



Identification of biomarkers associated with the rearing practices, carcass characteristics, and beef quality: An integrative approach

Mohammed Gagaoua, Valérie Monteils, Sébastien Couvreur, Brigitte Picard

► To cite this version:

Mohammed Gagaoua, Valérie Monteils, Sébastien Couvreur, Brigitte Picard. Identification of biomarkers associated with the rearing practices, carcass characteristics, and beef quality: An integrative approach. *Journal of Agricultural and Food Chemistry*, 2017, 10.1021/acs.jafc.7b03239 . hal-01588029

HAL Id: hal-01588029

<https://hal.science/hal-01588029>

Submitted on 22 Sep 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Identification of biomarkers associated with the rearing practices, carcass characteristics and beef quality: an integrative approach

Mohammed Gagaoua¹, Valérie Monteils¹, Sébastien Couvreur², Brigitte Picard^{1*}

¹ UMR1213 Herbivores, INRA, VetAgro Sup, Clermont université, Université de Lyon, 63122 Saint-Genès-Champanelle, France

² URSE, Université Bretagne Loire, Ecole Supérieure d'Agriculture (ESA), 55 rue Rabelais, BP 30748, 49007 Angers Cedex, France

***Corresponding author:** Dr. Brigitte Picard

Email: brigitte.picard@inra.fr ; phone: +33 4 73 62 40 56. Fax: +33 4 73 62 46 39

Abstract

Data from birth to slaughter of cull cows allowed using PCA-based approach coupled with the iterative *K*-means algorithm the identification of three rearing practices classes. The classes were different in their carcass characteristics. Old cows raised mainly on pasture have better carcass characteristics, while having an equivalent tenderness, juiciness, flavor, intramuscular fat content and pHu to those fattened with hay or haylage. The *Longissimus thoracis* muscle of the cows raised on pasture (with high physical activity) showed greater proportions of IIA fibers at the expense of the fast IIX ones. Accordingly, the meat of these animals have better color characteristics. Superoxide dismutase (SOD1) and α B-crystallin quantified by Dot-Blot were the only other biomarkers to be more abundant in “Grass” class compared to “Hay” and “Haylage” classes. The relationships between the biomarkers and the six carcass and eleven meat quality traits were investigated using multiple regression analyses per rearing practices. The associations were rearing practice class and phenotype trait-dependent. ICDH and TP53, were common for the three classes, but the direction of their entrance was different. In addition, rearing practices and carcass traits were not related with Hsp70-Grp75 and μ -calpain abundances. The other relationships were specific for two or one rearing practices class. The rearing practices dependency of the relationships was also found with meat quality traits. Certain proteins were for the first time related with some beef quality traits. MyHC-IIX, PGM1, Hsp40, ICDH and Hsp70-Grp75 were common for the three rearing practices classes and retained to explain at list one beef quality trait. A positive relationship was found between PGM1 and hue angle irrespective of rearing practices class. This study confirms once again that production-related traits in livestock are the result of sophisticated biological processes finely orchestrated during the life of the animal and soon after slaughter.

Key words: Cull cows, Rearing practices, Carcass characteristics, Meat quality, Biomarkers.

37 Introduction

38 In beef production systems, meat comes from various types of animals, of different breeds
39 and ages, which have been also managed and finished using different rearing practices.¹⁻³
40 Beef quality properties are well known to be impacted by these factors and also others related
41 to slaughtering and carcass handling practices.⁴⁻⁶

42 Today, consumers are (re)requesting stable sensory beef quality traits, namely meat color,
43 tenderness, juiciness and flavor attributes. Thus, stakeholders of meat chain must be able to
44 deliver and guarantee high-quality products to reduce perceived risk and gain consumer
45 confidence and, at the same time, producers must provide a viable farm holding, by increasing
46 animal and carcass performances or the added-value. This is a challenging task due to the
47 nature of beef itself. Beef is biochemically dynamic, hence it is naturally susceptible to
48 variation in acceptability which prompted the use of innovative and high-throughput
49 processing technologies for its characterization.⁷ Numbering among the innovative methods
50 that have been introduced to identify biomarkers of muscle food quality attributes are ‘-
51 omics’ based research. Proteomics approach was extensively implemented for better
52 understanding of several aspects of muscle to meat conversion in relation to different but
53 individual rearing practices factors. They have provided a broader and deeper understanding
54 of how these factors could contribute to meat quality variations,^{8,9} but less has been done in
55 relation with carcass characteristics. Consequently, the use of high-throughput processing
56 technologies for joint management of carcasses and meat quality will be very useful for the
57 beef sector, since numerous countries are seeking powerful tools that can help the
58 stakeholders to allow both a high i) remuneration for the farmers which is based on the
59 properties of the carcasses and ii) satisfaction of the consumer expectations in terms of beef
60 quality traits.

61 Proteomic investigations have further confirmed and elucidated previous concepts and
62 provided dozens of protein biomarkers belonging to heat shock stress, energy metabolism,
63 structure, oxidative stress, apoptosis, signaling and proteolysis pathways to be involved.⁹
64 These protein biomarkers may help beef stakeholders seeking better marketing options
65 through efficient beef production systems. These would also help to determine which rearing
66 practices and animal traits, as well as live-animal performances and carcass characteristics,
67 need to be improved to reach the targeted market specifications. Thus, there is a need to go
68 beyond the stage of identifying protein markers between proteomics data and the animals’
69 traits or phenotypes of interest. In this study, we report the use of Dot-Blot¹⁰ as a quantitative

approach for the quantification of protein biomarkers related to meat quality of Protected Designation of Origin (PDO) Maine-Anjou. This PDO exclusively included animals from the Rouge des prés breed which originated in the northwestern part of France. This area is excellent for beef production as it has both grassland and tillable land. The Rouge des Prés breed was the second among the 4 breeds allowed to be used in France for PDO meat production¹⁰. The PDO Maine-Anjou is composed of around 80% of cows, younger than 10 years of age, having calved at least once and a minimal carcass weight of 380 kg. Steers over 30 months of age with carcass weight of 400 kg minimum can also be found (20%). PDOs are of special economic importance for the valorization of local breeds as it is a dual-purpose breed, used for both beef and milk. The specifications of animal products under PDO label are also paid increasing attention in Europe (European Union Regulation (EEU) 2081/92). These labels are meant to support diversity in agricultural production, satisfy consumers, and protect product names against fraud and imitation. Thus, there is a great interest and economical value as an initiative of the breeders association, for the implementation of high-throughput techniques, likely omics techniques, for meat quality management of PDO meat to guarantee high quality for the consumers^{10,11}. In this study, the biomarkers were associated in addition to other muscle characteristics measured using conventional methods, with carcass properties and meat quality according to the rearing practices of PDO Maine-Anjou cows. Furthermore, the main objective was also to address an innovative approach by identifying the cows according to their rearing practices, which is valued not only the muscle, carcass, sensory and technological quality of meat but also the whole life of the animal affecting the beef quality obtained under different production systems. For this purpose, multiple regression analyses were performed to relate each carcass and meat trait with the muscle characteristics according to the rearing practices identified. A focus on the use of biomarkers to understand the relationships with carcass and meat quality potential was further explored.

Materials and methods

Animals, handling, slaughtering and sampling

A total of 110 French PDO Maine-Anjou cows originated in the northwestern part of France were collected from a cooperative of livestock farmers located in the department of Maine-et-Loire. The rearing practices of each animal were surveyed by a questionnaire.¹¹ Briefly, the questionnaire included information about **i**) the finishing period [part of hay, haylage and/or grass in the finishing diet (% w/w); daily and global amount of concentrate (kg); fattening duration (days); physical activity (% days out)] and **ii**) the animal

characteristics [Animals with meat or dairy-ability; birth month/season ; birth weight (kg); age at weaning (month); weaning duration (week); age of first calving; number of calving; suckling value (0-10) and age at slaughter]. Before slaughter, all animals were food deprived for 24 h and had free access to water. All the animals were slaughtered in the same commercial abattoir (Elivia, Lion d'Angers, 49220, France), stunned using captive-bolt pistol prior to exsanguination and dressed according to standard commercial practices. Slaughtering was performed in compliance with the French welfare regulations and respecting EU regulations (Council Regulation (EC) No. 1099/2009). After slaughter, the carcasses were characterized and graded according to the European beef grading system (CE 1249/2008). Thus, information for each carcass were measured, namely hot carcass weight (HCW, kg), EUROP conformation score (EUROP grid), weight of the whole ribeye, 12th rib characterization by *Longissimus* muscle weight, muscle carcass weight, carcass fat weight and fat to muscle ratio (% w/w). The carcasses were not electrically stimulated and they were chilled at 4°C until 24 h *p-m*.

Muscle samples of *Longissimus thoracis* (LT, mixed fast oxido-glycolytic muscle) were excised from the right-hand side of the carcass of each animal 24 h *p-m* and four parts were distinguished. The first part of the samples was subsequently frozen in liquid nitrogen and kept at -80°C until analyzed for fiber characterization (fiber area, myosin heavy chains isoforms (MyHC) and metabolic enzymes activities) and for protein extractions for the quantification of the proteins by Dot-Blot. The second part of the sample was cut into pieces (1–2 cm cross-section), vacuum packed and stored at -20 °C until analyzed for intramuscular fat content. Another part was used for meat color measurement. The last part was cut into steaks (20 mm thick) and placed in sealed plastic bags under vacuum and kept at 4°C for ageing (14 days). Each loin sample was then frozen and stored at -20°C waiting sensory evaluation and shear force measurements.

