

Effect of sex and livestock production system on free amino acids content on LD muscle from “Galician mountain” foals

*Lorenzo, J.M., Purriños, L., Garcia-Fontan, M.C., Bermudez, R. and Franco, D.

¹ Meat Technology Centre of Galicia, Rúa Galicia N°4-Parque Tecnológico de Galicia, San Cibrao das Viñas, 32900 Ourense, Spain

*Corresponding author to provide phone: 0034988548277, fax: 0034988548277, e-mail: jmlorenzo@ceteca.net

Abstract- The effects of sex and livestock production system were studied on the content of free amino acids in *Longissimus dorsi* muscle from “Galician mountain” foals. A total of 33 foals of the “Galician Mountain” crossbreed were used in this study. Sex factor had no significant effect ($P>0.05$) in content of FAA, whereas livestock production system had significant effect ($P<0.05$) on content of glycine, taurine and hydroxiprolin. Males showed lower values of total FAA than females and in foals from semi extensive system we observed higher values than foals from freedom extensive system. Arginine was the most abundant FAA found in foal meat, followed by taurine, alanine and histidine.

Keywords: Amino acids, Foals, Livestock production system

I. INTRODUCTION

Meat quality is affected by many physical and (bio)chemical changes involved during the *post-mortem* conversion of muscle to meat (Valin, & Ouali, 1992). Muscle enzymes are responsible for most of these changes and muscle aminopeptidases contribute to the generation of free amino acids (FAA) in *post-mortem* which improve the nutritional value and can affect flavour as taste enhancers or precursors of aroma compounds. Studies of FAA and peptides in meat have focused mainly on the effects of fibre type (Aristoy, & Toldrà 1998; Cornet, & Bousset 1999), animal species (Nishimura, Rhue, Okitani, & Kato, 1988) and *post-mortem* conditioning (Feidt, Petit, Bruas-Reignier, & Brun-Bellut, 1996). These studies concluded that FAA and peptides could partly explain the differences in the taste of meat. Measurement of FFA can give some indication of the proteolytic activity within a tissue (Mullen, Stoeva, Laib, Gruebler, Voelter, & Troy, 2000).

The release of certain amino acids can have an influence on meat quality attributes such as drip loss, water holding capacity and the development of flavour (Rossi, Denoyer, & Berdagué, 1995). The nutritive quality can be defined as the one that assures the presence of nutritive components (proteins, essential amino acids, essential fatty acids, minerals, vitamins, and microelement) and their sufficient bio-availability. Nowadays, consumers want to know more about the nutritive quality of food, and in the case of meat, this depends on the nitrogenous components, basically protein of high biological value, FAA, dipeptides and other non-protein nitrogenous compounds (NPN).

Therefore the aim of this study was to assess the effect of management system (semi extensive system vs. freedom extensive system) and sex on content of free amino acids from LD of “Galician Mountain” foals.

II. MATERIALS AND METHODS

II.1. Experimental design and animal management

For this study, thirty-three foals of the “Galician Mountain” crossbreed were used. Eleven animals (6 females and 5 males) were obtained from experimental herd of Agricultural Research Centre of Mabegondo. Animals were together in a single group, with their mothers, at pasture until three months prior to slaughter. The last three months foals were finished in indoors. The concentrate ration consisted of hay silage *ad libitum* and three kilograms of concentrate (Eco-Feed[®]) per head per day. Animals belong to this herd were denominated as semi extensive system (SES).

The others twenty-three foals (11 females and 12 males) were obtained from Monte Cabalar (agricultural cooperative of “Galician Mountain”) located in a mountain (A Estrada, Spain). All foals were reared with their mothers in an extensive system in freedom regimen, according to an extensive production system on wood pasture. Animals belong to this herd were denominated as freedom extensive system (FES). All animals of this study were slaughtered at nine months years old.

