



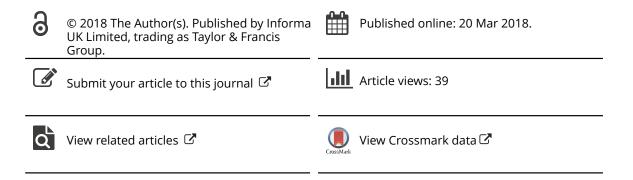
ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

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**To cite this article:** Elena Saccà, Willington Ojong Bessong, Mirco Corazzin, Stefano Bovolenta & Edi Piasentier (2018): Comparison of longissimus thoracis physical quality traits and the expression of tenderness-related genes between Goudali zebu breed and Italian Simmental × Goudali crossbreed, Italian Journal of Animal Science, DOI: <u>10.1080/1828051X.2018.1443290</u>

To link to this article: <u>https://doi.org/10.1080/1828051X.2018.1443290</u>



### PAPER

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# Comparison of *longissimus thoracis* physical quality traits and the expression of tenderness-related genes between Goudali zebu breed and Italian Simmental $\times$ Goudali crossbreed

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### ABSTRACT

The aim of this trial was to compare some meat physical quality traits and the expression of some tenderness-related genes between Goudali (G, Bos indicus) breed and Italian Simmental × Goudali (SimGoud, SG, Bos taurus x Bos indicus) crossbreed. Ten G and 12 SG bulls, aged  $31\pm0.49$  months, bred in the same farm and conditions in Western Highland Plateau Savannah of Cameroon were considered. Physical quality traits of longissimus thoracis (LT) muscle such as water losses, colour parameters and rheological properties were determined together with ultimate pH (pHu). The mRNA early post-mortem abundance of calpain-1 (Capn1), calpain-2 (Capn2), calpastatin (Cast), caspase 3 (Casp3), caspase 9 (Casp9), αB-crystallin (Cryab), heat shock proteins 27 (Hsp27), 40 (Hsp40) and 70 (Hsp70) was detected by gPCR. The Capn1, Cast, Hsp27 and Hsp70, early post-mortem protein content was determined by ELISA. The meat of G bulls had greater values of lightness (p < .05) and cooking loss (p < .05) than SG. The expression of Hsp70 in G was greater at mRNA (p < .05) level than SG. None of the other variables considered was significantly different between G and SG breeds. Whereas crossbreeding with Italian Simmental breed changed the colour and ameliorated the water retention capacity of G meat, tenderness and the expression of tenderness-related genes, both at mRNA and at protein level, were not significantly different between the pure local breed and the crossbreed. In comparison with pure Goudali, crossbreeding Goudali with Italian Simmental breed has a limited impact on meat traits in tropical conditions.

### Introduction

The Goudali (G) is a *Bos indicus* breed widespread in Cameroon, with good tolerance to heat, resistance to local parasites and high foraging ability (Nfor et al. 2014). However, these animals present limitations in carcass and meat quality. In the past, in order to improve carcase and growth performance of *Bos indicus* breeds, various successfully experiences of cross-breeding between *Bos indicus* and *Bos taurus* were conducted (Williams et al. 2001).

Considering meat quality, it is widely recognised that one of the most important characteristic for consumers is tenderness. In general, as the percentage of *Bos indicus* increases in crossbreeds, the variability of tenderness tends to increase and tenderness tends to decrease, although there are some contrary findings. Indeed, Frylinck and Heinze (2003) reported similar values of shear force between Simmental and Brahman loins, whereas Frylinck et al. (2009) showed higher values for Simmental *longissimus* muscle.

Many enzymatic systems are known to control the quality traits of meat and its tenderness in particular, among which the calpain system has been considered the most important. The quantity of calpains and the ratio between calpain-1 and its inhibitor calpastatin seems to be the cause for meat hardness of *Bos indicus* breeds (Wheeler et al. 1990). Becila et al. (2010) provided evidences that cell death in *post-mortem* muscle occurs via an apoptotic process, in which caspase 3 and caspase 9 operate. Although caspases are the main effectors of apoptosis, their importance in meat tenderisation process is still debated (Saccà et al. 2015a). For their physiological role as chaperones, anti-

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#### **ARTICLE HISTORY**

Received 23 November 2017 Revised 13 February 2018 Accepted 13 February 2018

### **KEYWORDS**

Bos indicus; Bos taurus  $\times$  Bos indicus; meat quality; gene expression; tenderness

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apoptotic and anti-oxidative factors, heat shock proteins are involved in the transformation of muscle to meat (Cassar-Malek and Picard 2016). Among these, alphaB-cristallyn and heat shock protein 27 (Hsp27) have been shown to be involved in myofibrillar proteins protection (Guillemin et al. 2011; Lomiwes et al. 2014) and Hsp27 was reported as related to tenderness (Bernard et al. 2007; Saccà et al. 2015a). Besides having a significant role in cell thermotolerance, Hsp70 has been shown to inhibit the apoptotic pathways and appears to be a good biomarker of low tenderness (Kregel 2002; Picard et al. 2014).