Muscle characteristics determination

The metabolic muscle type was determined by measuring glycolytic and oxidative enzyme activities of respectively lactate dehydrogenase (LDH; EC 1.1.1.27) and isocitrate dehydrogenase (ICDH; EC 1.1.1.42).¹² These enzymes representative of different steps of the glycolytic and oxidative pathways are currently used for the characterization of the metabolic types of beef muscles.⁵

The contractile properties of the muscle were determined by the quantification of the myosin heavy chains (MyHC) isoforms according to the method of Picard *et al.*¹³ using an adequate electrophoresis protocol. Thus, three isoforms of myosin Heavy Chain (MyHC) isoforms were quantified using this technique. Briefly, the myofibrillar proteins were extracted using a buffer containing 0.5 M NaCl, 20 mM Na Pyrophosphate, 50 mM Tris, 1 mM EDTA and 1 mM Dithiothreitol. After homogenization of approximately 100 mg of frozen muscle in 5 mL of the extraction buffer using a Polytron (x22 000) and an incubation time of 5 min at 4°C on ice, the sample was subjected to a centrifugation at 5000 x g for 10 min at 4°C. Following centrifugation, the supernatant was diluted 1:1 (v/w) with glycerol at 87% and stored at -20°C until use. The samples were then mixed with an equal volume of loading buffer (basic 2 x Laemmli) containing 4% SDS (w/v), 125 mM Tris (pH 6.8), 20% glycerol (v/v), 10% β-mercaptoethanol (v/v) and 0.02% pyronin Y (w/v), incubated at room temperature for 10 min and then heated (70°C) in a water bath for 10 min. The protein extracts (10 µg) were loaded per well onto 0.75-mm-thick gels and were separated using 9.2% polyacrylamide gels. The lower running buffer consisted of 50mM Tris (base), 75mM glycine and 0.05% w/v SDS. The upper running buffer was at 2x the concentration of the lower running buffer and β-mercaptoethanol (0.07% v/v) was added. Ten micrograms of protein extracts were loaded per well onto 0.75-mm-thick gels mounted on a Mini-Protean II Dual Slab Cell electrophoretic system (Bio-Rad). The migration was carried out at a constant voltage of 70 V for 30 hours at 4°C. Controls of bovine muscle containing three (MyHC-I, IIa and IIx) or four (MyHC-I, IIa, IIx and IIb) muscle fibers were added at the extremities of each gel.¹⁰ After migration, the gels were fixed in 30% (v/v) ethanol and 5% acetic acid (v/v) and then stained with colloidal Coomassie Blue R250 for 24 h. Gels were destained in a 30% ethanol (v/v) and 5 % acetic acid (v/v) solution until the background was sufficiently cleared. After staining in a Coomassie Blue dye solution, the gels were scanned and the proportions of the different MyHCs bands were quantified by densitometry with ImageQuant Software 5500 (Amersham Biosciences/GE Healthcare) and expressed in percentage. The quantification of the bands revealed no existence of MyHC-IIb isoform in PDO Maine-Anjou breed, thus only MyHC-I, IIa and IIx isoforms are reported.

The muscle mean cross sectional fiber area for all the animals was determined on 10-µm thick sections cut perpendicular to the muscle fibers with a cryotome.¹⁴ Between 100 and 200 fibers in each of two different locations in the muscle were used to determine the mean fiber area (in µm²) by computerized image-analysis.

The protein concentrations of the protein extracts used in this study were determined according to the dye binding method of Bradford¹⁵ using the Bio-Rad Protein assay (Bio-Rad Laboratories Inc.). Bovine serum albumin (BSA) at a concentration of 1 mg/mL was used as standard, and the protein content was calculated from the standard curve.

The relative abundance of 21 protein biomarkers was evaluated using the immunological Dot-Blot technique.^{16,17} The biomarkers belong to 6 biological pathways:

1) *heat shock proteins* [α B-crystallin, Hsp20, Hsp27, Hsp40, Hsp70-1A, Hsp70-1B, Hsp70-8 and Hsp70-Grp75];

2) *metabolism* [Enolase 3 (ENO3) and Phosphoglucomutase 1 (PGM1)];

3) *structure* [α -actin, Myosin binding protein H (MyBP-H), Myosin light chain 1F (MyLC-1F) and Myosin heavy chain IIx (MyHC-IIx)];

4) *oxidative stress* [Superoxide dismutase [Cu-Zn] (SOD1), Peroxiredoxin 6 (PRDX6) and Protein deglycase (DJ1)];

5) *proteolysis* [μ -calpain and m-calpain] and

6) *apoptosis and signaling* [Tumor protein p53 (TP53) and H2A Histone (H2AFX)].

The conditions retained and suppliers for all primary antibodies dilutions and details of the protocol were according to our previous studies.^{10,17} An antibody was considered specific against the studied protein when only one band at the expected molecular weight was detected by western blot. Briefly, the protein extracts (15 μ g) of each muscle sample were spotted in quadruplicate on a nitrocellulose membrane with the Minifold I Dot-Blot apparatus from Schleicher & Schuell Biosciences (Germany) in a random order on the 96-spot membrane. In addition, a mixed standard sample (15 μ g) was deposited for data normalization. The Dot-Blot membranes were air-dried for 5 min, blocked in 10% PBS milk buffer at 37°C for 20 min, and then incubated to be hybridized with the specific primary antibody of each protein. Subsequently, the membranes were incubated at 37°C for 30 min with the anti-mouse fluorochrome-conjugated LICOR-antibody IRDye 800CW (1 mg/mL). Infrared fluorescence detection was used for quantification of the relative protein abundances. Subsequently, the membranes were scanned using the Odyssey NIR imager (LI-COR Biosciences), with an 800 nm laser, a 169 μ m spatial resolution and a fixed gain of 5. Finally, the Dot-Blot images were quantified with GenePix PRO v6.0 (Axon). Each dot volume was calculated as the total dot intensity from which the median local background value multiplied by the dot area was subtracted. Because Dot-Blot offers the possibility of replicates, a data-prefiltering approach was implemented to eliminate outlier values mainly due to dust. The exclusion technique of

outliers was based on the Medium Absolute Difference (MAD) and applied before repeated values were averaged. Finally, to make the data comparable between assays, the data were normalized using a regression-approach based on the used mix standard specific for each muscle. Thus, the relative protein abundances were based on the normalized volume and were all expressed in arbitrary units (A.U).

Meat quality evaluation

For muscle ultimate pH evaluated at 24h *post-mortem*, the measurements were conducted by inserting a glass electrode into each muscle sample using a Hanna pH meter (HI9025, Hanna Instruments Inc., Woonsocket, RI, USA) suitable for meat penetration.¹⁰ The measurements were done between the 6th and 7th rib. For that, five measurements were made and the average value was used. The pH meter was calibrated at chilling temperature (average temperature 3.5°C) using pH 4 and pH 7 buffers.

For surface meat color determination, a portable colorimeter (Minolta CR400, Konica Minolta, Japan) was used to measure the L^* , a^* and b^* color coordinates of the muscle cuts according to Gagaoua *et al.*¹⁰ The parameters Chroma (C^*), related to the intensity of color, and hue angle (h^*), related to the change of color from red to yellow, were further calculated using the following equations:

$$C^* = [(a^{*2} + b^{*2})^{1/2}] \quad (1)$$

$$h^* = [\text{ATAN}^{-1}(b^*/a^*)] \quad (2)$$

The measurements were done in three replicate series, at nine different locations on each steak to obtain three measures for each muscle cut, and an average value was used for analysis.

For intramuscular fat (IMF) content, a Dionex ASE 200 Accelerated Solvent Extractor (Dionex Corporation, Sunnyvale, CA, USA) was used. From 1 ± 0.001 g of meat powder were placed in a 22 mL extraction cell previously prepared with a cellulose filter and silicon balls. The extraction was performed with petroleum ether at a temperature of 125 °C and a pressure of 103 bar. The extract, containing fat content and petroleum ether, was collected and transferred in an evaporation vial previously weighted (± 0.001 g). After 15 min of evaporation, the vial was placed 17h in a drying oven at 105°C and then weighted (± 0.001 g) to determine the IMF content in the meat sample.

For sensory beef assessment the protocol recently described by Gagaoua *et al.*⁶ was used. Briefly, steaks all aged for 14 days were thawed, without stacking or overlapping, at 5°C in vacuum packs for 48 h before cooking and sensory assessment at 55 °C. One hour before sensory assessment, the meat samples were cut into approximately 1.50 cm thick steaks and grilled on a double grooved plate griddle (SOFRACA, Morangis, France) heated to 300°C for 30 min before cooking. Steaks were heated for 2 min until the end-points temperature of 55°C in the geometric center of the steak was reached (measured using a temperature probe (Type K, HANNA HI 98704, Newark, USA)). After grilling, each steak was cut into 20 mm cubes that were immediately served to 12 panelists chosen according to the criteria described by Gagaoua *et al.*⁶ Briefly, the expert panelists, grouping 6 males and 6 females, were trained in accordance with the ISO standards ISO/TC 34 (ISO_8586, 2012) and were selected based on their sensory sensitivity, appropriate training and experience in sensory testing. Formal evaluation allowed selection of those panelists capable of making consistent and repeatable sensory assessments of meat products that is, making comparative judgements both within a session and from one session to another. The continuous accuracy and precision of these panelists have been already assured by regular training sessions and subsequent evaluation of performance, according to the ISO 8588-1 standards. For the present study, we have used panelists with several years experience of sensory evaluation of meat and meat products.

The panelists rated the steaks on a 10-cm unstructured line scale (from 0 to 10) measured in mm for the following attributes: global tenderness (0 – extremely tough, 10 – extremely tender), juiciness (0 – extremely dry, 10 – extremely juicy) and beef flavor intensity (0 – extremely weak, 10 – extremely strong). The sessions were carried out in a sensory analysis room equipped with individual booths under artificial red light to reduce the influence of the appearance of the samples. At each session, a monadic presentation of 6 samples was done, each sample being selected in random order. Each tasting booth was equipped with computer terminals linked to a fileserver running a sensory software program (Fizz v 2.20 h, Biosystemes, Couternon, France) that facilitated the direct entry of assessor ratings, which were later formatted in Excel. Within each sensory session, scores were averaged across panelists for each steak, and the means were used in the statistical analyses.

For objective tenderness, the Warner-Bratzler shear force (WBSF) was measured according to Lepetit and Culioli,¹⁸ Briefly, from the sample cuts cooked as previously described, two to five 1 x 1 x 4 cm cores per steak sample were removed parallel to the longitudinal orientation of the muscle fiber of LT muscle. WBSF was assessed using an

Instron 5944 two or three times per core in order to obtain around 10 repetitions per sample. Force at rupture during shear compression testing was expressed in N/cm².

