UNDERSTANDING OF BEEF TENDERNESS VARIABILITY BASED ON THE CONTINUUM DATA USING CHEMOMETRICS: A PROOF-OF-CONCEPT STUDY

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I. INTRODUCTION

Beef tenderness is influenced by several intrinsic and extrinsic factors [1], which are measurable from the farmgate to meat levels. In the large literature, there is scarcity in studies that have evaluated simultaneously the effects of the continuum data, from farmgate to meat, on beef qualities. Accordingly, we hypothesized that considering this continuum would characterize sufficiently the main factors driving the desirable beef qualities [2]. This study deals with these aspects and intends to consider dozen variables of the continuum data by the means of chemometrics, *i.e.,* partial least squares (PLS) and principal component regressions (PCR), as easy and straightforward multivariate methods to relate the multi-dimensional nature of the factors, to beef tenderness. Thus, this trial provides a proof-of-concept concerning the statistical selection of the most influencing factors at different levels for accurate understanding of beef tenderness variability.

II. MATERIALS AND METHODS

Seventy one young bulls of three pure breeds; 21 Aberdeen Angus, 25 Limousin, and 25 Blond d'Aquitaine as detailed in Gagaoua *et al.* [3] were used. After slaughter (~15 min), *Longissimus thoracis* (LT) and *Semitendinosus* (ST) muscles were excised for laboratory analyses [3, 4]. The carcasses stored at ~3°C until 24 h *p-m* were characterized [5]. Then, samples were taken from both muscle cuts and aged for 14 days at ~3°C before sensory evaluation of steaks grilled at 55 °C. A total of 46 variables at 4 levels of the continuum were recorded. 1) Farmgate (q_X = 8): rearing factors by slaughter age (months), initial body weight before fattening (BW, kg), final BW (kg), dry matter intake (DMI, kg DM/day), forage and concentrate in percentage (in the DM diet), average daily gain (ADG) (kg/day), and feed efficiency (kg/kg DM). 2) Slaughterhouse (q_X = 8): carcass characterization by the weights in kg of the eviscerated animal, carcass and 6th rib. The dressing% was computed and the % of muscle and fat tissue carcass weights were estimated [5]. The carcasses were graded under the EUROP carcass grading scheme for carcass conformation and fatness scores [5]. 3) Muscle (q_X = 25): by enzyme activities [ICDH, PFK, COX and CS] and protein biomarkers quantification using Dot-Blot [heat shock proteins (αB-crystallin, Hsp20, Hsp27, Hsp40, Hsp70-1A/B, Hsp70-8, and Hsp70-Grp75); muscle fiber structure (α-actin, MyLC-1F, CapZ-β, MyBP-H, MyHC-I, -II and -IIx); metabolism (ENO3, LDH-B and MDH1); oxidative resistance (DJ-1, Prdx6 and SOD1) and proteolysis (μ-calpain)] as described in Picard *et al.* [4]. 4) Meat ($q_{Y.X}$ = 5): by sensory beef tenderness evaluated by a trained panel [4], color (*L*a*b**) and pHu traits [5]. For statistical analysis, a core model was first computed using multivariate regression analysis under SAS 9.4 [5] to consider the fixed effects of breed, replicate and their interaction for each variable in a stepwise manner. Then, projections to latent structures by means of PLS was used to examine how the set of explanatory variables (q_X = 45) was related to beef tenderness in each muscle. The filter method with the variable importance in the projection (VIP) was used for variable selection [6]. Thus, after the first model run including 45 X-variables, those with a VIP < 1 were eliminated. A second model run with the remaining variables was done. Subsequently, a PCR was performed on the retained variables for calibration and to identify the direction of the relationships between the X-variables and beef tenderness.