II.2. Sample collection

Animals were conventionally slaughtered at a commercial abattoir (Council Directive of the European Union 95/221EC). Immediately after slaughter, carcasses were weighed and chilled at 4 °C in a cold chamber for 24 h. At this point, the *Longissimus dorsi* (LD) muscle was extracted from the left half of each carcass, between the fifth and the tenth rib. Samples were taken immediately to the laboratory under refrigerated conditions. Samples were packed under vacuum conditions (99%) (FRIMAQ, V-900, Spain) and were stored at 4 °C for 4 days.

II.3. Analytical methods

II.3.1. Chemicals and chromatographic instrumentation

AccQ.Fluor reagent kit (AQC, borate buffer) and AccQ.Tag Eluent A concentrate were acquired from Waters (Milford, MA, USA). Acetonitrile (MeCN), disodium ethylenediaminetetraacetic acid (EDTA), phosphoric acid, sodium acetate trihydrate, and sodium azide were from Baker (Phillipsburg, PA, USA); triethylamine (TEA) was purchased from Aldrich (Milwaukee, WI, USA). Amino acid standards, taurine and hydroxyproline were from Sigma (St. Louis, MO, USA).

HPLC systems used were a Waters system Alliance 2695 with a 2475 scanning fluorescence detector. Empower 2 software (Waters, Milford, MA, USA) was used to control system operation and results management.

II.3.2. Derivatization of standards and samples and chromatographic analysis

10 µl of sample was buffered to pH 8.8 (AccQ.Fluor borate buffer) to yield a total volume of 100 µl. Derivatization was initiated by the addition of 20 µl of AccQ-Fluor reagent (3 mg/ml in MeCN). Reaction of the AQC with all primary and secondary amines was rapid and excess reagent was hydrolyzed within 1 min. Completion of hydrolysis of any tyrosine phenol modification was accelerated by heating for 10 min at 55°C.

Separations were carried out using a Water AccQ-Tag column (3.9 mm x 150 mm with a 4 µm of particle size) with a flow-rate of 1.0 mlmin⁻¹ and performed at 37 °C. The gradient profile and composition of the mobile phase was adapted from methodology developed by Vandelen and Cohen, (1997). Detection was accomplished by fluorescence with excitation at 250 nm and emission at 395 nm.

Amino acids were identified by retention time using an amino acid standard to which taurine and hydroxiprolin were added.

II.4. Statistical analysis

For the statistical analysis of the results, data were analyzed using the SPSS (version 15.0, USA). One-way analysis of variance (ANOVA) was used to analyze the effect of sex and slaughtered age on FAA content studied in the work. The least squares mean (LSM) were separated using Duncan's t-test. All statistical test of LSM were performed for a significance level $\alpha < 0.05$.

III. RESULTS AND DISCUSSION

The content of FAA is show in Table 1. Sex factor had no significant effect ($P > 0.05$) in content of FAA, whereas livestock production system had significant effect ($P < 0.05$) on content of glycine, taurine and hydroxiprolin.

Table 1. Content of free amino acids of *Longissimus dorsi* muscle from “Galician Mountain” foals. Effect of sex and livestock production system