Statistical analysis

The collected data concern the animal life and fattening period of 110 PDO Maine-Anjou cows data ($p = 16$), carcass characteristics ($p = 7$), muscle properties ($p = 27$) and ribeye steak (*Longissimus thoracis*) quality traits ($p = 11$).

Rearing practices classes were created using an innovative statistical approach based on principal component analysis (PCA). We first projected the 16 variables related both to the ***fattening period*** [part of hay, haylage and/or grass in the finishing diet (% w/w); amount of concentrate (daily and overall in kg); fattening duration (days) and physical activity (% days out)] and ***animal characteristics*** [Animals with meat or milk-ability; birth season ; birth weight (kg); age of weaning (month); weaning duration (week); age of first calving; number of calving; suckling value (0-10) and age at slaughter]. Afterwards, a *K*-means cluster analysis ($k = 3$) via the variability explained by PCA allowed the clustering of the animals into distinct clusters. *K*-means clustering is a widely used classification method that generates a specific number of classes (nonhierarchical). This method assumes a certain number of clusters, k , fixed *a priori* and produces a separation of the objects into nonoverlapping groups coming from Euclidean distances minimized at each step of an iterative procedure¹⁰. We proceeded by using the variability explained by the axes of the PCA with eigenvalues >1.0 after Z-scores calculation.¹⁰ Five factors with eigenvalues >1.0 were extracted on the basis of the scree plot and evaluation of the factor loading matrix after orthogonal rotation. To check the suitability of the factorial model, the Kaiser-Meyer-Olkin (KMO) test for sampling adequacy was used. Three clusters representing three different rearing practices classes were identified and named to simplify the discussion as follow: Class 1= “**Hay class**”; Class 2 = “**Grass class**” and Class 3 = “**Haylage class**”, respectively. The use of *K*-means provides an important justification for PCA-based data reduction and allows good separation by grouping the animals according to their similarities in terms of their properties.

To determine the significance of differences between the least squares means of the animal life and fattening period, carcass, muscle and meat quality traits between the three rearing practices classes, PROC GLM procedure of SAS was performed. The significant differences were performed using Tukey's test at a significance level of $P < 0.05$.

For better understandings of the relationships and hence of mechanisms, simple and multiple regression analyses were performed using the PROC REG of SAS to create best regression equations for meat quality traits and carcass characteristics (as dependent variables, y) using the protein muscle characteristics and protein biomarkers (as independent variables, x) per rearing practice class. For that, the option “optimal model” was chosen to produce the model with the highest r^2 value. We maximized at 4 the number of the explanatory variables to be retained in the models to meet to the principle of parsimony [one variable each ten observations (animals)]. Regression coefficients and significance of each retained variable are calculated for the models. The absence of collinearity was systematically verified for each regression model, by producing condition indices and variance proportions using the COLLIN procedure of SAS. Variables were identified as collinear if they possessed both a high condition index >10 and a proportion of variation >0.5 for two or more traits.⁵

Results and Discussion

The present study aimed to **i)** separate into classes using an innovative multivariate analysis a total of 110 PDO Maine-Anjou cows according to their rearing practices applied throughout their live; and **ii)** compare these classes for their carcass, meat quality and muscle characteristics. In addition, this study aims also **iii)** to relate for the first time a list of biomarkers quantified by Dot-Blot and other muscle characteristics, with carcass and meat quality traits according to the rearing practices of PDO Maine-Anjou and explore the consistencies and differences.

Characteristics of the three identified rearing practices classes

The data concerning the succession of main management events from birth to slaughter of cows allowed, using PCA-based approach coupled with the iterative K -means algorithm, the identification of three rearing practices (**Figure 1**). These multivariate analyses shows that the cows with similar characteristics were clustered together as can be seen on the ellipses of the insert at the upper left corner of **Figure 1**. The first class (hereafter “**Hay class**”) grouped 44 young and light cows with the lowest age of weaning and finished mainly with hay, moderately active, with low calving and milking ability (**Table 1**). The second class (hereafter “**Grass class**”) was very different from the two other classes, grouped 30 old cows with the highest age at weaning, the highest number of calving and suckling score and finished under grass diet, with greater physical activity **Table 1**. The third class (hereafter “**Haylage class**”) grouped 36 old cows, with higher birth weight, finished with haylage, and characterized by

low activity **Table 1**. PCA has been numerously used to characterize muscle foods as this method point out quickly the relationships among the variables themselves. This method was recommended to reduce a whole set of related parameters of animal and meat where coherent patterns can be detected more clearly.¹⁹ Hence, plotting of the five axis with eigenvalues > 1.0 on a Cartesian axis revealed that, on the whole, the cows might be discriminated.

Differences and similarities in carcass, meat quality and muscle characteristics

The carcass characteristics of the cows for the three rearing practices classes are summarized in **Table 2**. Cows within the Grass class had different carcass characteristics in comparison to those of Hay and Haylage classes. The only parameter that is not different is the fat to muscle ratio. It is recognized that management practices, such as rearing method, can alter carcass characteristics through differences in finishing practices and diet.²⁰ Nevertheless, it is known that the specific effects on carcass are not easy to evaluate.²¹ In this study, the old animals reared under Grass had the highest carcass conformation, as well as hot carcass, muscle and carcass fat weights. Muscle protein turnover and better vascularization may be played a great role in the high muscularity of the animals as it is known to be highly dependent on energy intake and protein supply.²² Further, in spite of their high age, the cows of Grass class gave carcass properties that are economically advantageous for breeders whom, according to the EUROP classification, will be paid more. Earlier investigations observed that cattle finished on pasture can grade as well as feedlot cattle.²³ We may then speculate that fattening the animals with the same amount of concentrate ($P > 0.05$) but managing them differently, induced significant differences in favor of the cows of Grass class in comparison with the Haylage class. Thereby, one can suppose that the farmers by their expertise were able to identify the importance to keep the cows in the herd because they fit with the objectives of PDO Maine-Anjou: reproductive performance, suckling ability and good skeletal and muscular development, as confirmed in this study (**Table 1** and **2**). Moreover, this study showed clearly that the Grass class is more suitable for carcass properties of PDO Maine-Anjou and there is a need to encourage farmers to maintain this practice to produce added-value beef. These might also satisfy consumers requesting pasture meat, perceived as environmentally friendly. Still, little information is available on the effect of the feeding or rearing system on the proportion of various tissues, thus carcass properties, in the studied breed, and this work is the first to provide these comparisons in addition to a recent preliminary work by our group on these animals.²⁴

The comparison of the meat eating quality properties between the three rearing practices classes are given in **Table 3**. The levels of ultimate pH did not differ. The values were within the normal range for cattle^{10,25} where the mean values in each individual were all below the threshold of 6.0.

Tenderness evaluated using instrumental (WBSF) or trained sensory panels was not different between the rearing practices (**Table 3**). Similarly, juiciness, a texture attribute positively related irrespective of cooking temperature and country panel with tenderness⁶ was not different. Texture is one of the most important component of meat quality.⁸ Our results emphasize that the cows of the three rearing practices classes, characterized by differences in slaughter age, carcass weights and individual (zoo-technical) characters, led to the same meat texture properties. These findings may be partly explained by the ageing period of meat cuts (14 days), which is common for high-quality beef.²⁶ Moreover, French *et al.*²⁷ found in line to our findings, no difference in meat quality with respect to shear force between cattle finished on different regimen practices, including those of pasture. Thus, these results speculate that irrespective of the differences in rearing practices and carcass properties, the same texture properties of PDO Maine-Anjou ribeye steaks are achieved.

Intramuscular fat (IMF) content and beef flavor scores were not different between the classes. These variables are closely related and known to affect the palatability of meat cuts.⁵ In contrast to our findings, finishing practices were reported to influence these two traits, namely grass feeding.²⁸ The similar IMF contents in this work may be explained by the high fat deposition in the studied breed which is not influenced by the rearing practice in this experiment. On another hand and in agreement to earlier investigations,^{5,29} none to weak effects of IMF on sensory traits were found in this study (data not shown). A meta-analysis by our group showed that IMF accounted for a small percentage (4 to 5%) of the variation observed in palatability traits in *Longissimus thoracis* muscle of cows and young bulls.²⁹

Beef color is the first attribute evaluated by consumers and is the major factor that determines and influences the decisions of meat purchase at the point of sale.³⁰ In the present study, there is no difference in lightness (L^*) between the meat of the 3 classes. Whereas, the meat of the animals raised on pasture (Grass class) was redder in color than of Hay and Haylage classes (**Table 4**). Causes of this effect are difficult to evaluate because several factors, not a specific one, are responsible for these differences. In agreement to earlier reports, Dunne *et al.*³¹ reviewed that, due to higher physical activity as it is the case in this study (**Table 1**), animals reared on pasture have higher concentrations of myoglobin in

response to the high oxidative fibers.¹² It was also reported that beef color is influenced by factors such as carcass fatness, animal age and carcass weight, all were the highest as described above in Grass class than the two other rearing practices.²¹

Concerning muscle properties of the three classes, among the biomarkers analyzed, only 4 muscle proteins were different ($P < 0.05$, **Table 4**). MyHC-IIa, Superoxide dismutase (SOD1) and α B-crystallin were more abundant in Grass class. MyHC-IIx level was the lowest in this class, as confirmed by both electrophoresis and Dot-Blot analyses (**Table 4**). The results highlight a switch to more oxidative fibers (IIA fibers) at the expense of IIX fibers in grass class (**Figure 2b**), a finding in agreement to previous studies.¹² The higher levels of SOD1 and α B-crystallin in Grass class are coherent with earlier studies reporting higher abundances of SOD1 and α B-crystallin in oxidative fibers.^{32,33} This may be partly due to the grass diet and high physical activity (~98% vs. ~49% for Hay and ~27% for Haylage classes) of the cows, allowing hence the development of intermediate rather than fast contractile properties.

