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BIOCHEMICAL CHARACTERIZATION OF MEAT OF CALABRESE × LARGE WHITE PIG REARED IN OUTDOOR OR INDOOR HOUSING SYSTEM: PRELIMINARY RESULTS

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ABSTRACT

This work reports some results concerning protein and lipid profiles of pork from 12 subjects of Calabrese Ancient Local Genetic Type (ALGT) × Large White (LW) crosses reared in outdoor or indoor housing system. Aim of this paper is to examine the effect of two housing systems on protein and lipid profiles. Analysis of the proteome of the water soluble fraction (sarcoplasmic) was carried out on meat samples obtained from muscle *Caput Longum Tricipitis Brachii* (CloTB). The analysis covered a total of 12 samples and 24 two-dimensional gel electrophoresis maps (2 for each sample), which were digitized for subsequent image analysis and used for spot identification by MALDI-TOF mass spectrometry. The comparison of 24 two-dimensional maps was performed by Image Master 2D-Platinum software in order to establish the relative intensity, expressed as vol%, of each spot for each gel. Image analysis produced 12 common spots in all maps analyzed. The housing system did not influence the relative abundance (vol%) of 12 spots detected. These proteins, identified by peptide mass fingerprint, are grouped according to their biological function as metabolic, cellular defense and transport protein. Inter and intra-muscular fatty acid profiles of CloTB was determined by gas-chromatography. No significant differences for the fatty acids content were observed between the two housing systems suggesting that intrinsic factors, such as the genetic asset, may be more important than extrinsic factors.

Key words: fatty acids / MALDI-TOF fingerprint / Sarcoplasmic proteins / ancient local genetic type / housing system

1 INTRODUCTION

To safeguard and valorise the animal farm biodiversity, especially the Ancient Local Genetic Types (ALGT) inserted in the microbiosphere of a particular geographical area, the application of the connected strategies of the 'omic' science [genomics, epigenomics, proteomics, metabolomics (lipidomics, glycomics, etc.)] allows to increase the knowledge of ALGT and gives semantic elements for the improvement of the quality and safety of food according to an innovative vision identified as 'geography of the health.' Proteomic analysis defines the identity, the structure and the relative abundance of proteins in a given cell type in a specific set of conditions and it contributes to understand better the factors that affect the muscular development and function, muscular *ante* and *post-mortem* metabolism as well as meat quality (Bouley *et al.*, 2004; Bendixen, 20005; Lametsch *et al.*, 2006). Proteomic studies identified levels of proteins which change according to some meat properties (colour, drip loss and shear force), different rearing environments and "race" (Hwang *et al.,* 2004; Sayd *et al.*, 2006; Kwasiborski *et al.*, 2009). Previous studies on pigs (Matassino *et al.*, 2010) evidenced the influence of the breed on protein expression as well as the different metabolic activity between pigs differing in coat color (white or black); in fact, they use glycolytic and oxidative pathway in a different way. These differences could represent sources of molecular characterization for ancient and modern breed or crossbreed traceability. The characterization of fat,

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in particular the intramuscular fat, can also contribute to define the nutritional and extranutritional quality of the meat (Matassino *et al.* 2010). This quality depends by intrinsic genetic and epigenetic factors (Matassino *et al.*, 2011) and by extrinsic factors, in particular animal's diet.

2 MATERIAL AND METHODS

The analyses were carried out on 12 samples of CloTB muscle from 12 different Calabrese × Large White subjects. The pigs, reared according to two different housing systems, were fed with the same formulation until 30 kg (on average) live weight and with another formulation until slaughter (on average at 116 kg). The available area per head was equal to 4.6 m^2 and 45 m^2 for indoor (ID) and outdoor (OD) system, respectively. The slaughter was carried out in a single establishment and the carcass maturation was carried out in refrigerator at a temperature of 2–4 °C for a period of about 72 hours.

