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HEAT SHOCK PROTEIN DYNAMICS IN DARK-CUTTING BEEF: IMPLICATIONS IN THE PROCESS OF MEAT TENDERIZATION AND POTENTIAL EARLY BIOMARKERS OF MEAT QUALITY

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I. INTRODUCTION

In cattle, pre-slaughter stress induces oxidative stress, contributing to the development of dark, firm, and dry (DFD) beef, also known as dark-cutting beef. This condition is characterised by a high ultimate pH, an unappealing dark colour and reduced shelf life. It is well established that cellular stress after animal's slaughter induces the expression of Heat Shock Proteins (HSPs), molecular chaperones that are essential for maintaining cellular homeostasis and controlling apoptosis, which influences the meat tenderization process [1]. Under stress conditions, HSPs protect proteins from aggregation and can translocate to the Z-disk of sarcomeres where they may function as muscle structure stabilizers [2]. The aim of this study was to compare the expression levels of HSP27, HSP70, and HSP90 in the sarcoplasmic (soluble cytosolic components) and myofibrillar (which contains membrane, nuclear structures, cytoskeleton and contractile proteins) fractions of muscle from normal quality (CONTROL) and DFD beef and to evaluate their potential as biomarkers for early detection of DFD beef.

II. MATERIALS AND METHODS

The pH_{24} of the *Longissimus Thoracis et Lumborum* (LTL) muscle was monitored in 1133 carcasses from 'Asturiana de los Valles' young bulls slaughtered in commercial abattoirs across Asturias (Spain). Sixteen carcasses were classified as DFD (pH_{24}>6.2). Additionally, 16 carcasses with similar characteristics (weight, sex, age, origin) but with normal pH_{24} values (5.4 to 5.6) were selected as CONTROL samples. Muscle samples (10g) were collected from the LTL at 24 hours *post-mortem* (*pm*) and stored at -80°C. At 48 hours *pm*, a portion from the LTL between the 10th and 13th ribs was taken for beef quality assessment. The sarcoplasmic and myofibrillar protein fractions were extracted, and the expression of HSP27, HSP70, and HSP90 was analysed in both fractions by Western blot [3]. Additionally, beef toughness was evaluated at 3, 7 and 14 days *pm* using the Warner-Bratzler shear force (WBSF) test. Differences in HSPs expression between CONTROL and DFD samples were assessed using an independent samples t-test and the effects of sample type, *pm* time and their interaction on WBSF values were analysed using a general linear model (SPSS v.22).

III. RESULTS AND DISCUSSION

DFD beef exhibited an atypical tenderization pattern, with significantly lower WBSF values ($P < 0.01$) compared to CONTROL beef, which remained constant throughout the *pm* period (45.2 to 46.1 N, Table 1). In contrast, CONTROL beef showed a progressive decrease in WBSF values over time (from 79.3 to 56.8 N, $P < 0.01$), which indicates a normal meat tenderization process.

Table 1 – Effect of pH_{24} sample type (CONTROL vs DFD) and *post-mortem* (*pm*) time on meat toughness.

Variable	Time *pm*	CONTROL (5.4 ≤ pH_{24} ≤ 5.6)	DFD (pH_{24} ≥ 6.2)	SEM	P-value
WBSF (N)	3 days	79.3c	45.2	5.2	0.000
	7 days	65.7b	45.7	4.3	0.000
	14 days	56.8a	46.1	3.4	0.004

In columns, different letters indicate significant differences ($P < 0.05$) between *pm* times. SEM: standard error of the mean. WBSF: Warner-Bratzler shear force test.

At 24h *pm*, DFD beef showed significantly higher expression levels of HSP27 (4-fold, $P \leq 0.001$), HSP70 (1.7-fold, $P < 0.01$), and HSP90 (3-fold, $P < 0.001$) in the sarcoplasmic fraction compared to CONTROL beef (Table 2). It is known that several pre-slaughter stressors, such as dietary restriction, oxidative stress or heat stress, may trigger the production of HSPs, which could be the reason of their increased abundance in the sarcoplasmic fraction of DFD beef in this study. These findings align with previous results reported in the LTL muscle of Simmental crossbreed

bulls [1]. In contrast, the expression of HSPs in the myofibrillar fraction was significantly higher in CONTROL beef for HSP27 (1.9-fold, $P \leq 0.01$), HSP70 (2.5-fold, $P < 0.001$), and HSP90 (3-fold, $P < 0.005$).

Table 2 – Expression of Heat Shock Proteins in sarcoplasmic and myofibrillar fractions of CONTROL and DFD beef (mean±SEM in arbitrary units of optical density) at 24 *h post-mortem (pm)*.

Variable	Sarcoplasmic fraction				Myofibrillar fraction			
	CONTROL	DFD	SEM	P-value	CONTROL	DFD	SEM	P-value
HSP27	96.8	392.3	12.0	0.000	77.4	41.1	12.6	0.010
HSP70	89.7	150.1	19.9	0.008	76.1	29.9	7.3	0.000
HSP90	318.3	944.7	135.5	0.001	120.0	36.9	25.4	0.005

SEM: standard error of the mean

Under normal conditions, HSPs are mainly found free and unbound in the cytosol. However, in muscle tissues exposed to stress, HSPs can translocate and accumulate in damaged structures, such as the Z-disks, to maintain their stability and integrity [2]. Thus, after slaughter, HSPs may bind to myofibrils to protect cellular structures from damage, which could explain the higher levels of HSPs observed in the myofibrillar fraction of CONTROL beef. In contrast, most of the HSPs in DFD beef are retained in the sarcoplasmic fraction. These differences could be due to several factors that affect their diffusion capacity: 1) The compromised structural integrity of DFD beef, which begins a process of structural deterioration at early *pm* stages. This structural instability may facilitate the release of HSPs into the sarcoplasmic fraction during the process of sample preparation for protein extraction; 2) The previously mentioned higher synthesis of HSPs in DFD animals, likely due to their increased susceptibility to pre-slaughter stress; 3) A reduced efficiency of HSPs translocation from the sarcoplasmic to the myofibrillar fraction in the DFD muscle, possibly due to the higher pH_{24}. This is consistent with findings by Pulford et al. [5], who observed that small HSPs (HSP20 and HSP27) become more soluble as the cellular pH approaches 6, which is close to their isoelectric point, thereby increasing their solubility in the sarcoplasmic fraction.
These alterations in HSP localization in the muscle cell of DFD beef, particularly their reduced presence in the myofibrillar fraction, could lead to structural disorganization and increased degradation, making myofibrils become more vulnerable to cellular proteases. This weakens the myofibrillar lattice and accelerates muscle degradation, negatively affecting beef tenderization and contributing to the atypical soft texture of DFD beef.

IV. CONCLUSION

This study demonstrated that DFD beef exhibits an atypical *pm* tenderization profile and altered HSPs expression pattern at 24h *pm.* The significant overexpression of HSP27, HSP70, and HSP90 in the sarcoplasmic fraction, coupled with their reduced presence in the myofibrillar fraction, suggests impaired translocation of these proteins from cytosol to myofibrilar structures in DFD beef. This impairment likely reduces their protective role of the myofibrillar structures, leading to accelerated muscle degradation. Consequently, the overexpression of HSPs in the sarcoplasmic fraction at 24h *pm* may serve as potential biomarker for the early identification of DFD beef.

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