Relationships between protein biomarkers with carcass characteristics per rearing practices class

The relationships between the biomarkers and carcass traits were investigated using multiple regression analyses per rearing practice, as the explanatory power of the global regression equations were very low (**Table S1-supplementary data**). Thus, the regression coefficients and significance of the proteins retained in each explaining equation by rearing practice are given in **Tables 5 – 7** for Hay, Grass and Haylage classes, respectively. A summary of these associations, which aimed to better understand the relationships, and underpinning mechanisms are given in **Figure 2a**. To our knowledge, proteomic studies reporting links between protein biomarkers and carcass properties are still scarce and this study is the first to show the abundances of biomarkers that may be affected according to different rearing practices. Meanwhile, the findings confirm the previous investigations³⁴ reporting the dependency of the relationships between biomarkers and phenotypes, which are in this study, rearing practices and carcass trait-dependent.

For each rearing practices class, 14 biomarkers (common or different) entered in the regression equations of the 6 carcass characteristics studied (**Tables 5 – 7 and Figure 2a**). The explained variability ranged from 24 to 62% according to the carcass properties and the rearing practices classes. Two proteins only, ICDH and TP53, were common for the three classes and entered in at least one carcass trait for each rearing practices class. ICDH,

representative of oxidative metabolism, had negative and positive coefficient for conformation score for Hay and Haylage classes, respectively. TP53, a protein representative of apoptosis, entered negatively and positively in HCW equations of Hay and Grass classes, respectively. These inverted relationships, highlight the complexity of the effect of rearing practices on the development of muscle and carcass properties, and thereby those of protein abundances, as earlier postulated in several studies by our group for muscles, meat quality traits and breeding practices.^{5,26,33,34} Further, it must be recognized that the lack of consistency would be a consequence of the impact of animal performance effects and the existence of individual animal performances x rearing practice interactions.

Irrespective of rearing practice, Hsp70-Grp75 and μ -calpain were not associated with any of the carcass traits. These two proteins have a common denominator, Ca^{2+} , as they are both involved on its trafficking from the endoplasmic reticulum (ER) to the sarcoplasm and mitochondria.¹⁷ It seems that rearing practices and carcass traits are not related with these two biomarkers as for meat quality traits.

Other relationships were specific for two or one rearing practices class (**Figure 2a**). SOD1, Hsp70-1A and MyLC-1F involved in carcass traits of Hay and Haylage classes. Hsp70-1B, MyBP-H, H2AFX, Hsp20 and Prdx6 entered in the regression equations of Grass and Hay classes. MyHC-IIx, Hsp40, α -actin, PGM1 and ENO3 entered in the regression equations of Grass and Haylage classes. The other proteins were related with carcass traits in one rearing practices class only, likely α B-crystallin, m-calpain, LDH and MyHC-IIa for Hay class; Hsp70-8 and fiber area for Grass class; and finally DJ-1, MyHC-I and Hsp27 for Haylage class. The analysis of these relationships by rearing practices class with a focus on the relevant proteins only, *i.e.*, proteins that are retained in the equations more than twice for carcass traits, was highlighted in the following.

For Hay class, MyBP-H and α B-crystallin retained 4 and 3 times to explain the carcass traits, respectively (**Figure 3a** and **Table 5**). MyBP-H was negative in the regression equations of conformation, ribeye, m. *longissimus* and hot carcass weights. MyBP-H has significant effects on length, thickness, and lateral alignment of myosin filaments.³⁵ Located in the A-band of the myofibrils, it is closely related with the thick filaments, however its primary association is with the fibers of fast twitch muscles.³⁵ The relationship of α B-crystallin with ribeye, muscle and fat carcass weights agree with the importance of Hsp in the regulation of intermediate filament network, likely desmin, involved in the structural integrity and function of muscle cells and hence, those of carcass traits.³⁶ In pig, Hou *et al.*³⁷ reported

$\alpha\beta$ -crystallin implication in the regulation of skeletal muscle growth and body size. Small Hsp proteins were found in several studies to be also related with fat accumulation in bovine skeletal muscle. For example, Kim *et al.*³⁸ pointed out that Hsp27 has a negative correlation with fat deposition in agreement with our findings. For instance, it is difficult to discuss more there relationships. Nevertheless, they offer some important perspectives.

For Grass class, MyHC-IIx, H2AFX, MyBP-H and Hsp40 were the proteins mostly related with the carcass traits (**Figure 2a** and **Table 6**). The first three proteins in addition to ICDH were retained in the model of *Longissimus* muscle weight (LMW) and explained 62% of variance. The negative relationship with MyHC-IIx agree with the findings discussed above, and suggest that more the animals are grazed, more are the oxidative properties of the muscle, herein by ICDH showed with a strong regression coefficient ($\beta = 0.53$; $P < 0.001$). As expected, the impact of MyHC-IIx is more pronounced within ribeye and muscle carcass weights (**Table 6**). Similarly, an earlier study revealed that the protein content in skeletal muscle is affected by the regulation of *MyHC* expression when steers are grazed.³⁹ MyBP-H, common to Hay class, was further retained in the regression equations of ribeye, *Longissimus* muscle and fat carcass weights of Grass class. These findings support its involvement in carcass traits and may partly explain the inverse relationships found within Grass and Hay rearing practices class, described above to differ in their contractile properties. The precise role of MyBP-H in carcass composition has not yet been described and we suggest this biomarker to play a pivotal role in muscularity development under a marked rearing practice effect. The relation with Hsp40, agrees well with the results found for Hay class, involving cellular protein trafficking and stress defense. Accordingly, our group was the first to report Hsp40 expression during cattle muscle development.⁴⁰ In the present study, we show for the first time links between Hsp40 and carcass traits in a rearing practices dependency manner. Hsp40 is a co-chaperone that would assist the regulation of complex formation between Hsp70 and client proteins using rapid and transient interaction by activating ATP hydrolysis.⁴¹ Changes in Hsp40 expression might further reflect a shift in muscle metabolism.⁴⁰ Our findings agree to this proposal. Thus, the rearing practices effect and specificity on Hsp40 abundance is of great significance, as the cows of Grass class showed a clear orientation of muscle metabolism towards the oxidative type.¹² The involvement of H2AFX in conformation, *Longissimus* muscle and fat carcass weights highlights the high level of regulation that occurs during animal growth and carcass development of cows reared under Grass class. In Hay class, H2AFX was negatively related with ribeye and fat carcass weights.

This study is the first to show these relationships. We may suggest H2AFX to play a pivotal role in the balance between different biological processes. This histone was described to bind to DNA and to various enzymes and protein entities, thereby modulating many cellular pathways⁴². H2AFX was further reported to be involved in the underlying mechanisms of muscle to meat conversion, namely of tenderness.^{16,42, 43}

For Haylage class, MyHC-I, PGM1 and Hsp27 were the main proteins involved in different carcass traits (**Figure 2a** and **Table 7**). The first two proteins reflect both contractile and metabolic properties of the muscle. Their involvement together with that of Hsp27 agrees with the results described above for Hay and Grass classes. It seems that the retained proteins and their direction differ between classes but they share common biological pathways that would underpin muscle growth and carcass development. These proteins reveal a great role of energy metabolism, heat stress and signaling pathways. Moreover, the findings highlight specific sophisticated mechanisms behind adaptation of muscle and carcass to each rearing practices management. This agrees with the previously published literature indicating that physiology of growth in meat animals is an orchestrated process characterized by fine coordination of cell proliferation and increase in cell size in organs, skeletal muscle, bone, and adipose tissue from birth to market weight (for review see ²²). The involvement of Hsp27 supports the relationships found with carcass traits at both protein^{36,38} and gene levels.⁴⁴ Hsps protect proteins of the cytoskeleton and have the ability to collaborate to control the redox status.⁴¹ In this work, the explaining regression equations confirmed mechanisms reported in certain studies exploring relationships with few proteins or genes. For example, the equation of fat carcass weight retained MyHC-I and Hsp70-1A (positive) and DJ-1 and SOD1 (negative). The positive involvement of both MyHC-I and Hsp70-1A agrees with the specific expression of large Hsp70 in slow-twitch fibers.⁴¹ More precisely, these authors reported that the inducible form of Hsp70 is constitutively expressed in rat muscles comprised of type I muscle fibres, but not in those comprised of type IIb fibres.⁴¹ The oxidative properties of cells are continuously affected along these mechanisms, which may explain the implication of the two anti-oxidant proteins (DJ-1 and SOD1). These results need further investigations since little is known about these proteins in bovine muscle in relation to carcass, according to the rearing practices of meat animals.

Associations between muscle biomarkers with meat quality traits per rearing practice class

The relationships between the biomarkers and meat quality traits were performed using the same approach discussed above. Similarly, the regression coefficients and significance of the proteins retained in each equation by rearing practices class are given in **Tables 5 – 7** for Hay, Grass and Haylage classes, respectively. A summary of these associations is given in **Figures 2b,c** for each rearing practices class. A total of 17 biomarkers for Hay class, and 18 for both Grass and Haylage classes, were entered in the regression equations of the 11 meat quality traits (**Tables 5 – 7** and **Figure 2b**). Furthermore, 3 to 5 carcass traits were entered in the equations by class. These later did not explain a great variability compared to biomarkers, unless for fat carcass weight which as expected, strongly and positively affected intramuscular fat irrespective of rearing practices class. The explained variance of the regression equations ranged from 20 to 80% (**Tables 5 – 7**). The findings support the numerous studies highlighting the relationships between protein biomarkers identified using proteomic studies and meat quality traits as recently reviewed in two comprehensive reviews^{9,42} The implementation of high-throughput analytical tools over the last two decades was an important step toward a better understanding of the complex biological systems that define muscle to meat conversion.^{8,16,17} The results reveal the rearing practice dependency of the relationships. Certain proteins were for the first time related with some beef quality traits, namely juiciness, beef flavor and color parameters.