A crossbreeding programme between the African Goudali and the Italian Simmental (IS, *Bos taurus*) breeds was initiated in the year 2008 (Bessong et al. 2011) to obtain the SimGoud (SG) crossbreed, with the positive result of increasing meat yield (Bessong et al. 2018).

The aim of this trial was to compare some physical meat quality traits and the expression, at mRNA and at protein level, of some tenderness-related genes between G breed and SG crossbreed, to evaluate the effect of the crossbreeding in tropical pastures conditions on important aspects of meat quality.

### **Materials and methods**

### Animals and treatments

The experimental procedures followed the requirements of the European Community Directive 2010/63/ EU regarding the protection of animals used for experimental and other scientific purposes. Twentytwo male bulls, aged  $31 \pm 0.49$  months, of two genotypes, 10G and 12 SG crossbred, born and reared in Cameroon (Latitude 060 42' North, Longitude 0100 25' East), in the same conditions, were considered. The SG bulls were F1-crossbred originated using G cows and semen of IS bulls, whereas the G bulls originated from pure G cows and bulls (Bessong et al. 2018). All the experimental animals were fed by grazing natural herbage of the Western Highland Plateau Savannah, plus NaCl supplementation. At the end of the trial, the animals were initially moved on-foot for 208 km and then were moved by an animal transport truck for 306 km. After the journey, to allay stress, the animals were rested for five weeks at the Douala Cattle market lairage. During this period, they grazed every day from sunrise to sunset on native pastures, close to the market. After this, the animals were slaughtered in the slaughterhouse at Douala, following standard industrial procedures.

### Sample collection and physical measurements

Five-g samples of longissimus thoracis (LT) muscle were obtained within 30 min of slaughter, frozen in liquid N<sub>2</sub> and stored at -80 °C until proteomic and transcriptomic analysis. After chilling for 24 h, the LT muscle was extracted from the carcass. The ultimate pH (pHu) was measured by a pH-meter (HI 8424; Hanna Instruments, Padua, Italy). Approximately, 100 g of muscle was cut and weighed; after that, the cuts were suspended for 24 h at 4°C and then weighed again to determine the drip loss (48 h of ageing). After 48 h of ageing at 4°C, the colour of muscle was measured using a portable spectrophotometer Minolta CM 2600d (Konica Minolta, Tokyo, Japan). The values recorded, according to the standard conditions of the Commission International d'Eclairage (CIE 1976) included L\* (Lightness), a\* (Redness) and b\* (Yellowness). Samples of muscle were aged at 4°C for 5 days after slaughtering until cooking loss determination. Slices of muscle of 2 cm thickness were cooked in plastic bags, according to the procedure described by Honikel (1998). The cooking loss (5 days of ageing) was calculated by difference between the weight before and after cooking and expressed as a percentage of the initial weight. After two days at 4°C (7 days of ageing), the Warner-Bratzler Shear Force test was performed on cooked meat. Cylinders of 15 mm in diameter were sheared perpendicularly of muscle fibres using a Warner-Bratzler (WB) device, mounted on a Lloyd TA Plus texture analyser (Lloyd, Borgnor Regis, UK). From the WB deformation curves, two parameters were recorded: the peak yield force (PY, in N) and the final yield (FY, in N). The first peak is taken as a measurement of the myofibrillar component of tenderness and the second is taken as a measurement of the connective tissue component of tenderness (Møller 1981).

### RNA content analysis

RNA content analysis was performed in accordance with Corazzin et al. (2013). Briefly, total RNA was extracted using RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Concentration and purity of RNA extracted were assessed using spectrophotometer NanoDrop 2000c (Thermo Fisher Scientific, Waltham, MA). Integrity of RNA (the presence of intact 18S and 28S rRNA bands) and absence of genomic DNA were assessed by agarose gel (1% in Tris-borate-EDTA buffer [TBE]) electrophoresis, comparing with molecular

