CHARACTERIZATION OF RETAIL HORSE-MEAT IN NORTHERN SPAIN: CHEMICAL COMPOSITION AND FATTY ACID PROFILE

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Abstract – Horse-meat survey was performed in six northern Spanish regions in order to characterize its nutritional quality (chemical and fatty acid (FA) composition). Samples were collected in spring (n=41) and winter (n=41) of 2013. Regional effect was significant for muscle fat content. Higher fat content was associated with higher mono-unsaturated FA and lower polyunsaturated FA (PUFA). The total accumulation of conjugated linoleic acids, non-conjugated dienes and total *trans*-FAs was lower in these monogastric herbivores compared to ruminants. Differences in the long-chain PUFA content of horse-meat between seasons appear to be associated with the type of feeding and genetic differences of horses between specific regions.

Key Words – long chain PUFA, n-3, *trans*-FA.

I. INTRODUCTION

Horse-meat consumption has been associated with the prehistoric human. Horse tissues might have been a valuable source of essential fatty acids (FA) for the Upper Paleolithic people, time where plants and marine foods availability were minimal due to recurrent glaciations [1]. The horse, as a monogastric herbivore, has been shown to have the ability to transfer quite efficiently polyunsaturated fatty acids (PUFA) from diet into meat [2]. In general, its FA profile has been defined as 'healthy' due to its high content in essential and other long-chain (LC)-PUFAs [3-5]. Due to its characteristic fat metabolism [6], it has been observed that horse fat contains higher linoleic (18:2n-6) and linolenic (18:3n-3) acid contents [2, 6], but less conjugated linoleic acids (CLA) [7] in comparison to meats from ruminant species. Moreover, it has been observed that a thorough characterization of horse tissue is lacking in the literature, especially as it relates to the identification and quantification of minor fatty acids. Therefore, the objective of the present study was to characterize the chemical and fatty acid composition of northern Spanish horse-meat available at retail level.

II. MATERIALS AND METHODS

The survey consisted of collecting horse-meat (loin steaks; n=82) from butcher-shops and large grocery stores in six northern Spanish regions, at both sides of the Cantabric Mountains (Basque Country, Navarra, Cantabria, Asturias, Galicia, Castilla y León). Horse-meat was collected in the spring (n=41; 5-7 samples per region) from animals that would likely have entered the feedlot in the fall, while animals slaughtered in early winter (n=41) would likely have suckled their mothers and grazed in mountain areas from spring to late autumn.

Normalized procedures were used for dry matter [8], crude protein [9], ether extract [10] and ash determinations [11] of *Longissimus thoracis et lumborum* (LTL) muscle. Lipids were extracted from 1.5 g of freeze-dried LTL using chloroform-methanol (2:1, v/v) [12]. Lipid aliquots (10 mg) from each steak were methylated separately using acid (methanolic HCl) and base (sodium methoxide) reagents [13]. For quantitative purposes, internal standards (13:0 and 23:0) were added. Fatty acid methyl esters (FAME) were analyzed by GC/FID using a 100 m SP2560 column [14]. To determine CLA isomers and other co-eluting FAMEs, samples were subjected to a second GC/FID analysis using a 100 m ionic liquid SLB-IL111 column [15]. For identification, several reference standards (NuCheck Prep Inc., Supelco, Matreya and Larodan), fractions of FAMEs obtained by Ag^+- solid phase extraction

[14, 16], and retention times and elution orders reported in the literature [17-22] were used. Statistical analysis was carried out using IBM SPSS Statistics 22 for Windows.

III. RESULTS AND DISCUSSION

The chemical composition of LTL is presented in Table 1. Interaction was significant for moisture and season effect was significant for ash content where percentages were highest in samples collected in spring. The ether extract was significantly affected by region and differences were primarily associated with the diversity in local breeds, sex, age [23, 24], or production system [25].

Table 1 Effect of season on the chemical composition (%) of horse-meat samples (n = 41/season).

Composition	Season		SEM	*P value*		
	Spring	Winter		S	R	S*R
Moisture	73.9	72.9	0.181	0.007	0.068	0.045
Crude Protein	23.2	23.7	0.181	0.427	0.935	0.131
Ether extract	1.99	1.97	0.136	0.832	0.001	0.764
Ash	1.63	1.31	0.0350	0.000	0.376	0.380

SEM: standard error of the mean; S: season; R: region; S*R; season x region interaction.

Nutritionally interesting individual, group and ratios of FAs (mg/100g of fresh meat) are represented in Table 2. On absolute basis, no season effect was found for the total FAME (average of 1981mg/100g of meat), while in general, only few differences were found. Docosapentaenoic acid (22:5n-3) was significantly higher in samples collected in winter compared to spring samples.