III. RESULTS AND DISCUSSION

Out of the 45 independent variables included in the PLS models, 31 (for LT) and 30 (for ST) had a VIP < 1.0 and were eliminated. This improved the explained variation (R^2X) and the powerful of link with tenderness (R^2Y). The PCR models explained 67 and 65% of tenderness variability of LT and ST muscles, respectively (Table 1). For LT and among the 14 retained variables, 3 were from the farmgate level, 1 from the slaughterhouse level and 10 were protein biomarkers. For ST, 15 variables were retained and 3 of them were rearing factors, 5 carcass characteristics and 7 protein biomarkers. Seven variables from the 3 levels were common for both muscles. Among them, 4 shared the same direction, namely dry matter intake, HSP20 and

Table 1. Ranking of the retained variables, according to their VIP, of the second PLS meat tenderness model in *Longissimus thoracis* (LT) and *Semitendinosus* (ST) muscles of young bulls. The regression coefficients (*β*) from PCR models of the variables belonging to each level of the continuum are given including the standard errors (SE).

Continuum data (LT muscle)	PLS (R^2= 55%)		PCR (R^2 = 67%)		Continuum data (ST muscle)	PLS (R^2= 49%)		PCR (R^2 = 65%)	
	Rank	VIP	*β*	SE		Rank	VIP	*β*	SE
Farmgate level: Rearing factors									
DMI, kg DM/day	4	1.69	***+0.23***	0.12	ADG, kg/d	1	2.30	-0.70	0.96
Initial body weight before fattening, kg	9	1.23	-0.03	0.13	Feed efficiency, kg/kg DM	3	1.85	+0.88	0.81
Forage, %	14	1.01	+0.25	0.15	***DMI, kg DM/day***	6	1.69	***+0.63***	0.47
Slaughterhouse level: carcass characteristics									
Conformation score, 1 – 15 scale	13	1.11	**+0.22**	0.22	Dressing, %	2	1.85	-0.21	0.15
					Muscle carcass, %	4	1.83	-0.34	0.59
					Fat carcass, %	5	1.75	+0.42	0.61
					Conformation score	7	1.44	**-0.24**	0.17
					Fatness score, 1 – 15 scale	11	1.29	+0.10	0.16
Muscle level: protein biomarkers									
HSP20, arbitrary units (AU)	2	1.87	***+0.25***	0.14	***HSP20, AU***	14	1.15	**+0.02**	0.13
HSP40, AU	11	1.16	**-0.17**	0.15	**HSP40, AU**	15	1.00	**+0.22**	0.14
HSP70-1B, AU	3	1.81	***-0.15***	0.20	***HSP70-1B, AU***	10	1.31	***-0.27***	0.13
HSP70-8, AU	5	1.62	-0.14	0.16	***MyBP-H, AU***	12	1.21	***+0.16***	0.14
Myosin Heavy Chain-I, AU	6	1.47	-0.18	0.17	MLC-1F, AU	13	1.17	+0.07	0.14
MyBP-H, AU	7	1.42	***+0.15***	0.14	α-actin, AU	8	1.37	-0.01	0.13
CapZ-β, AU	8	1.31	-0.33	0.14	**ENO3, AU**	9	1.36	**-0.10**	0.14
Citrate synthase (CS), µmol min^{-1} g^{-1}	1	2.65	+0.15	0.12					
ENO3, AU	10	1.20	**+0.11**	0.18					
PFK, µmol min^{-1} g^{-1}	12	1.13	-0.02	0.14					

The variables from the different levels of the continuum that are in **bold character** highlight those retained in the models of both muscles; and those in ***bold and italic character*** share the same direction (- or +) in the PCR models of the two muscles.

MyBP-H (all positively) and HSP70-1B (negatively). Based on the PCR regression coefficients and rank of all the independent variables in the PLS models, the most influential were protein biomarkers for LT muscle, and rearing factors and carcass characteristics for ST muscle. The statistical approach allowed efficient selection of the relevant explanatory variables from a large list (~15/45). It highlighted also the main drivers of tenderness that belong to different levels of the continuum by providing the positive and negative impacts. The results showed the involvement of different mechanisms in tenderness determinism of the two muscles.

IV. CONCLUSION

This trial highlighted the importance of studying the effects of multiple combined factors on beef tenderness rather than simply evaluating the links within each level or factor separately. We propose the implementation of this approach to explore accurately meta-data that come from several experiments and group thousand animals, to provide decision tools for beef sector seeking to manage beef tenderness variability.

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