| 21.8 36.7 | PA | | |
| Type II, % | 75.2 73.0 | NS MR SD G R |
| 68.1 78.2 | | | |
| 54.2 63.3 | | | |

Notes: Values shown in this table are equivalent to the medians of the measured parameters. Medians followed by different superscript letters in the same row (a–d) indicate significant differences among genotypes (p < 0.05). FL, Florida; GU, del Guadarrama; MA, Majorera; PA, Palmera; PY, Payoya; RE, Retinta; TF, Tinerfeña; VE, Verata; NS, natural suckling; MR, milk replacer; SD, % of type fibre standard deviation; G, genotype; R, rearing method.

Table 2. Morphology differences between Type I and Type II fibres.

| Type I Fibre type | SD | Fixed effects Fibre type |
|-------------------|----|----------------------------|
| Area, μm² | 184.9 | 223.9 | 104.8 | <0.001 |
| Area polygon, μm² | 187.5 | 225.2 | 100.9 | <0.001 |
| Perimeter, μm | 50.7 | 56.8 | 13.3 | <0.001 |
| Maximum diameter, μm | 18.6 | 21.1 | 5.2 | <0.001 |
| Minimum diameter, μm | 12.2 | 13.1 | 3.5 | <0.001 |
| Mean diameter, μm | 15.3 | 16.7 | 3.9 | <0.001 |
| Maximum radius, μm | 9.9 | 11.2 | 2.8 | <0.001 |
| Minimum radius, μm | 5.7 | 6.1 | 1.7 | <0.001 |
| Maximum:minimum ratio | 1.7 | 1.8 | 0.9 | <0.001 |

Note: Values shown in this table are equivalent to the medians of the measured parameters.

The rearing method has been reported to have a minor effect on muscle fibre populations in cows Maltin et al. (2001). Thus, changes in energy consumption by calves before weaning cannot be observed at 18 months of age due to the compensatory growth (Brandstetter et al. 1998). The reduced age and body weight at slaughter in the present study are those commonly used in goat kid meat production in Southern Europe. Consequently, these animals do not have enough time to develop a compensatory growth. This fact may explain the large changes in muscle fibre populations caused by the rearing method. These results are in agreement with those described by Brandstetter et al. (1998) in calves before weaning. Therefore, it is hypothesised that reduced energy level in milk replacer compared to goat milk may have caused the differences in muscle fibre populations among rearing method groups.

Breed effect on muscle fibres Type I and Type II (Table 3 and 4) morphological characteristics was similar, displaying VE, RE, GU and PY bigger muscle fibres than TF, PA, MA and FL. Şirin et al. (2017) observed differences in type II fibres number within Turkish sheep breeds. However, the diameter of the fibres in LD muscle did not show variations between the six sheep breeds studied. Muscle fibres characteristics are affected not only by genotype but also by maternal nutrition during gestation. Fahey et al. (2005) stated that poor maternal nutrition before muscle fibre formation can alter the muscle fibre development in lamb foetuses. This could be the explanation of found differences.

Although it has been reported similarities between beef and dairy breeds for muscle fibres size at adult weights Juirie et al. (2007), recent transcriptomics studies have demonstrated differences in growth potential between beef and dairy breeds (Ciecierska et al. 2020). As explained above, the VE,
Table 4. Genotype and rearing method effects on Type II morphology parameters.

| Breed | FL | GU | MA | PA | PY | RE | TF | VE |
|-------|----|----|----|----|----|----|----|----|
| Area  | 215.2<sup>a</sup> | 226.5<sup>b</sup> | 207.5<sup>b</sup> | 231.2<sup>b</sup> | 257.8<sup>b</sup> | 228.2<sup>b</sup> | 199.8<sup>b</sup> | 237.7<sup>b</sup> |
| Area polygon | 209.6<sup>a</sup> | 220.8<sup>b</sup> | 202.6<sup>b</sup> | 226.0<sup>b</sup> | 251.5<sup>b</sup> | 222.3<sup>b</sup> | 190.7<sup>b</sup> | 267.3<sup>b</sup> |
| Perimeter | 55.9<sup>a</sup> | 56.5<sup>b</sup> | 56.7<sup>b</sup> | 58.8<sup>b</sup> | 59.8<sup>b</sup> | 56.6<sup>a</sup> | 52.9<sup>b</sup> | 62.4<sup>b</sup> |
| Maximum diameter | 21.1<sup>a</sup> | 21.0<sup>b</sup> | 20.8<sup>b</sup> | 21.7<sup>b</sup> | 21.1<sup>a</sup> | 21.1<sup>a</sup> | 19.6<sup>b</sup> | 23.5<sup>b</sup> |
| Minimum diameter | 12.4<sup>a</sup> | 13.1<sup>bc</sup> | 12.4<sup>b</sup> | 13.0<sup>cd</sup> | 14.5<sup>c</sup> | 13.3<sup>c</sup> | 12.6<sup>c</sup> | 14.5<sup>c</sup> |
| Medium diameter | 16.4<sup>a</sup> | 16.8<sup>cd</sup> | 16.2<sup>a</sup> | 17.0<sup>b</sup> | 17.9<sup>bc</sup> | 16.9<sup>a</sup> | 15.9<sup>a</sup> | 18.4<sup>a</sup> |
| Maximum radius | 11.1<sup>a</sup> | 11.1<sup>a</sup> | 11.3<sup>a</sup> | 11.6<sup>bc</sup> | 11.7<sup>b</sup> | 11.1<sup>a</sup> | 10.3<sup>a</sup> | 12.4<sup>a</sup> |
| Minimum radius | 6.0<sup>c</sup> | 6.2<sup>c</sup> | 5.7<sup>d</sup> | 6.1<sup>b</sup> | 6.8<sup>b</sup> | 6.2<sup>d</sup> | 5.9<sup>c</sup> | 6.8<sup>b</sup> |
| Max./Min. radius | 1.8<sup>a</sup> | 1.7<sup>bc</sup> | 1.9<sup>b</sup> | 1.8<sup>b</sup> | 1.6<sup>b</sup> | 1.7<sup>c</sup> | 1.7<sup>d</sup> | 1.7<sup>d</sup> |