2.1 PROTEIN PROFILE

Soluble muscle protein fraction was extracted with phosphate buffer 10mM at pH 7.0. The first dimension (IEF-IPG) was carried out by Ettan IPGphor II (GE Healthcare) using Immobiline DryStrips gel pH 3-10NL (18 cm) rehydrated with a solution of 8 M Urea, 0.5% CHAPS, 0.2% DTT, 0.5% IPG Buffer. The second dimension was carried out in accordance with the procedure of O'Farrell (1975) in polyacrylamide gradient gel electrophoresis (T = 9–18% and C = 2.5%) by using Ettan Twelve System (GE Healthcare). Two gels were produced per sample giving 24 gels in total. The gels were stained with Coomassie Brilliant Blue G250 and image analysis of two-dimensional maps (2-DGEm) was performed by software Image Master 2D-Platinum (GE Healthcare) quantifying in volume % (vol%) the expression level of each spot. For one spot, the mean of two values (corresponding to the gels in duplicate) was calculated. Data relative to 12 common spots of samples were elaborated by ANCOVA (package SPSS, version 15.0) before correction for live weight after a feed fasting period of 12–24h and refrigerated carcass weight. The spots were digested *in situ* with trypsin according to the procedure of Shevchenko *et al.* (1996) and tryptic digests were analyzed with Ettan MALDI-Tof/PRO mass spectrometer (GE Healthcare).

Table 1: *Spots identified by MALDI-Tof mass spectrometry: comparison between housing systems*

Spot, N	Protein identity	Indor vs Outdoor
554	DJ-1	n.s.
553		
648	Thioredoxin	n.s.
527	Adenylate kinase	n.s.
538		n.s.
398	Enolase	n.s.
505	Triosephosphate isomerase	n.s.
638	H-FABP	n.s.
645	Haemoglobin β-chain	n.s.
614	Myoglobin	n.s.
620		
616		

2.2 LIPIDIC PROFILE

Lipids were extracted by Folch method (Folch *et al.* 1957); subsequently, lipid fraction was trans-esterificated in hexane with a solution of KOH in methanol (0.1N). Fatty acid methyl esters were separated by GC-FID (Thermo Electron Corporation).

Covariance analysis was carried out on dry matter (dm) data (data corrected for live weight after a fasting period of 12–24h and refrigerated carcass weight). Comparison between housing systems was performed for singular fatty acid, SFA (saturated fatty acids), UFA (unsatu-

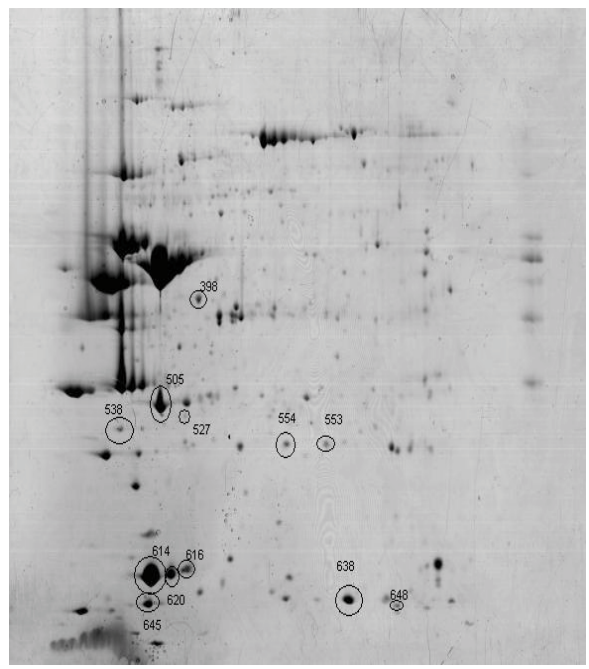

Figure 1: *Representative gel image of soluble protein fraction of CloTB muscle*

rated fatty acids); MUFA (monounsaturated fatty acids) and PUFA (polyunsaturated fatty acids).