| Gene <sup>a</sup> | Primers sequence <sup>b</sup> , 5' to 3' | Amplicon length, bp | Accession Number | Efficiency | R <sup>2</sup> |
|-------------------|--|---------------------|------------------|------------|----------------|
| Capn1             | F: AACCGGATCCGGAATTACCTGTCCATCTTC        | 282                 | NM_174259        | 0.979      | 0.986          |
|                   | R: GTAACCACTTAAACAAGTCAAAGGTCACCA        |                     |                  |            |                |
| Capn2             | F: CGAGGACATGCACACCATTGGCTTCG            | 314                 | NM_001103086.1   | 0.968      | 0.997          |
|                   | R: TCCTCGCTGATGTCAATCTCGTCAATGTTG        |                     |                  |            |                |
| Cast              | F: CACAGAAGCCAAGGCTATTCC                 | 87                  | NM_001030318     | 0.978      | 0.997          |
|                   | R: TTTACAGCCTGTTTTTTGTGTCTTTTC           |                     |                  |            |                |
| Casp3             | F: AGAACTGGACTGTGGTATTGAGA               | 167                 | NM_001077840.1   | 0.957      | 0.996          |
|                   | R: CACAAAGAGCCTGGATGAAC                  |                     |                  |            |                |
| Casp9             | F: CCTGTGGTGGAGAGCAGAAAG                 | 134                 | NM_001205504.1   | 0.945      | 0.980          |
|                   | R: CATCTGGCTCGTCAATGGAA                  |                     |                  |            |                |
| Cryab             | F: CCGCCTCTTTGACCAGTT                    | 134                 | NM_174290        | 0.991      | 0.997          |
|                   | R: AGAGGCCAGTGTCAATCC                    |                     |                  |            |                |
| Hsp27             | F: CGTTGCTTCACTCGCAAATA                  | 210                 | NM_001025569.1   | 0.936      | 1.000          |
|                   | R: TACTTGTTTCCGGCTGTTCG                  |                     |                  |            |                |
| Hsp40             | F: GGACTGACCATTGCTGCTG                   | 138                 | NM_001034458.1   | 0.984      | 0.998          |
|                   | R: CAAACCCACCTCTGTAATAGC                 |                     |                  |            |                |
| Hsp70-1A          | F: AACAAGATCACCATCACCAACG                | 274                 | NM_00174550      | 1.015      | 0.996          |
|                   | R: TCCTTCTCCGCCAAGGTGTTG                 |                     |                  |            |                |
| Cyclophilin       | F: GGATTTATGTGCCAGGGTGGTGA               | 119                 | NM _00178320     | 0.992      | 0.998          |
|                   | R: CAAGATGCCAGGACCTGTATG                 |                     |                  |            |                |
| β-actin           | F: CTCTTCCAGCCTTCCTTCCT                  | 177                 | NM_00173979      | 0.961      | 0.997          |
|                   | R: GGGCAGTGATCTCTTTCTGC                  |                     |                  |            |                |
| GAPDH             | F: TCATCCCTGCTTCTACTGGC                  | 177                 | NM_001034034     | 0.974      | 0.999          |
|                   | R: CCTGCTTCACCACCTTCTTG                  |                     |                  |            |                |
| RPLPO             | F: CAACCCTGAAGTGCTTGACAT                 | 226                 | NM_001012682     | 0.987      | 0.999          |
|                   | R: AGGCAGATGGATCAGCCA                    |                     |                  |            |                |

Table 1. Primers sequence, amplification product and amplification efficiency of genes in exam and reference genes.

<sup>a</sup>Capn1: calpain-1; Capn2: calpain-2; Cast: calpastatin; Casp3: caspase 3; Casp9: caspase 9; Cryab: αB-crystallin; Hsp27: heat shock protein 27; Hsp40: heat shock protein 40; Hsp70: heat shock protein 70. Cyclophilin, β-actin, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), Ribosomal Protein Large P0 (RPLP0): reference genes.

<sup>b</sup>F: Forward; R: Reverse.

weight standards. To obtain cDNA, the iScript cDNA Synthesis kit (BioRad, Hercules, CA) was used according to the manufacturer's protocol and with a volume of RNA solution to have 50 ng/µL RNA final concentration. A qualitative PCR was carried out to validate primers pair specificity for all the genes in exam (calpain-1 [Capn1], calpain-2 [Capn2], calpastatin [Cast], caspase 3 [Casp3], caspase 9 [Casp9], αB-crystallin [Cryab], heat shock proteins 27 [Hsp27], 40 [Hsp40], 70 [Hsp70] and reference genes [Table 1]). The amplification was performed using the Bio-Rad CFX96 system (BioRad) including 1 cycle of 3 min at 95 °C, 40 PCR cycles of 15 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C, then 1 min at 95 °C, followed to a melt curve of 55-95 °C with 0.5 °C increments every 5 s. Amplicons length (Table 1) was verified by agarose gel (1.5% in TBE buffer) electrophoresis, comparing with molecular weight standards. The quantitative PCR (qPCR) was performed using the same instrument, volumes and conditions of the qualitative PCR, for each sample, in triplicate. The relative gene expression was calculated according to the efficiency-corrected method (Pfafll 2001). Among considered genes, only  $\beta$ -actin and GAPDH were treated as reference genes and used for normalisation of gPCR data, since they showed the highest stability (M < 1.50; Vandesompele et al. 2002). Data were presented in fold-change ratio having G breed as reference.