Notes: Values shown in this table are equivalent to the medians of the measured parameters. Medians followed by different superscript letters in the same row (a-f) indicate significant differences among genotypes (p < 0.05). FL, Florida; GU, del Guadarrama; MA, Majorera; PA, Palmera; PY, Payoya; RE, Retinta; TF, Tinerfeña; VE, Verata. NS, natural suckling; MR, milk replacer. G, genotype; R, Rearing method.

Table 5. Color and pH of the longissimus thoracis muscle of kids reared with milk replacer (MR) or natural suckling (NS).

| Breed | R | FL | GU | MA | PA | PY | RE | TF | VE | B |
|-------|----|----|----|----|----|----|----|----|----|----|
| pH  | MR | 5.54 | 5.75 | 5.84 | 6.38 | 5.78 | 5.54 | 6.05 | 5.75 | 0.0001 |
| NS  | 5.49 | 5.71 | 5.86 | 5.97 | 5.64 | 5.47 | 5.84 | 5.83 | 0.0001 |
| L*  | MR | 58.75 | 50.84 | 52.5 | 58.61 | 54.45 | 55.85 | 47.94 | 50.32 | 0.0001 |
| NS  | 55.69 | 48.66 | 54.42 | 54.81 | 49.15 | 53.33 | 56.99 | 53.77 | 0.0001 |
| C*  | MR | 12.34 | 9.48 | 11.83 | 14.66 | 13.79 | 15.82 | 14.09 | 11.60 | 0.0001 |
| NS  | 12.91 | 12.06 | 16.40 | 13.24 | 11.52 | 13.83 | 13.70 | 13.18 | 0.0001 |

Notes: R, rearing method; NS, natural suckling; MR, milk replacer. FL, Florida. GU, del Guadarrama. MA, Majorera; PA, Palmera; PY, Payoya; RE, Retinta; TF, Tinerfeña; VE, Verata; B, breed; L*, Lightness; C*, chroma.

RE, GU and PY breeds can be considered hardy meat breeds; the other four breeds used in the present study (FL, MA, PA and TF) are considered dairy breeds. Although the PY breed is also considered a dairy breed, the breeding selection started a few years ago, which could explain the similar results obtained in this breed compared to the meat goat breeds. Postnatal muscle growth is run by hypertrophy phenomena (Brameld et al. 2008). The muscle fibre hypertrophy is due to the fusion of satellite cells with muscle fibres. The results obtained by Ciecierska et al. (2020) confirmed that the expression profile of several groups of genes is common in beef breeds at the level of proliferating satellite cells but differs from those observed in cattle dairy breeds.

As described above, the effect of the rearing method (NS vs MR) on the morphological characteristics of muscle fibres Type I and Type II was similar. Goat kids from the MR group showed smaller fibres than NS animals. These differences were not observed by Argüello et al. (2005) in goat kids from the Majorera breed. However, in other species (i.e. cattle), energy restriction causes decreased cross-sectional area Oddy et al. (2001).

In addition, muscle fibre characteristics may influence pH and meat colour according to Joo et al. (2013). Rapid discoloration occurs in muscles that contain greater proportions of type I muscle fibres (Joo et al. 2013). In our study, VE, RE, PY and GU kids showed a higher proportion of Type I fibres, but this was not correlated with an increased lightness. Ripoll et al. (2019) concluded that the colour of skeletal muscles depends on the rearing system and is strongly modulated by the goat breed. Furthermore, Şirin et al. (2017) found a positive correlation between the diameter of type I muscle fibres and pH and number of type II fibres and L* in Turkish native sheep breeds. In the present study, differences in pH values between rearing methods and genotype were not very significant although some small differences were observed in Palmera, Payoya, and Tinerfeña kids reared with milk replacers. Associations between morphological fibre characteristics and quality traits of meat among the different breeds should be further studied to obtain solid results.

In conclusion, both genotype and rearing method have a great effect on muscle fibre populations as well as on fibre morphology. Considering the energy intake and production system orientation (meat vs dairy), the main explanations for the observed results.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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