Among the biomarkers, five were common for the three rearing practices class and retained to explain at list one beef quality trait. These are MyHC-IIx, PGM1, Hsp40, ICDH and Hsp70-Grp75. From these, PGM1 was positively related with h^* irrespective of rearing practice class. This robust relationship was earlier reported in cattle and identified PGM1 to be positively related with a^* and h^* .¹⁰ This is in line with the idea that in the early *post-mortem* period, glycolysis will continue, thereby affecting meat color and other sensory qualities. PGM1 catalyzes the production of 3-phosphoglycerate and ATP from 1.3-bisphosphoglycerate during aging and the phosphorylation of PGM1 would play an important role in pH drop and hence of meat quality traits development (for review see ⁸).

The other metabolic enzyme, ICDH, entered positively with WBSF of Grass and Hay classes, was further positively related with pHu of Hay and Haylage classes. ICDH involved in the tricarboxylic acid (TCA) cycle was already identified to play a great role in *post-mortem* beef metabolism. This agrees with the above results comparing classes and describing relationships with carcass traits, supported mainly by the entrance of MyHC-IIx fibers in the regression equations numerous times.

The results about heat shock proteins are in line with the great role that these chaperones would play in muscle to meat conversion.^{16,17,45} Hsp40 was negative in the regression equations of meat color coordinates of Hay (with C^* and h^*), Grass (with b^*) and Haylage (with L^*) classes. It was further positively related with juiciness (for Hay class) and tenderness (for Haylage class). The primary studies have shown negative relationships between *DNAJ1* (the gene coding for Hsp40 protein) expression in *Longissimus thoracis* muscle and tenderness.⁴⁶ Later on, this protein was reported by our group to be related with beef color.^{10,17} Moreover, the central role of the large Hsp70-Grp75 in color and sensory meat qualities was recently proposed.¹⁷ In the present study, Hsp70-Grp75 was positive in C^* of Hay class, negative and positive respectively with juiciness and pHu for Grass class, and positive with L^* for Haylage class. After animal bleeding, apoptosis is induced and in response, Hsp proteins are recruited to impede the onset of apoptosis and to chaperone the unfolding of muscle proteins.⁸ We assume that HSPs have essential roles in *post-mortem* skeletal muscle since they contribute to proper conformation of proteins and preservation of their biological functions. They act as molecular chaperones during protein assembly, protein folding and unfolding, and in the refolding of damaged proteins, thus maintaining cell homeostasis.⁴⁵ Hsp70-Grp75, a 75 kDa glucose regulated protein retained in the three classes, is the only mitochondrial Hsp present in nearly all cellular compartments and may affect muscle to meat conversion in different ways.^{17,47} Further Hsp proteins were also involved but in just two or one rearing practice. For example, α B-crystallin was as described above for carcass traits positively related with IMF for Haylage class and negatively with juiciness, L^* and b^* for Hay class. Hsp70-8 entered positively in beef flavor and negatively as expected in sensory tenderness and C^* equation of Hay class and positively with juiciness and a^* for Grass class. Hsp27 was negative with pHu in Hay class as described by Sevane *et al.*⁴⁴ and positive with WBSF and negative with a^* and b^* for Grass class. Hsp20, another small Hsp, was negative with C^* for Grass class and positive with L^* for Hay class. Finally, Hsp70-1A was specific for Haylage class only and related negatively with pHu. Comparisons concerning most of these relationships were extensively reported for different beef quality traits, namely tenderness. The reader would refer to recent reviews in the field.^{9,42}

The other similarities found in this study are the positive relation of H2AFX with h^* for both Grass and Hay classes. This study is the first to found this relation. It was further involved negatively in beef flavor and ultimate pH and positively with b^* for grass class. H2AFX is an histone involved in the cellular response to DNA damage, notably during

oxidative stress, which may explain its relationships with these traits where oxidation would play an important role. This protein was proposed by our group using *in silico* to be related with beef tenderness.¹⁶

From the other antioxidant proteins reported, Prdx6 was positive with C^* for both Grass and Haylage classes. It was also negatively and positively related with h^* and a^* in the same classes, respectively. These findings are in line with our recent investigations on beef color^{10,17} and those of other laboratories.⁴⁸ Prdx6 is expressed in nearly all tissues including skeletal muscle, and protects cells against oxidative stress. In addition, it was positively related and in agreement to Guillemín *et al.*¹⁶ with both tenderness and juiciness in Haylage class. SOD1, a member of antioxidant enzymes, entered in both Hay and Grass classes. It was negative in the regression equations of juiciness and beef flavor for Hay class, and positive with WBSF and a^* for Grass class. SOD1 is an antioxidant, which allows a fast dismutation of O^{2-} to O_2 and H_2O_2 . Accordingly, Grabež *et al.*⁴⁹ reported great abundance of SOD1 in tough meat. The authors suggest that stronger oxidative stress may cause ROS accumulation in tough meat and reduce cell protection.

Structural proteins were also retained in several regression equations, but were specific for each quality trait and the direction of their entrance is not always the same. The first example concern α -actin negatively and positively related with WBSF of Haylage and Grass classes, respectively. Meat tenderness is achieved primarily through ageing, during which structural changes occur in the muscle owing to complex mechanisms involving pH and ionic strength, combined with the action of cellular proteolytic systems.⁸ In this study, both μ -calpain and m-calpain, two proteolytic systems, were related with meat quality traits, but rearing practice and trait-dependent. For example, m-calpain was retained negatively and positively in L^* equations for Haylage and Hay classes, respectively. Furthermore, m-calpain was negatively related with WBSF and positively with sensory tenderness for Hay class. For further interpretation of the involvement of both proteolytic and structure proteins and their influence on meat quality, the reader would refer to the comprehensive reviews published recently.^{8,50}

Overall, taking all of the results together, this study allowed us to explore the protein biomarkers relationships with Grass, Hay and Haylage rearing practices of PDO Maine-Anjou beef. This study confirms that many production traits or production-related traits in livestock are the result of sophisticated biological processes finely orchestrated during the life of the animal and soon after slaughter. These results may serve as a first guideline of the relationships between muscle proteome with carcass and beef quality traits according to the

620 rearing practices. Furthermore, these protein biomarkers related to the carcass traits offer
621 scientific community a starting point from which to study the complex protein-networks
622 managing muscle to meat conversion that underlie economically important phenotype traits.
623 In this study, direct relationships using a consistent number of animals and biomarkers in
624 comparison to proteomic studies investigating generally little animals allowed confirming the
625 earlier statements and reveals for the first time direct relationships with carcass traits.
626 Furthermore, the most original in this integrative approach is that we have associated for the
627 first time myriad proteins belonging to several biological pathways in relation to carcass
628 qualities that were not often considered in the same study. The relationships were also with
629 eleven meat quality traits grouping texture, flavor, pH and color, according to the rearing
630 practices of the animals.

Notes

The authors declare no competing financial interest.

Funding

Project number S3-23000846 funded by Auvergne Rhône-Alpes Region and FEDER.

Acknowledgments

The financial support given to MG from S3-23000846 project is highly acknowledged. The authors would thank also “Pays de Loire Region”, the defense Trade Union of PDO Maine-Anjou, Adema and Elivia for the use of data. The authors thank the many colleagues involved in this project, namely Guillain Le Bec and Ghislain Aminot from SICA Rouge des Prés and Didier Micol from INRA for their assistance in data collection, the management and slaughter of animals, muscle sampling, and biochemical measurements. Many thanks to Nicole Dunoyer and Jeremy Huant for their technical assistance concerning the biomarkers quantifications by Dot-Blot.

Supporting Information

Table S1. Described the multiple regression analyses of carcass characteristics and meat quality traits for the 110 m cows as one dataset using protein biomarkers. The Supporting Information is available free of charge on the ACS Publications website.

References

1. Schreurs, N. M.; Garcia, F.; Jurie, C.; Agabriel, J.; Micol, D.; Bauchart, D.; Listrat, A.; Picard, B., Meta-analysis of the effect of animal maturity on muscle characteristics in different muscles, breeds, and sexes of cattle. *Journal of Animal Science* **2008**, *86*, 2872-2887.
2. Prache, S.; Gatellier, P.; Thomas, A.; Picard, B.; Bauchart, D., Comparison of meat and carcass quality in organically reared and conventionally reared pasture-fed lambs. *Animal* **2011**, *5*, 2001-9.
3. Soulat, J.; Picard, B.; Leger, S.; Monteils, V., Prediction of beef carcass and meat traits from rearing factors in young bulls and cull cows. *J Anim Sci* **2016**, *94*, 1712-26.
4. Hocquette, J. F.; Botreau, R.; Legrand, I.; Polkinghorne, R.; Pethick, D. W.; Lherm, M.; Picard, B.; Doreau, M.; Terlouw, E. M. C., Win-win strategies for high beef quality, consumer satisfaction, and farm efficiency, low environmental impacts and improved animal welfare. *Animal Production Science* **2014**, *54*, 1537-1548.
5. Gagaoua, M.; Terlouw, E. M. C.; Micol, D.; Hocquette, J. F.; Moloney, A. P.; Nuernberg, K.; Bauchart, D.; Boudjellal, A.; Scollan, N. D.; Richardson, R. I.; Picard, B., Sensory quality of meat from eight different types of cattle in relation with their biochemical characteristics. *Journal of Integrative Agriculture* **2016**, *15*, 1550-1563.