# Enzyme-linked immunosorbent assay (ELISA) analysis

### Proteins extraction and quantification

Protein extraction for Capn1 and Cast analysis was made according to Geesink and Koohmaraie (1999), experiment 1, using pre-rigor extraction buffer, with 10  $\mu$ L of Protease Inhibitor Cocktail (P8340, Sigma-Aldrich, Saint Louis, MO). Protein extraction for Hsp27 and Hsp70 analysis was made according to Pulford et al. (2008), using 10  $\mu$ L of Protease Inhibitor Cocktail (P8340, Sigma-Aldrich) in the extraction buffer. Protein concentration of muscle extracts was determined by the method of Bradford (1976) using Bradford reagent (Sigma-Aldrich).

Quantification of Capn1 and Cast was made according to Doumit et al. (1996), whereas quantification of Hsp27 and Hsp70 was made according to Lomiwes et al. (2014), using 96 well Costar high binding polystyrene plates (Corning Inc., 3590). The primary antibodies used were mouse monoclonal antibodies: anti-calpain antibody (C0355, Sigma-Aldrich) diluted 1 µg/mL for Capn1; anti-calpastatin antibody (C270, Sigma-Aldrich) diluted to 1:5000 for Cast; anti-Hsp27 antibody (Hytest 4HSP27, Hytest, Turku, Finland) and anti-Hsp70 (Hytest 4HSP70; Hytest), each diluted to 1:10,000, for Hsp27 and Hsp70, respectively. The secondary antibody utilised was a goat anti-mouse lgG

| muscle belongin                                   | ig to              | Goudali            | (G) and          |  |  |  |
|---|--------------------|--------------------|------------------|--|--|--|
| Simmental $	imes$ Goudali (SG) crossbreed cattle. |                    |                    |                  |  |  |  |
| Trait <sup>1</sup>                                | G                  | SG                 | SEM <sup>2</sup> |  |  |  |
| Colour  |                    |                    |                  |  |  |  |
| L*  | 36.8ª              | 32.2 <sup>b</sup>  | 1.04             |  |  |  |
| a*  | 11.1               | 14.3               | 0.97             |  |  |  |
| b*  | 14.0               | 14.9               | 0.80             |  |  |  |
| pHu   | 5.61               | 5.58               | 0.021            |  |  |  |
| WBSF  |                    |                    |                  |  |  |  |
| PY (N)  | 52.9               | 60.1               | 2.21             |  |  |  |
| FY (N)  | 48.1               | 53.1               | 2.03             |  |  |  |
| Water losses                                      |                    |                    |                  |  |  |  |
| Drip loss, %                                      | 3.85               | 2.97               | 0.272            |  |  |  |
| Cooking loss, %                                   | 36.64 <sup>b</sup> | 32.80 <sup>a</sup> | 0.682            |  |  |  |

Table 2. Characteristics of longissimus thoracis

<sup>1</sup>pHu: Ultimate pH; WBSF: Warner Bratzler Shear Force; PY: Peak yield force; FY: Final yield force; L\*: Lightness; a\*: Redness; b\*: Yellowness.

<sup>2</sup>SEM: Standard Error of the Mean.

\*,\*\**p* < .05.

Horseradish Peroxidase (HRP) conjugate (A4416, Sigma-Aldrich). The absorbance was measured at 450 nm using a Tecan Sunrise plate reader (Tecan Group Ltd., Männedorf, Switzerland). Relative quantity of Capn1, Cast, Hsp27 and Hsp70 was measured by optical density. Data were presented as fold-change ratio having G breed as reference.

### Statistical analysis

Stability of genes expression was assessed using R software (v 3.2.1; R core team, Vienna, Austria), package SLqPCR (Kohl 2007). The other statistical analyses were performed using SPSS for Windows (v. 7.5.21; SPSS Inc., Chicago, IL). The normality of the data distribution was tested using Shapiro–Wilk test. Where appropriate, non-parametrically-distributed data were transformed for parametric testing. The effect of genotype on the characteristics of *longissimus thoracis* muscle was evaluated by one-way ANOVA considering the genotype (G vs. SG) as fixed factor. The effect of genotype (G vs. SG) as fixed factor and the age of the animals as covariate. Data are presented as mean ± standard error.

### **Results and discussion**

### Physical characteristics of meat

Table 2 presents the characteristics of LT muscle of G and SG. G showed a greater lightness (L\*) value with respect to SG (p < .05). The L\* value of SG crossbreed is not so far from that declared by Corazzin et al. (2012; 33.6) for IS bulls, but below the lowest value reported by Muchenje et al. (2009a; 33.2) that considered both European and African breeds in their review.

The lightness of G appeared high for a Bos indicus breed, as showed by other authors (Bressan et al. 2011; 32.3; Nfor et al. 2014; 29.5 on average), even if Strydom (2008) reported greater values of L\* (39.0) for a Bos indicus crosses (Brahman) with respect to Simmental crosses (37.8). Moreover, Gama et al. (2013) found a reduction of lightness as effect of crossbreeding between Bos indicus and Bos taurus bred at pasture. Taylor (2004) suggested that differences in fibre characteristics found between breeds would rather relate to differences in meat colour between breeds than to differences in meat tenderness. Although we did not give the characterisation of muscle fibre composition in G and SG, we can suppose a higher presence of large, white fibres in G type (Strydom 2008) that can lead to a brighter colour and a higher water loss. Redness (a\*) and yellowness (b\*) were similar between genotypes (p > .05). The redness in G was greater than the values reported by Nfor et al. (2014) for zebu breeds (7.2 on average), but lower than redness recorded by Bressan et al. (2011) for Bos indicus breeds (19.8). In any case, our values of redness, both for G and for SG, were within the limits showed by Muchenje et al. (2009a; 11.1 to 23.6).