	Sex		Livestock production system		Significance		SEM
	Males	Females	SES	FES	LPS	Sex	
Animales	(n=16)	(n=15)	(n=9)	(n=22)			
Chemical Composition							
Water	76.07	76.42	75.43	76.53	**	n.s.	0.18
Protein	21.35	20.94	20.61	21.32	*	n.s.	0.14
Amino acids							
Histidine	12.89±3.64	14.53±9.65	17.88±9.68	11.90±5.64	n.s.	n.s.	1.57
Isoleucine	1.65±0.94	1.91±0.74	1.87±0.44	1.76±0.96	n.s.	n.s.	0.19
Leucine	2.83±1.79	3.15±1.42	2.90±0.76	3.06±1.84	n.s.	n.s.	0.36
Lysine	3.26±1.43	3.89±1.80	4.19±1.90	3.35±1.49	n.s.	n.s.	0.37
Methionine	1.78±0.70	1.83±0.65	1.53±0.28	1.93±0.75	n.s.	n.s.	0.15
Phenylalanine	1.75±1.19	1.88±0.89	1.78±0.42	1.84±1.21	n.s.	n.s.	0.24
Valine	2.25±1.11	2.54±0.91	2.40±0.67	2.42±1.13	n.s.	n.s.	0.23
Arginine	215.30±61.98	254.38±61.85	257.42±63.16	227.66±63.65	n.s.	n.s.	13.90
Alanine	16.07±4.00	17.24±7.57	18.46±7.20	15.91±5.67	n.s.	n.s.	1.37
Aspartic acid	0.25±0.19	0.31±0.24	0.41±0.24	0.22±0.18	n.s.	n.s.	0.04
Glutamic acid	2.92±1.41	3.25±2.08	3.70±1.69	2.83±1.81	n.s.	n.s.	0.40
Glycine	6.27±2.66	7.51±2.73	8.84±2.62	6.09±2.33	*	n.s.	0.53
Proline	2.61±1.22	2.92±1.04	2.74±0.75	2.80±1.27	n.s.	n.s.	0.26
Serine	2.88±1.88	4.19±2.51	4.20±2.84	3.34±2.05	n.s.	n.s.	0.49
Tyrosine	1.83±1.02	2.19±0.90	2.32±0.50	1.89±1.09	n.s.	n.s.	0.21
Taurine	19.69±9.72	22.90±13.48	29.97±15.45	17.50±7.31	*	n.s.	2.31
Hydroxiprolin	0.28±0.14	0.35±0.33	0.57±0.35	0.21±0.05	***	n.s.	0.04

Results expressed as mg/100 g muscle

Significance: *** ($P < 0.001$), ** ($P < 0.01$), * ($P < 0.05$), n.s. = $P \geq 0.05$

SES: Semi extensive system

FES: Freedom extensive system

LPS: Livestock production system

SEM: Standard error of mean

The total FAA fell in a range of values between 294 and 360 mg FFA/100 grams of fresh meat. Males showed lower values of total FFA than females and in foals from SES we observed higher values than foals from FES.

Arginine was the main FAA found in foal meat, followed by taurine, alanine and histidine. Arginine represented about 74% of total FAA. Taurine and β -alanine are non-protein compounds and thus their contents will not increase when muscle is transformed to meat (Cornet, & Bousset, 1999).

Histidine was the most abundant FAA within non-essential FAA fraction represented about 4% of total FAA. We found slight higher values of taurine in all samples studied. Taurine, play an important biological role because accumulation of taurine is necessary to regulate the function of the eyes, heart, brain and central nervous system (Schuller-Levis, & Park, 2003). Taurine required by the human body is synthesized from the dietary sulphur amino acids methionine and cysteine; a baby's metabolism is not able to do this. Taurine is also highly recommended for heart diseases like congestive heart failure, which occurs when the cardiac muscle cannot pump the blood efficiently enough (Azuma, Takihara, & Awata, 1985).

Foals from FES showed lower values of glycine and taurine (6.09 vs. 8.84 and 17.50 vs. 29.97, respectively). However, in foals from FES we observed lower values of hydroxiprolin (0.21 vs. 0.57). This result agrees with those reported in a previous study (data not published) where foals from SES showed higher values of collagen (1.10 vs. 1.07%) than foals from FES.

IV. CONCLUSIONS

Sex factor had no significant effect ($P>0.05$) in content of FAA, whereas livestock production system had significant effect ($P<0.05$) on content of glycine, taurine and hydroxiprolin. Arginine was the main FAA found in foal meat, followed by taurine, alanine and histidine.

ACKNOWLEDGEMENTS

Authors are grateful to FEADER (Project 2007-03) for the financial support. Special thanks to CIAM and Monte Cabalar for the foal samples supplied for this research.

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