6. Gagaoua, M.; Micol, D.; Picard, B.; Terlouw, C. E.; Moloney, A. P.; Juin, H.; Meteau, K.; Scollan, N.; Richardson, I.; Hocquette, J. F., Inter-laboratory assessment by trained panelists from France and the United Kingdom of beef cooked at two different end-point temperatures. *Meat Sci* **2016**, *122*, 90-6.
7. Almeida, A. M.; Bassols, A.; Bendixen, E.; Bhide, M.; Ceciliani, F.; Cristobal, S.; Eckersall, P. D.; Hollung, K.; Lisacek, F.; Mazzucchelli, G.; McLaughlin, M.; Miller, I.; Nally, J. E.; Plowman, J.; Renaut, J.; Rodrigues, P.; Roncada, P.; Staric, J.; Turk, R., Animal board invited review: advances in proteomics for animal and food sciences. *Animal* **2015**, *9*, 1-17.
8. Ouali, A.; Gagaoua, M.; Boudida, Y.; Becila, S.; Boudjellal, A.; Herrera-Mendez, C. H.; Sentandreu, M. A., Biomarkers of meat tenderness: present knowledge and perspectives in regards to our current understanding of the mechanisms involved. *Meat Sci* **2013**, *95*, 854-70.
9. Picard, B., Gagaoua, M., & Hollung, K. (2017). Chapter 12: Gene and protein expression as a tool to explain/predict meat (and fish) quality. In P. Purslow (Ed.), *New Aspects of Meat Quality: From Genes to Ethics* (pp. 321-354). Netherlands: Elsevier Science.
10. Gagaoua, M.; Couvreur, S.; Le Bec, G.; Aminot, G.; Picard, B., Associations among Protein Biomarkers and pH and Color Traits in *Longissimus thoracis* and *Rectus abdominis* Muscles in Protected Designation of Origin Maine-Anjou Cull Cows. *J Agric Food Chem* **2017**, *65*, 3569-3580.
11. Couvreur, S., Le Bec, G., Micol, D., Aminot, G., Picard, B., PDO Maine-Anjou Culled cow characteristics and finishing practices influence meat quality. In: 20^{èmes} *Rencontres Recherches Ruminants*, **2013**, 165-168, Paris.
12. Jurie, C.; Ortigues-Marty, I.; Picard, B.; Micol, D.; Hocquette, J. F., The separate effects of the nature of diet and grazing mobility on metabolic potential of muscles from Charolais steers. *Livestock Science* **2006**, *104*, 182-192.
13. Picard, B.; Barboiron, C.; Chadeyron, D.; Jurie, C., Protocol for high-resolution electrophoresis separation of myosin heavy chain isoforms in bovine skeletal muscle. *Electrophoresis* **2011**, *32*, 1804-1806.
14. Oury, M. P.; Dumont, R.; Jurie, C.; Hocquette, J. F.; Picard, B., Specific fibre composition and metabolism of the rectus abdominis muscle of bovine Charolais cattle. *BMC Biochem* **2010**, *11*, 12.
15. Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **1976**, *72*, 248-54.
16. Guillemin, N.; Bonnet, M.; Jurie, C.; Picard, B., Functional analysis of beef tenderness. *J Proteomics* **2011**, *75*, 352-65.
17. Gagaoua, M.; Terlouw, E. M.; Micol, D.; Boudjellal, A.; Hocquette, J. F.; Picard, B., Understanding Early Post-Mortem Biochemical Processes Underlying Meat Color and pH Decline in the *Longissimus thoracis* Muscle of Young Blond d'Aquitaine Bulls Using Protein Biomarkers. *J Agric Food Chem* **2015**, *63*, 6799-809.
18. Lepetit, J.; Culioli, J., Mechanical properties of meat. *Meat Science* **1994**, *36*, 203-237.
19. Karlsson, A., The Use of principal component analysis (PCA) for evaluating results from pig meat quality measurements. *Meat science* **1992**, *31*, 423-33.
20. Fiems, L. O.; Campeneere, S. D.; De Smet, S.; Van de Voorde, G.; Vanacker, J. M.; Boucqué, C. V., Relationship between fat depots in carcasses of beef bulls and effect on meat colour and tenderness. *Meat science* **2000**, *56*, 41-47.

21. Priolo, A.; Micol, D.; Agabriel, J., Effects of grass feeding systems on ruminant meat colour and flavour. A review. *Anim. Res.* **2001**, *50*, 185-200.
22. Moloney, A. P.; McGee, M., Chapter 2 - Factors Influencing the Growth of Meat Animals A2 - Toldra', Fidel. In *Lawrie's Meat Science (Eight Edition)*, Woodhead Publishing: 2017; pp 19-47.
23. Bowling, R. A.; Smith, G. C.; Carpenter, Z. L.; Dutson, T. R.; Oliver, W. M., Comparison of Forage-Finished and Grain-Finished Beef Carcasses1. *Journal of Animal Science* **1977**, *45*, 209-215.
24. Gagaoua, M.; Picard, B.; Couvreur, S.; Le Bec, G.; Aminot, G.; Monteils, V. *Rearing practices and carcass and meat properties: a clustering approach in PDO Maine-Anjou cows*, In *Proceedings of the 63rd International Congress of Meat Science and Technology*, Cork, Ireland, 13-18/08/2017, **2017**; Troy, D.; McDonnell, C.; Hinds, L.; Kerry, J., Eds. Wageningen Academic Publishers: Cork, Ireland, 2017; pp 97-98.
25. Jeremiah, L. E.; A.K.W, T.; Gibson, L. L., The usefulness of muscle color and pH for segregating beef carcasses into tenderness groups. *Meat science* **1991**, *30*, 97-114.
26. Renand, G.; Picard, B.; Touraille, C.; Berge, P.; Lepetit, J., Relationships between muscle characteristics and meat quality traits of young Charolais bulls. *Meat science* **2001**, *59*, 49-60.
27. French, P.; O'Riordan, E. G.; Monahan, F. J.; Caffrey, P. J.; Mooney, M. T.; Troy, D. J.; Moloney, A. P., The eating quality of meat of steers fed grass and/or concentrates. *Meat science* **2001**, *57*, 379-86.
28. Varela, A.; Oliete, B.; Moreno, T.; Portela, C.; Monserrat, L.; Carballo, J. A.; Sánchez, L., Effect of pasture finishing on the meat characteristics and intramuscular fatty acid profile of steers of the Rubia Gallega breed. *Meat science* **2004**, *67*, 515-522.
29. Chriki, S.; Renand, G.; Picard, B.; Micol, D.; Journaux, L.; Hocquette, J. F., Meta-analysis of the relationships between beef tenderness and muscle characteristics. *Livestock Science* **2013**, *155*, 424-434.
30. Mancini, R. A.; Hunt, M. C., Current research in meat color. *Meat science* **2005**, *71*, 100-121.
31. Dunne, P. G.; Monahan, F. J.; Moloney, A. P., Current perspectives on the darker beef often reported from extensively-managed cattle: Does physical activity play a significant role? *Livestock Science* **2011**, *142*, 1-22.
32. Hollander, J.; Fiebig, R.; Gore, M.; Bejma, J.; Ookawara, T.; Ohno, H.; Ji, L. L., Superoxide dismutase gene expression in skeletal muscle: fiber-specific adaptation to endurance training. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* **1999**, *277*, R856-R862.
33. Picard, B.; Gagaoua, M.; Micol, D.; Cassar-Malek, I.; Hocquette, J. F.; Terlouw, C. E., Inverse relationships between biomarkers and beef tenderness according to contractile and metabolic properties of the muscle. *J Agric Food Chem* **2014**, *62*, 9808-18.
34. Ellies-Oury, M.-P.; Gagaoua, M.; Saracco, J.; Chavent, M.; Picard, B., Biomarker Abundance in Two Beef Muscles Depending on Animal Breeding Practices and Carcass Characteristics. *JSM Bioinformatics, Genomics and Proteomics* **2017**, *2*, 1013.
35. Gilbert, R.; Cohen, J. A.; Pardo, S.; Basu, A.; Fischman, D. A., Identification of the A-band localization domain of myosin binding proteins C and H (MyBP-C, MyBP-H) in skeletal muscle. *J Cell Sci* **1999**, *112 (Pt 1)*, 69-79.
36. Ghosh, J. G.; Houck, S. A.; Clark, J. I., Interactive sequences in the stress protein and molecular chaperone human α B crystallin recognize and modulate the assembly of filaments. *The International Journal of Biochemistry & Cell Biology* **2007**, *39*, 1804-1815.

37. Hou, X.; Yang, Y.; Zhu, S.; Hua, C.; Zhou, R.; Mu, Y.; Tang, Z.; Li, K., Comparison of skeletal muscle miRNA and mRNA profiles among three pig breeds. *Molecular Genetics and Genomics* **2016**, *291*, 559-573.
38. Kim, N. K.; Cho, S.; Lee, S. H.; Park, H. R.; Lee, C. S.; Cho, Y. M.; Choy, Y. H.; Yoon, D.; Im, S. K.; Park, E. W., Proteins in longissimus muscle of Korean native cattle and their relationship to meat quality. *Meat science* **2008**, *80*, 1068-73.
39. Shibata, M.; Matsumoto, K.; Hikino, Y.; Oe, M.; Ojima, K.; Nakajima, I.; Muroya, S.; Chikuni, K., Influence of different feeding systems on the growth performance and muscle development of Japanese Black steers. *Meat science* **2011**, *89*, 451-456.
40. Cassar-Malek, I.; Guillemin, N.; Hocquette, J. F.; Micol, D.; Bauchart, D.; Picard, B.; Jurie, C., Expression of DNAJA1 in bovine muscles according to developmental age and management factors. *Animal* **2011**, *5*, 867-74.
41. Liu, Y.; Steinacker, J. M., Changes in skeletal muscle heat shock proteins: pathological significance. *Front Biosci* **2001**, *6*, D12-D25.
42. Picard, B.; Gagaoua, M., Chapter 11 - Proteomic Investigations of Beef Tenderness. In *Proteomics in Food Science: from farm to fork*, Academic Press: 2017; pp 177-197.
43. Gagaoua, M.; Terlouw, E. M. C.; Monteils, V.; Couvreur, S.; Picard, B. In *Stress proteins in cull cows: relationships with transport and lairage durations but not with meat tenderness*, Proceedings of the 63rd International Congress of Meat Science and Technology, Cork, Ireland, 13-18/08/2017, 2017; Troy, D.; McDonnell, C.; Hinds, L.; Kerry, J., Eds. Wageningen Academic Publishers: Cork, Ireland, 2017; pp 427-428.
44. Sevane, N.; Armstrong, E.; Wiener, P.; Pong Wong, R.; Dunner, S., Polymorphisms in twelve candidate genes are associated with growth, muscle lipid profile and meat quality traits in eleven European cattle breeds. *Molecular Biology Reports* **2014**, *41*, 4721-4731.
45. Lomiwes, D.; Farouk, M. M.; Wiklund, E.; Young, O. A., Small heat shock proteins and their role in meat tenderness: a review. *Meat science* **2014**, *96*, 26-40.
46. Bernard, C.; Cassar-Malek, I.; Le Cunff, M.; Dubroeucq, H.; Renand, G.; Hocquette, J. F., New indicators of beef sensory quality revealed by expression of specific genes. *J Agric Food Chem* **2007**, *55*, 5229-37.
47. Gagaoua, M.; Terlouw, E. M. C.; Picard, B., The study of protein biomarkers to understand the biochemical processes underlying beef color development in young bulls. *Meat science* **2017**, *134*, 18-27.
48. Wu, W.; Gao, X.-G.; Dai, Y.; Fu, Y.; Li, X.-M.; Dai, R.-T., Post-mortem changes in sarcoplasmic proteome and its relationship to meat color traits in M. semitendinosus of Chinese Luxi yellow cattle. *Food Research International* **2015**, *72*, 98-105.
49. Grabez, V.; Kathri, M.; Phung, V.; Moe, K. M.; Slinde, E.; Skaugen, M.; Saarem, K.; Egelanddal, B., Protein expression and oxygen consumption rate of early postmortem mitochondria relate to meat tenderness. *J Anim Sci* **2015**, *93*, 1967-79.
50. Erthjerg, P.; Puolanne, E., Muscle structure, sarcomere length and influences on meat quality: A review. *Meat science* **2017**, *132*, 139-152