The pHu of all the animals was below the value of 5.87 considered the cut-off between normal and darkcutting beef carcasses (Page et al. 2001). The five weeks of rest before slaughter seem to be effective in reducing the stress of the travel from the farm to the slaughterhouse and to reconstitute the glycogen stores. Similarly to our trial, other authors did not find pH differences between *Bos taurus* and *Bos indicus* (Bressan et al. 2011; 5.86 on average) or between these genotypes and their crosses (Gama et al. 2013). Also Muchenje et al. (2008, 2009b) did not find pHu differences between *Bos taurus* and *Bos taurus* × *Bos indicus* breeds (5.7 on average).

Concerning the rheological parameters, differences between G and SG were not found (p > .05), neither for the myofibrillar nor for the connective component of the shear force. To this regards, the literature shows controversial results. Some authors highlighted that Bos taurus gives more tender meat compared to Bos indicus (Wheeler et al. 1990), conversely, Strydom (2008) and Frylinck et al. (2009) reported higher WBSF for Simmental crosses in comparison with Brahman crosses (Bos indicus) and Nguni crosses (Bos taurus africanus). Du Plessis and Hoffman (2007) also reported no differences in shear force resistance of three days aged loin muscle from Nguni, Brahman and Simmental crosses aged between 18 and 30 months, showing that breed effect on meat shear force was not significant in natural pasture of South African arid subtropics. Also in our condition (tropical climate, natural pasture feeding, high physical activity) differences between genotypes have not been highlighted. Moreover, the tenderness of SG bulls may have been reduced by a food restriction suffered during transfer and lairage time, when the pasture allowance was likely not sufficient to satisfy their feeding requirements. Indeed, as reported for the largest group of bulls comprising the animals of this experiment (Bessong et al. 2018), it is interesting to note that the more performing SG bulls showed a marked loss of weight during transfer and lairage time (6.1%), while G ones, during this period, were able to increase their body weight of 4.4%.

The values of WBSF for G (PY, maximum peak vield) are much lower than the values reported for the same breed by Nfor et al. (2014; 112 N), probably because of the lower average age and better-controlled management conditions of animals in our study. Corazzin et al. (2012) reported values of WBSF of 47.9 N for IS breed reared in stable, whereas our values of WBSF were 56.9 N on average between G and SG. The slightly higher shear force values in loin of bulls finished on pasture may have been also due to increased collagen cross-linking associated with increased exercise. In any case, our values were fully ranged between limits reported by Muchenje et al. (2009a), both at 2 and at 21 days of ageing. In a number of studies in South Africa (De Bruyn 1991; Frylinck and Heinze 2003; Marais 2007), the poor tenderness quality of Simmental meat was questioned. In particular, De Bruyn (1991) found no significant differences between shear force resistance of Afrikaner, Bonsmara, Charolais and Hereford meat, whereas the toughness values were significantly higher for Brahman and Simmental meat. Moreover, Frylinck et al. (2009) hypothesised that, under specific environmental challenges, both Simmental and Bos indicus contributed to the poor tenderness of the crossbreed meat.

Muchenje et al. (2008, 2009b) found no differences in drip and cooking losses of *longissimus* muscle meat between *Bos Taurus*, *Bos indicus* × *Bos Taurus* and *Bos taurus africanus*. On the contrary, in our trial, cooking losses were different between the two genotypes, with SG having lower values than G (p < .05). The low water loss of fresh meat is considered a positive commercial characteristic. Even if the pH was comparable between the two genotypes, the differences in meat colour and water losses could be related to a different muscle fibres type composition, as already observed. The value of cooking loss for G was slightly lower than the values reported by Nfor et al. (2014) for the Cameroonian *Bos indicus* breeds (38.4% on average). Conversely, the values of cooking loss were greater for both genotypes than those showed by Corazzin et al. (2012) in young bulls of Simmental breed (30.4%).