Table 3 includes the percentage of several representative individual and groups of FAs. In percentages, season effect was not significant in total saturated (SFA) or branched-chain FAs. Palmitic (16:0, 25.7%) and stearic (18:0, 6.15%) acids were the most abundant SFAs. Total monounsaturated FA (MUFA) content (%) was significantly higher in samples collected in spring (35.5%) than in winter (32.3%) and this was influenced by both the major *cis*- and *trans*-MUFAs. The total *cis*-MUFAs content tended to be higher in spring, but the 9*c*-18:1 content (%) was significantly higher in spring than in winter. Elaidic acid (9*t*-18:1) was the major *trans*-18:1 isomer in horse-meat followed by vaccenic acid (11*t*-18:1), and both of them were significantly higher in samples collected in spring. The composition of *trans*-18:1 isomer is very different in ruminants where 11*t*- or 10*t*-18:1 predominate depending on diet.

Table 2 Effect of season on the FA composition (mg/100g) of horse-meat samples (n = 41/season).

FAME	Season		SEM	*P value*
	Spring	Winter		
Total FAME	1956	2005	114.8	0.864
SFA	716.9	745.5	45.51	0.818
BCFA	7.322	6.875	0.3557	0.592
MUFA	731.6	733.2	59.92	0.950
trans-MUFA	4.944	4.469	0.2246	0.207
cis-MUFA	726.6	728.7	59.71	0.953
PUFA	418.3	457.2	15.27	0.191
n-6	280.6	299.7	10.11	0.286
18:2n-6	243.2	259.4	9.406	0.331
20:4n-6	23.67	25.38	0.6681	0.167
n-3	131.7	151.6	10.76	0.387
18:3n-3	101.9	114.8	9.606	0.536
22:5n-3	12.82	15.56	0.5061	0.003
CLA	1.852	1.611	0.1224	0.278
trans-FA	7.534	7.084	0.3192	0.420

SEM: standard error of the mean; SFA: saturated FA; BCFA: branched-chain FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; CLA: conjugated linoleic acid; NC-: no conjugated; *trans*-FA: all FA with at least one double bond in *trans* configuration.

The PUFA content (%) was significantly higher in samples collected in winter (26.9%) than in spring (23.7%). Significant differences were also observed between collection regions ($P<0.001$, data not reported). Amongst regions, the higher fat content was generally associated with higher MUFA and lower PUFA contents. Significant differences were found for some PUFA between seasons particularly for 20:3n-6, while all LC-PUFAs in the n-3 group (20:5, 22:5 and 22:6n-3) were significantly higher in winter compared to spring collection. Consequently, significantly higher P/S values were observed. Accumulation of n-3 PUFAs in horse tissues was reported in animals fed under extensive conditions (grass feeding) [1, 5], which agrees with our finding of higher n-3 PUFA levels in samples collected in

early winter from animals that presumably grazed in mountain areas till late fall.

The CLA content of horse-meat was low, and highest in samples collected in spring. Rumenic acid (9*c*,11*t*-18:2) was the major isomer which represented 42% of the total CLA. The total accumulation of non-conjugated dienes and *trans*-FAs was also low compared to ruminants. In horses, fermentation of PUFA and absorption of their metabolites is limited and occurs in the post-absorptive region of the gut [26, 27].

Table 3 Effect of season on the FA composition (%) of horse-meat samples (n = 41/season).

FAME	Season		SEM	*P value*
	Spring	Winter		
SFA	37.0	36.6	0.228	0.243
16:0	26.0	25.4	0.289	0.244
18:0	6.21	6.09	0.154	0.595
BCFA	0.405	0.396	0.0170	0.972
MUFA	35.5	32.3	0.975	0.049
trans-MUFA	0.267	0.236	0.00564	0.001
9*t*-18:1	0.0847	0.0706	0.00240	0.000
11*t*-18:1	0.0298	0.0249	0.00115	0.016
cis-MUFA	35.2	32.1	0.977	0.052
9*c*-16:1	5.54	5.56	0.233	0.996
9*c*-18:1	25.8	22.9	0.755	0.022
PUFA	23.7	26.9	0.930	0.034
n-6	15.8	17.2	0.512	0.122
18:2n-6	13.5	14.7	0.405	0.109
20:2n-6	0.245	0.250	0.00764	0.766
20:3n-6	0.368	0.454	0.0236	0.039
20:4n-6	1.44	1.61	0.0865	0.306
n-3	7.64	9.35	0.593	0.088
18:3n-3	5.78	6.87	0.481	0.179
20:5n-3	0.317	0.491	0.0374	0.005
22:5n-3	0.804	1.06	0.0633	0.014
22:6n-3	0.219	0.290	0.0191	0.020
CLA	0.0932	0.0768	0.00303	0.003
NC-dienes	0.0810	0.0776	0.00224	0.372
Trienes	0.109	0.124	0.00608	0.136
trans-FA	0.410	0.386	0.0100	0.148
P/S	0.640	0.739	0.0283	0.028
n-6/n-3	3.03	2.86	0.239	0.673

SEM: standard error of the mean; SFA: saturated FA; BCFA: branched-chain FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; CLA: conjugated linoleic acid; NC-: no conjugated; *trans*-FA: all FA with at least one double bond in *trans* configuration.

IV. CONCLUSION

Overall, horse-meat is characterized by low total fat content and a relatively good source of n-3 PUFAs. Differences in the LC n-3 PUFA content of horse-meat between seasons appears to be associated with the type of feeding and genetic differences in horses between specific regions.

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