Figure captions

Figure 1. Principal component analysis showing both the loading and score plots of the variables used to discriminate between the 110 cows. The rearing practice classes 1 – 3 (ellipses of the insert at the upper left corner of PCA) were highlighted in blue, green and red respectively. The variables in bold character are significantly different ($P < 0.05$) between classes and those in italic are not different as depicted in **Table 1**.

Figure 2. A summary of the entrance frequency of the protein biomarkers in the regression equations of **A)** carcass characteristics and **B)** meat quality traits within rearing practice classes from **Tables 5 – 7**. The regression equations of meat quality traits (**B**) were built by including **C)** carcass characteristics as explanatory variables (FCW: Fat Carcass weight; LMW: *Longissimus* muscle weight; MCW: muscle carcass weight; HCW: hot carcass weight; EUROP CS: EUROP carcass score and RW: ribeye weight). The relevance of the proteins in the traits of each rearing practice are presented by a red gradient color from none (0) with white to the high (4) in intense red.

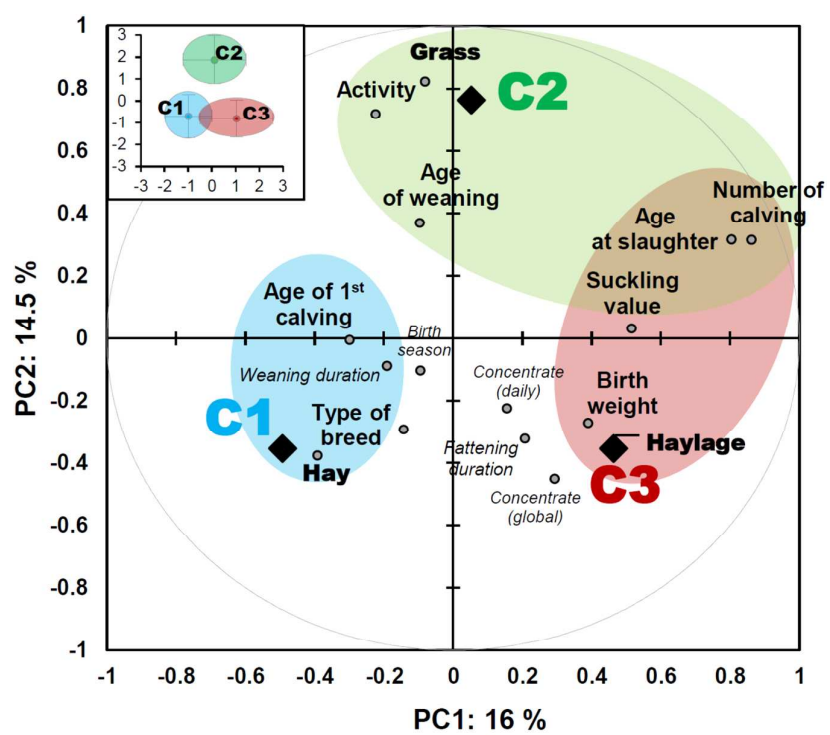


Figure 1.

**Meat quality traits regressed
with protein biomarkers (B) and
carcass characteristics (C)**

Figure 2.

Table 1. Comparison of the three rearing practices identified according to variables related to animal life and fattening period.

Variables	Class 1 ^a “Hay” (n = 44)	Class 2 “Grass” (n = 30)	Class 3 “Haylage” (n = 36)	SEM ^b	p-value ^c
Birth weight (kg)	49.3 ^b	48.3 ^b	52.2 ^a	0.49	**
Age of weaning (month)	6.9 ^b	7.6 ^a	7.2 ^{a,b}	0.10	*
Weaning duration (week)	7.7	9.8	9.1	0.90	ns
Age at first calving (month)	33.5 ^a	32.1 ^{a,b}	31.3 ^b	0.39	*
Number of calving	2.1 ^b	3.7 ^a	3.4 ^a	0.20	***
Suckling score (/10)	5.6 ^b	6.4 ^a	5.9 ^{a,b}	0.13	*
Fattening duration (day)	98.9	91.8	104	2.87	ns
Haylage diet (%)	3.6 ^b	6.1 ^b	75.4 ^a	3.54	***
Hay diet (%)	84.6 ^a	28.8 ^b	19.7 ^b	3.58	***
Grass diet (%)	11.8 ^b	65.0 ^a	4.9 ^b	3.08	***
Daily concentrate diet (kg)	7.8	7.6	7.7	0.20	ns
Global concentrate diet (kg)	760	683	757	23.37	ns
Activity (%)	49.1 ^b	93.9 ^a	26.7 ^c	4.43	***
Age at slaughter (month)	57.9 ^b	76.1 ^a	72.2 ^a	2.37	**

^a Least square means in the same row for class effect not followed by a common letter (a-c) differ significantly: $P < 0.05$.

^b Standard error of mean

^c Significances : ns: not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Table 2. Comparison of the three rearing practices identified according to variables related to carcass characteristics.

Variables	Class 1 ^a “Hay” (n = 44)	Class 2 “Grass” (n = 30)	Class 3 “Haylage” (n = 36)	SEM ^b	p-value ^c
Hot carcass weight (HCW, kg)	428 ^b	459 ^a	435 ^b	3.46	***
EUROP conformation score (EUROP CS)	4.8 ^{a,b}	5.1 ^a	4.5 ^b	0.08	*
Ribeye weight (g) (RW)	2956 ^b	3333 ^a	2903 ^b	61.10	**
<i>Longissimus</i> muscle weight (LMW)	370 ^b	407 ^a	345 ^c	7.57	**
Muscle carcass weight (MCW)	1811 ^b	2026 ^a	1773 ^b	38.60	*
Fat carcass weight (FCW)	551 ^b	650 ^a	553 ^b	18.23	*
Fat to muscle ratio (% w/w)	30.9	32.8	31.2	0.97	ns

^a Least square means in the same row for class effect not followed by a common letter (a-c) differ significantly: $P < 0.05$.

^b Standard error of mean

^c Significances : ns: not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Table 3. Comparison of the three rearing practices identified according to variables related to meat quality traits.

Variables	Class 1 ^a “Hay” (n = 44)	Class 2 “Grass” (n = 30)	Class 3 “Haylage” (n = 36)	SEM ^b	p-value ^c
Ultimate pH	5.59	5.60	5.60	0.01	ns
Lightness (<i>L</i> *)	39.5	39.9	39.6	0.22	ns
Redness (<i>a</i> *)	8.65 ^b	9.27 ^a	8.67 ^b	0.12	*
Yellowness (<i>b</i> *)	7.00 ^b	8.31 ^a	7.24 ^b	0.14	***
Chroma (<i>C</i> *)	11.2 ^b	12.5 ^a	11.3 ^b	0.15	**
Hue angle (<i>h</i> *)	38.9 ^b	41.6 ^a	39.8 ^b	0.47	*
Tenderness (0 – 10 scale)	4.5	4.6	4.4	0.05	ns
Juiciness (0 – 10 scale)	3.3	3.2	3.3	0.04	ns
Beef flavor (0 – 10 scale)	4.7	4.7	4.7	0.03	ns
Warner-Bratzler shear force (N/cm ²)	44.6	42.8	45.8	1.07	ns
Intramuscular fat content (% w/w)	15.7	17.6	16.4	0.59	ns

^a Least square means in the same row for class effect not followed by a common letter (a-c) differ significantly: $P < 0.05$.

^b Standard error of mean

^c Significances : ns: not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Table 4. Comparison of the three rearing practices identified according to variables related to muscle characteristics.