### RNA and protein expression of considered genes

The values of mRNA expression for G and SG LT muscles are shown in Table 3. Many proteins systems are known to control the quality traits of meat and its tenderness in particular, among which the calpain system has been considered the most important. It consists of two calcium-dependent proteases, Capn1 and their competitive Capn2 and inhibitor Cast. Concerning the mRNA expression of calpains and Cast our results are in accordance with the rheological measurements. In fact, no significant differences occurred between the two genotypes for both shear force values and the expression levels of genes related with tenderness. The ELISA analysis (Table 4) confirmed the lack of difference in the expression for both Capn1 and Cast between the two breeds that was

**Table 3.** Fold change ratio in relative mRNA expression of genes in *longissimus thoracis* muscle belonging to *Goudali* (G) and *Simmental*  $\times$  *Goudali* (SG) crossbreed cattle.

| Geneª      | G   | SG    | SEM <sup>b</sup> |
|------------|-----|-------|------------------|
| Capn1      | 1   | 0.84  | 0.329            |
| Capn2      | 1   | 0.81  | 0.380            |
| Cast       | 1   | 1.21  | 0.282            |
| Capn1/Cast | 1   | 0.94  | 0.234            |
| Casp3      | 1   | 0.69  | 0.361            |
| Casp9      | 1   | 0.74  | 0.267            |
| Cryab      | 1   | 0.71  | 0.310            |
| Hsp27      | 1   | 0.64  | 0.335            |
| Hsp40      | 1   | 0.86  | 0.213            |
| Hsp70      | 1** | 0.41* | 0.348            |

<sup>a</sup>Capn1: calpain-1; Capn2: calpain-2; Cast: calpastatin; Casp3: caspase 3; Casp9: caspase 9; Cryab:  $\alpha$ Bcrystallin; Hsp27: heat shock protein 27; Hsp40: heat shock protein 40; Hsp70: heat shock protein 70.

<sup>b</sup>SEM: Standard Error of the Mean.

\*,\*\**p* < .05.

**Table 4.** Fold change ratio in relative protein quantity of *longissimus thoracis* muscle belonging to *Goudali* (G) and *Simmental*  $\times$  *Goudali* (SG) crossbreed cattle.

| Protein <sup>a</sup> | G | SG   | SEM <sup>b</sup> |
|----------------------|---|------|------------------|
| Capn1                | 1 | 1.03 | 0.081            |
| Cast                 | 1 | 1.02 | 0.025            |
| Capn1/Cast           | 1 | 1.00 | 0.084            |
| Hsp27                | 1 | 1.37 | 0.271            |
| Hsp70                | 1 | 0.87 | 0.075            |

<sup>a</sup>Capn1: calpain-1; Capn2: calpain-2; Cast: calpastatin; Hsp27: heat shock protein 27; Hsp70: heat shock protein 70.

<sup>b</sup>SEM: Standard Error of the Mean.

highlighted by mRNA analysis. Some authors reported a higher Cast activity and/or a lower Capn1 expression in Bos indicus than Bos taurus, resulting in lower rates and amount of post-mortem tenderisation (Wheeler et al. 1990). The lack of difference between SG and G in the expression of Capn and Cast genes, sustained by the absence of difference in share force values, may be related to the extreme environmental factors (climate, feeding regime and management stresses) that characterised our experimental conditions and that, as already discussed, may have particularly affected the more performing and demanding SG genotype during the transfer and lairage time. Similarly, Marais (2007) considering Brahman, Nguni and Simmental crosses highlighted a failure in the correspondence between the genotypic makeup and the phenotypic expression of tenderness genes caused by environmental factors.

In recent years, Becila et al. (2010) provided evidences that cell death in *post-mortem* muscle occurs via an apoptotic process. Casp3 and Casp9 are involved in the intrinsic pathway of apoptosis. In spite of their central role in apoptosis, the involvement of caspases in meat tenderisation, by means of proteolytic degradation, is still debated (Saccà et al. 2015a, 2015b). In our trial, no significant differences were found between G and SG in the expression of Casp3 and Casp9 mRNA (Table 3), in line with Capn and Cast genes expression.

The anoxic state that characterises post-mortem condition also activates cell survival processes, such as the increase in the concentration of several Hsp. Because of their anti-apoptotic function, their protective actions on myofibrillar proteins and their anti-oxidative role, Hsp have been proposed to have a potential role in meat tenderisation process (Cassar-Malek and Picard 2016). In particular, alphaB-crystallin and Hsp27 are known to stabilise and protect myofibrillar proteins (Lomiwes et al. 2014). Concerning the expression of Hsp genes at mRNA level, the two genotypes showed not significant differences (Table 3), except for Hsp70 that is more expressed in G (p < .05). The ELISA analysis (Table 4) confirmed the lack of difference in the expression of Hsp27 between the two breeds, but G showed only numerically higher Hsp70 protein abundance than SG. Because of the environmental adaptation, zebu breeds of cattle are better able to regulate body temperature in response to heat stress than European breeds (Beatty et al. 2006). Hsp70 is abundantly induced in response to cellular stress, possibly due to its function to preserve proteins (Gagaoua et al. 2015). Moreover, Hsp70 has a significant role in cell thermotolerance and animal survival (Kregel 2002). Hsp70 has been also reported as a good marker of meat hardness regardless of the type of muscle and the breed considered (Picard et al. 2014), but in our study, it seems to be more related to environmental stress resistance.