3 RESULTS AND DISCUSSION

3.1 PROTEIN PROFILE

Table 1 shows 12 common proteins of examined subjects and the significance of comparisons between housing systems. Figure 1 reports a two-dimensional map of sarcoplasmic proteins identified. Proteins can be grouped according to their biological function and some proteins present different isoforms (Adenylate kinase, DJ-1 and Myoglobin). Enolase, triosephosphate isomerase and adenylate kinase are involved in energy metabolism; H-FABP haemoglobin beta chain, myoglobin are transport proteins; DJ-1 and thioredoxin have antioxidant activity. The housing system did not influence the relative abundance (vol%) of proteins.

3.2 LIPID PROFILE

The effect of housing system on fatty acids profile of ClotB muscle is reported in Table 2. It shows that palmitic acid content (C16:0), the most abundant SFA, was 26.4% and 26.2% for ID and OD system, respectively. Total MUFA content was equal to 43,3% and 44,4% for ID and OD system, respectively. Among MUFAs, the oleic content had the highest value for both indoor (35.9%) and outdoor (37.3%) housing. PUFA content was higher in indoor (13.3%) than outdoor housing system (12.1%). Between housing systems no significant effect was observed for fatty acids SFA,UFA, MUFA and PUFA content. Serrano *et al.* (2013) reported that barrows and gilts responded slightly different to space allocation in respect

Table 2 *Effect of housing systems on fatty acids profile of ClotB muscle*

	Housing system						
	Indoor			Outdoor			ID *vs* OD
Fatty acid	Value	σ	c.v. (%)	Value	σ	c.v. (%)	P
C12:0	0.12	0.02	17.74	0.11	0.00	3.17	0.98
C14:0	1.77	0.28	15.90	1.71	0.05	2.76	0.94
C16:0	26.37	1.97	7.48	26.22	0.71	2.69	0.95
C17:0	0.28	0.06	19.91	0.25	0.05	19.98	0.32
C18:0	11.75	0.85	7.20	12.17	0.51	4.17	0.43
C20:0	0.11	0.03	26.50	0.11	0.01	7.78	0.85
SFA	40.39	3.21	94.73	40.58	1.32	40.55	0.74
C14:1	0.10	0.03	26.20	0.10	0.06	63.41	0.95
C16:1	3.16	0.72	22.71	2.94	0.28	9.66	0.83
C18:1 (*n-9 trans*)	0.09	0.01	7.59	0.10	0.01	10.44	0.21
C18:1 (*n-9 cis*)	35.87	1.99	5.55	37.29	1.36	3.65	0.42
C18:1 (*n-12*)	3.70	0.40	10.85	3.69	0.16	4.45	0.19
C20:1	0.31	0.05	16.22	0.28	0.05	16.35	0.17
C22:1	0.05	0.01	23.06	0.05	0.01	18.25	0.39
MUFA	43.28	1.39	3.21	44.45	0.98	2.20	0.22
C18:2 (*n-6*)	10.75	1.33	12.36	10.04	1.03	10.30	0.67
C18:3 (*n-6*)	0.05	0.01	22.72	0.04	0.00	10.77	0.36
C18:3 (*n-3*)	0.59	0.13	22.46	0.63	0.03	5.15	0.86
C20:2	0.35	0.07	18.81	0.35	0.03	8.55	0.69
C20:3 (*n-6*)	0.17	0.04	25.17	0.15	0.04	25.13	0.92
C20:4 (*n-6*)	1.15	0.51	44.58	0.89	0.31	35.29	0.60
UFA	56,34	1.62	2.87	56.55	1.56	2.75	0.64
PUFA	13.33	1.89	14.19	12.10	1.35	11.14	0.48

to feed intake and unsaturation of backfat and they observed that MUFA content decreased with increased space allowance.

4 CONCLUSIONS

Within the observation field both proteomic and lipidomic approach did not show statistically significant differences; this means that intrinsic factors, such as the genetic asset, is more important than extrinsic factors, nevertheless, it is necessary to increase the number of subjects to confirm our results, especially in relation to the gender.

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