Variables	Class 1 ^a “Hay” (n = 44)	Class 2 “Grass” (n = 30)	Class 3 “Haylage” (n = 36)	SEM ^b	p-value ^c
<i>Contractile properties by myosin fiber characterization</i>					
Fiber area, μm^2	2885	2940	2894	61.8	ns
MyHC-I (%)	30.5	31.9	31.4	0.71	ns
MyHC-IIa (%)	55.0 ^b	61.2 ^a	54.8 ^b	1.22	*
MyHC-IIx (%)	14.5 ^a	6.9 ^b	13.8 ^a	1.34	*
<i>Metabolic properties by metabolic enzyme activities</i>					
LDH ($\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$)	713	693	708	10.4	ns
ICDH ($\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$)	1.02	1.07	1.03	0.03	ns
<i>Protein biomarkers quantified Dot-Blot (in arbitrary units)</i>					
<i>Heat shock proteins</i>					
α B-crystallin	207.5 ^b	252.5 ^a	217.3 ^b	8.04	*
Hsp20	159.3	159.8	176.6	4.35	ns
Hsp27	75.7	81.8	79.6	1.90	ns
Hsp40	129.9	126.5	135.1	2.01	ns
Hsp70-1A	120.1	115.4	124.7	2.51	ns
Hsp70-1B	192.4	174.4	187.9	4.73	ns
Hsp70-8	112.7	113.7	109.7	2.38	ns
Hsp70-Grp75	143.6	145.1	146.2	2.92	ns
<i>Metabolism</i>					
Enolase 3 (ENO3)	141.8	145.8	146.7	3.47	ns
Phosphoglucosmutase 1 (PGM1)	106.5	94.3	102.5	2.61	ns
<i>Structure</i>					
α -actin	129.4	120.1	119.6	3.87	ns
Myosin binding protein H (MyBP-H)	125.9	122.9	127.7	1.78	ns
Myosin light chain 1F (MyLC-1F)	60.7	61.3	62.2	1.24	ns
Myosin heavy chain IIx (MyHC-IIx)	99.1 ^a	78.2 ^b	92.3 ^a	2.63	*
<i>Oxidative stress</i>					
Superoxide dismutase [Cu-Zn] (SOD1)	94.1 ^b	118.5 ^a	96.4 ^b	3.63	*
Peroxiredoxin 6 (PRDX6)	105.8	102.4	111.2	1.67	ns
Protein deglycase (DJ1)	93.4	87.7	91.5	1.33	ns
<i>Proteolysis</i>					
μ -calpain	145.4	155.9	157.5	3.66	ns
m-calpain	96.3	94.0	98.7	1.21	ns
<i>Apoptosis and signaling</i>					
Tumor protein p53 (TP53)	114.8	119.5	122.1	1.82	ns
H2A Histone Family Member X (H2AFX)	99.8	97.1	99.2	2.14	ns

^a Least square means in the same row for class effect not followed by a common letter (a-c) differ significantly: $P < 0.05$.

^b Standard error of mean

^c Significances : ns: not significant; *: $P < 0.05$

Table 5. Multiple regression analyses of carcass characteristics and meat quality traits for Hay class using protein biomarkers and carcass characteristics.

Carcass quality parameters								Meat quality parameters										
#	Variables	HCW	EUROP CS	RW	LMW	MCW	FCW	WBSF	GT	JUIC	BF	IMF	pHu	L*	a*	b*	C*	h*
	R-squared	0.31**	0.41***	0.29**	0.33**	0.24*	0.40***	0.50***	0.46***	0.40***	0.22*	0.42***	0.52***	0.29*	0.26*	0.30*	0.20*	0.40***
6	αB-crystallin			-0.48**		-0.50*	-0.51***			-0.43*				-0.41*		-0.29*		
6	m-calpain	0.52**			0.61***			-0.47**	0.35*					0.50**			0.35*	
5	MyBP-H		-0.53***	-0.41*	-0.59***	-0.43*		0.37*										
3	SOD1						0.20*			-0.43**	-0.25*							
3	ICDH		-0.40**					0.34**					0.47***					
3	H2AFX			-0.31*			-0.46**											0.61**
3	TP53	-0.48*							0.57***			0.34*						
3	PGM1										-0.35*				-0.33*			0.35*
3	Fiber area							0.42**				-0.25*			-0.33*			
3	MyHC-IIx (%)												-0.24*		0.41*			-0.34*
3	Hsp40									0.75***							-0.37*	-0.51**
3	Hsp70-8								-0.32*		0.48*						-0.45*	
2	Hsp20					-0.35*								0.35*				
2	Hsp70-1A		-0.29*				0.25*											
2	LDH	0.38*			0.40*													
2	MyLC-1F			0.30*		0.34*												
1	Hsp27												-0.36**					
1	MyHC-IIx (AU)										-0.29*							
1	ENO3															0.54**		
1	Hsp70-Grp75																0.59*	
1	MyHC-IIa (%)	0.39*																
1	Hsp70-1B		0.69***															
1	Prdx6				-0.30*													
3	FCW								0.36**		0.18*	0.36**						
2	LMW												-0.28*			-0.57*		
2	MCW													-0.22*		0.50*		
2	HCW									0.30*					0.33*			

#: frequency of the entrance of the variables in the regression equations.

Abbreviations: WBSF: Warner-Bratzler Shear Force; GT: global tenderness; JUIC: juiciness; BF: beef flavor; IMF: intramuscular fat; L*: lightness; a*: redness; b*: yellowness; C*: Chroma; h*: hue angle; HCW: hot carcass weight; RW: ribeye weight; LMW: *Longissimus* muscle weight; MCW: muscle carcass weight; FCW: fat carcass weight.

Significances: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 6. Multiple regression analyses of carcass characteristics and meat quality traits for Grass class using protein biomarkers and carcass characteristics.

Carcass quality parameters								Meat quality parameters										
#	Variables	HCW	EUROP CS	RW	LMW	MCW	FCW	WBSF	GT	JUIC	BF	IMF	pHu	L*	a*	b*	C*	h*
	R-squared	0.49**	0.48**	0.52***	0.62***	0.59***	0.58***	0.50**	0.53***	0.56***	0.57***	0.80***	0.44**	0.42**	0.55***	0.43**	0.45**	0.52***
7	H2AFX		0.73***		0.40**		-0.43*				-0.47**		-0.49*			0.55**		0.67***
7	MyHC-IIx (AU)			-0.68***	-0.38*	-0.72***	-0.36*			-0.71**						0.64*	0.95**	
4	Hsp40			0.28*		0.31*	0.45**									-0.43*		
3	α-actin	0.83**						0.40*	0.27*									
3	Prdx6	-0.46*															0.89**	-0.37*
3	Hsp70-8					0.46*				0.76**					0.46**			
3	ICDH				0.53***			0.50**			-0.41*							
3	PGM1	-0.56**													-0.47**			0.49**
3	MyBP-H			0.47**	0.47**		0.63**											
3	μ-calpain												-0.60*	0.66**			0.28*	
3	Hsp27							0.45*							-0.36*	-0.71**		
2	Hsp20		-0.30*														-0.35*	
2	MyLC-1F										-0.61***	-0.16*						
2	Hsp70-Grp75									-0.58**			0.98***					
2	SOD1							0.47**							-0.31*			
2	MyHC-IIx (%)													-0.49*				-0.36*
2	MyHC-I (%)								0.51**				0.39*					
2	ENO3			0.35*		0.33*												
1	Fiber area		0.24*															
1	HSP70-1B		0.57**															
1	TP53	0.87***																
1	LDH																	
1	MyHC-IIa (%)									-0.87***				-0.28*				
3	RW								0.36*			-1.23***		-0.49**				
1	EUROP CS								-0.44**									
1	HCW										0.48**							
1	LMW											0.45**						
1	FCW											1.34***						

#: frequency of the entrance of the variables in the regression equations.
Abbreviations: **WBSF**: Warner-Bratzler Shear Force; **GT**: global tenderness; **JUIC**: juiciness; **BF**: beef flavor; **IMF**: intramuscular fat; **L***: lightness; **a***: redness; **b***: yellowness; **C***: Chroma; **h***: hue angle; **HCW**: hot carcass weight; **RW**: ribeye weight; **LMW**: *Longissimus* muscle weight; **MCW**: muscle carcass weight; **FCW**: fat carcass weight.
Significances: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 7. Multiple regression analyses of carcass characteristics and meat quality traits for Haylage class using protein biomarkers and carcass characteristics.

<i>Carcass quality parameters</i>								<i>Meat quality parameters</i>										
#	Variables	HCW	EUROP CS	RW	LMW	MCW	FCW	WBSF	GT	JUIC	BF	IMF	pHu	L*	a*	b*	C*	h*
	R-squared	0.30*	0.34**	0.38**	0.43**	0.37**	0.44**	0.33*	0.43**	0.44**	0.56***	0.69***	0.35**	0.46***	0.40**	0.53***	0.61***	0.35**
5	MyHC-IIx (AU)		0.36*								-0.23*				0.97***	0.81***	0.72***	
5	MyLC-1F	0.31*			-0.35*			-0.36*								0.26*	0.36**	
5	PGM1	0.36*		0.35*		0.40*					-0.58**							0.47*
4	Hsp40			-0.40*		-0.41*			0.44*					-0.46**				
4	PRDX6								0.44*	0.43**					0.34*		0.28*	
4	MyHC-I (%)	0.53*		0.37*	0.31*		0.50**											
3	α -actin	-0.54**						-0.34*		-0.53**								
3	TP53				-0.62***					-0.63***						0.32*		
3	HSP70-1A		-0.35*				0.45**						-0.30*					
3	ICDH		0.53**										0.47**		-0.36*			
3	Hsp27		-0.38*	0.55**		0.42**												
3	MyHC-IIa (%)														-0.49*	-0.78**	-0.80***	
2	ENO3				0.50**				-0.59**									
2	DJ1						-0.36*		-0.77***									
2	MyBP-H										0.31*							-0.44*
2	m-calpain										-0.33*			-0.31*				
1	SOD1						-0.29*											
1	MyHC-IIx (%)					-0.34*												
1	Hsp70-Grp75													0.47**				
1	μ -calpain											-0.38**						
1	α B-crystallin											0.48***						
1	Fiber area																	-0.20*
4	FCW											0.45***	-0.25*	0.40**				0.45**
3	MCW							0.48*		0.43**			0.37*					
2	LMW							-0.45*				-0.56***						

#: frequency of the entrance of the variables in the regression equations.

Abbreviations: **WBSF**: Warner-Bratzler Shear Force; **GT**: global tenderness; **JUIC**: juiciness; **BF**: beef flavor; **IMF**: intramuscular fat; **L***: lightness; **a***: redness; **b***: yellowness; **C***: Chroma; **h***: hue angle; **HCW**: hot carcass weight; **RW**: ribeye weight; **LMW**: *Longissimus* muscle weight; **MCW**: muscle carcass weight; **FCW**: fat carcass weight.

Significances: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

TOC