Moloto et al. (2017) underline that the complexity of the tenderisation process and the different metabolic pathways involved in the transformation of muscle to meat, lead to the difficulty to identify definite markers for meat tenderness. Moreover, in our study, the extreme environmental and breeding conditions may have influenced the expression of mRNA and proteins in muscle cell (Guillemin et al. 2011) and particularly that of the more performing and demanding SG bulls, leading to a flattening of the differences between the two genetic types.

### Conclusions

In conclusion, in comparison to pure G breed, the crossing between IS and G seems to have limited impact on meat traits, at least in tropical environment. Indeed, although it caused a reduction of cooking losses of meat, it had no effects on meat tenderness and on the expression of the related genes. Based on its greater Hsp70 expression, the local Goudali breed should respond better to the tropical climate, where it has evolved over the years, with respect to the SimGoud crossbreed.

### **Disclosure statement**

No potential conflict of interest was reported by the authors.

### Funding

The authors thank the Ministry of Livestock, Fisheries and Animal Industries of Cameroon, the University of Udine and the Friuli Venezia Giulia Regional Authorities for the support.

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### References

Beatty DT, Barnes A, Taylor E, Pethick D, McCarthy M, Maloney SK. 2006. Physiological responses of *Bos taurus* and *Bos indicus* cattle to prolonged, continuous heat and 374 humidity. J Anim Sci. 84:972–985.

- Becila S, Herrera-Mendez CH, Coulis G, Laba R, Astruc T, Picard B, Boudjellal A, Pelissier P, Bremaud L, Ouali A. 2010. Postmortem muscle cells die through apoptosis. Eur Food Res Technol. 231:485–493.
- Bernard C, Cassar-Malek I, Le Cunff M, Dubroeucq H, Renand G, Hocquette J-F. 2007. New indicators of beef sensory quality revealed by expression of specific genes. J Agric Food Chem. 5513:5229–5237.
- Bessong WO, Biondokin C, Silvestrelli L, Menta G, Piasentier E. 2011. Preliminary Results of a Crossbreeding Program of Autochthonous Goudali (Zebu) Cattle of Cameroon with Italian Simmental. In: Book of abstracts, 29th European Simmental Federation Congress. Udine, Italy: ANAPRI. p. 183–202.
- Bessong WO, Saccà E, Corazzin M, Sepulcri A, Piasentier E. 2018. Body and meat characteristics of young bulls from Zebu Goudali of Cameroon and its crosses with the Italian Simmental. Ital J Anim Sci. 17:240–249.
- Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72:248–254.
- Bressan MC, Rodrigues EC, Rossato LV, Ramos EM, Da Gama LT. 2011. Physicochemical properties of meat from *Bos taurus* and *Bos indicus*. R Bras Zootec. 40:1250–1259.
- Cassar-Malek I, Picard B. 2016. Expression marker-based strategy to improve beef quality. Scientific World J. 2016:2185323.
- Commission Internationale de l'Eclairage (CIE). 1976. Colorimetry. 2nd ed. Vienna, Austria: CIE.
- Corazzin M, Bovolenta S, Saccà E, Bianchi G, Piasentier E. 2013. Effect of linseed addition on the expression of some lipid metabolism genes in the adipose tissue of young Italian Simmental and Holstein bulls. J Anim Sci. 91:405–412.
- Corazzin M, Bovolenta S, Sepulcri A, Piasentier E. 2012. Effect of whole linseed addition on meat production and quality of Italian Simmental and Holstein young bulls. Meat Sci. 90:99–105.
- De Bruyn JF. 1991. Production and product characteristics of different cattle genotypes under feedlot conditions [D. Sc. (Agric.) thesis]. South Africa: University of Pretoria.
- Doumit ME, Lonergan SM, Arbona JR, Killefer J, Koohmaraie M. 1996. Development of an enzyme-linked immunosorbent assay (ELISA) for quantification of skeletal muscle calpastatin. J Anim Sci. 74:2679–2686.
- Du Plessis I, Hoffman LC. 2007. Effect of slaughter age and breed on the carcass traits and meat quality of beef steers finished on natural pastures in the arid subtropics of South Africa. S Afr J Anim Sci. 37:143–153.
- Frylinck L, Heinze PH. 2003. Evaluation of meat tenderness of indigenous South African and other beef breeds. Proceedings of the 11th International Meat Symposium. Irene, South Africa: Agricultural Research Council, Animal Nutrition and Animal Production Institute. p. 28–47.
- Frylinck L, van Wyk GL, Smith TPL, Strydom PE, van Marle-Köster E, Webb EC, Koohmaraie M, Smith MF. 2009. Evaluation of biochemical parameters and genetic markers for association with meat tenderness in South African feedlot cattle. Meat Sci. 83:657–665.
- Gagaoua M, Terlouw EM, Boudjellal A, Picard B. 2015. Coherent correlation networks among protein biomarkers

of beef tenderness: what they reveal. J Proteomics. 128: 365–374.

- Gama LT, Bressan MC, Rodrigues EC, Rossato LV, Moreira OC, Alves SP, Bessa RJB. 2013. Heterosis for meat quality and fatty acid profiles in crosses among *Bos indicus* and *Bos taurus* finished on pasture or grain. Meat Sci. 93:98–104.
- Geesink GH, Koohmaraie M. 1999. Technical note: a rapid method for quantification of calpain and calpastatin activities in muscle. J Anim Sci. 77:3225–3229.
- Guillemin N, Jurie C, Cassar-Malek I, Hocquette J-F, Renand G, Picard B. 2011. Variations in the abundance of 24 protein biomarkers of beef tenderness according to muscle and animal type. Animal. 5:885–894.
- Honikel KO. 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49:447–457.
- Kohl M. 2007. SLqPCR: Functions for analysis of real-time quantitative PCR data at SIRS-Lab GmbH. Jena, Germany: SIRS-Lab GmbH.
- Kregel KC. 2002. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J Appl Physiol. 92:2177–2186.
- Lomiwes D, Hurst SM, Dobbie P, Frost DA, Hurst RD, Young OA, Farouk MM. 2014. The protection of bovine skeletal myofibrils from proteolytic damage post mortem by small heat shock proteins. Meat Sci. 97:548–557.
- Marais GL. 2007. Evaluation of genetic and physiological parameters associated with meat tenderness in South African Feedlot cattle [M. Sc. Thesis]. South Africa: Department of Animal Wildlife Science, University of Pretoria.
- Møller AJ. 1981. Analysis of Warner-Bratzler shear pattern with regard to myofibrillar and connective tissue components of tenderness. Meat Sci. 5:247–260.
- Moloto KW, Frylinck L, Modika KY, Pitse T, Strydom PE, Koorsen G. 2017. Is there a possibility of meat tenderness protein-biomarkers on the horizon? Int J Agr Inn Res. 6:467–472.
- Muchenje V, Dzama K, Chimonyo M, Raats JG, Strydom PE. 2008. Meat quality of nguni, bonsmara and aberdeen angus steers raised on natural pasture in the Eastern Cape, South Africa. Meat Sci. 79:20–28.
- Muchenje V, Dzama K, Chimonyo M, Strydom PE, Hugo A, Raats JG. 2009a. Some biochemical aspects pertaining to beef eating quality and consumer health: a review. Food Chem. 112:279–289.
- Muchenje V, Dzama K, Chimonyo M, Strydom PE, Raats JG. 2009b. Relationship between pre-slaughter stress responsiveness and beef quality in three cattle breeds. Meat Sci. 81:653–657.
- Nfor BM, Corazzin M, Fonteh FA, Aziwo NT, Galeotti M, Piasentier E. 2014. Quality and safety of beef produced in Central African sub-region. Ital J Anim Sci. 13:392–397.
- Page JK, Wulf DM, Schwotzer TR. 2001. A survey of beef muscle color and pH. J Anim Sci. 79:678–687.
- Pfafll MW. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29:2002–2007.
- Picard B, Gagaoua M, Micol D, Cassar-Malek I, Hocquette J-F, Terlouw CEM. 2014. Inverse relationships between biomarkers and beef tenderness according to contractile and

metabolic properties of the muscle. J Agric Food Chem. 62:9808–9818.

- Pulford DJ, Fraga Vazquez S, Frost DF, Fraser-Smith E, Dobbie P, Rosenvold K. 2008. The intracellular distribution of small heat shock proteins in post-mortem beef is determined by ultimate pH. Meat Sci. 79:623–630.
- Saccà E, Corazzin M, Pizzutti N, Lippe G, Piasenter E. 2015a. Early *post-mortem* expression of genes related to tenderization in two Italian Simmental young bulls' skeletal muscles differing in contractile type. Anim Sci J. 86:992–999.
- Saccà E, Pizzutti N, Corazzin M, Lippe G, Piasentier E. 2015b. Assessment of calpain and caspase systems activities during ageing of two bovine muscles by degradation patterns of αll spectrin and PARP-1. Anim Sci J. 87: 462–466.
- Strydom PE. 2008. Do indigenous Southern African cattle breeds have the right genetics for commercial production of quality meat? Meat Sci. 80:86–93.

- Taylor RG. 2004. Muscle fibre types and meat quality. In: Jensen WK, Devine C, Dikeman M, editors. Encyclopedia of Meat Science. Bristol (UK): Elsevier Academic Press; p. 876–882.
- Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3:research0034.0031.
- Wheeler TL, Savell JW, Cross HR, Lunt DK, Smith SB. 1990. Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. J Anim Sci. 68:4206–4220.
- Williams JL, Aguilar I, Rekaya R, Bertrand JK. 2001. Estimation of breed and heterosis effects for growth and carcass traits in cattle using published crossbreeding studies. J Anim Sci. 88:460–466.