CHARACTERIZATION OF BEEF FATTY ACID PROFILE BY MULTIVARIATE ANALYSIS

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Abstract – Eleven parameters (ether extract, EE; SFA; MUFA; PUFA; n-3; n-6; PUFA/SFA, n-6/ n-3, h/H ratios; AI, TI indices) obtained from fat content and fatty acids profile of *longissimus thoracis* muscle of Piemontese (P), Friesian (F) and Limousin (L) breeds were considered. The data were analysed by hierarchical cluster analysis (HCA), principal component analysis (PCA) and canonical discriminant analysis (CDA). HCA produced three clusters. The first cluster was characterized by L animals (6/11), the second by P (8/10) the third by F (6/10); EE, SFA, MUFA contents, PUFA/SFA ratio, AI and h/H indices significantly differed between breeds. PCA showed that PC1 differentiates between fatty and lean meat; F had a higher EE, SFA and MUFA content and also unfavourables AI and TI indices; P beef showed a better PUFA/SFA and h/H ratios and an unfavourable n-6/n-3 ratio. CDA showed that MUFA, EE, SFA and PUFA/SFA, TI index, n-3, AI and h/H ratio were the most discriminating variables. The 80.6% of grouped cases were correctly classified; function 2 was able to distinguish P and L groups; P group has a better PUFA/SFA ratio, while L had a better TI and a higher n-3 content.

Key Words – Cattle breeds, nutritional quality, classification methods.

I. INTRODUCTION

Beef is often perceived as detrimental to health because of fat content and fatty acid composition. Saturated fatty acids (SFA) are harmful to health, as they tend to raise LDL-cholesterol level, while monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) tend to decrease it. The Department of Health of England recommends a PUFA/SFA ratio of at least 0.45 [1].

On the other hand, n-3 and n-6 are two groups of fatty acids with important functions in the organism. The n-3 can reduce LDL-cholesterol level in blood serum and may decrease blood pressure, hence preventing coronary heart disease and arteriosclerosis. Moreover, n-3 fatty acids decrease the risk of arrhythmia and thrombosis. n-6 fatty acids act completely in reverse. They promote inflammation, blood clotting and tumour growth. So a correct balance between n-6 and n-3 fatty acids is important. The Department of Health of England recommends 4.0 as the maximum ratio of n-6/n-3 fatty acids [1].

In order to obtain an overview of the relationships among different variables and group samples with homogeneous characteristics, three multivariate methods were used to analyse some parameters obtained from intramuscular fat content and fatty acids profile of Piemontese, Limousin and Friesian breeds. Piemontese breed has a widely spread double muscled phenotype and a very low level of fat, Limousin breed is characterized by moderate increase of muscularity, Friesian breed is an early maturing beef cattle with a more marked tendency in fattening.

II. MATERIALS AND METHODS

Samples of *longissimus thoracis* muscle were collected from young bulls of three breeds: double muscled Piemontese (n=10), Limousin (n=11) and Friesian (n=10). According to the different maturing degree, the average carcass weight was 430 kg for Piemontese, 382 kg for Limousin and 337 kg for Friesian breed.

Intramuscular fat content was analysed by petroleum ether extraction according to the Association of Official Analytical Chemists [2].

Fatty acid composition was determined after lipid extraction [3] and methylation procedure [4] by gas-chromatography (SHIMADZU - GC 17A), using a HP88 capillary column (100 m x 0.25 mm ID, 0.2 μm film thickness; J&W Scientific). Peaks were identified by comparing the retention

times with pure fatty acid methylester (FAME) standards (Matreya Inc., Pleasant Gap, PA, USA and Restek Corporation, Bellefonte, PA, USA). The fatty acid composition was expressed as mg/100 g of edible portion. In order to evaluate the nutritional value of intramuscular fat, n-6/n-3 and PUFA/SFA ratios were calculated. As lipid quality depends on the relative contents of particular groups of fatty acids, the atherogenic index (AI) and thrombogenic index (TI) were calculated according to Ulbricht and Southgate [5].

The Hypocholesterolemic/Hypercholesterolemic ratio (h/H) was calculated according to Fernández *et al*. [6]. C22:4 and C22:5 fatty acids were not included in the calculation of h/H index as were not detected in the present study.

The statistical analysis was performed by hierarchical cluster analysis (HCA), principal component analysis (PCA) and canonical discriminant analysis (CDA), using SPSS package for Windows [7]. Eleven parameters were considered: intramuscular fat content (EE), SFA, MUFA, PUFA, n-3, n-6; PUFA/SFA, n-6/n-3, h/H ratios; AI, TI indices.

III. RESULTS AND DISCUSSION

Table 1 shows the least square means of the 11 considered parameters.

The HCA using Ward's method produced three cluster (Fig. 1). Eleven animals were classified in cluster 1, twelve in cluster 2 and eight in cluster 3. The first cluster was characterized by L animals (6/11), the second by P (8/10), the third by F (6/10). The one-way ANOVA was performed to establish which variables significantly contributed to the discrimination between groups. The between groups differences were significant for EE, SFA, MUFA contents, PUFA/SFA ratio, AI and h/H indices. The Tukey post hoc-test revealed that EE, SFA and MUFA differentiate the three cluster through their cluster means. PUFA/SFA, AI and h/H only significantly differentiated cluster 1 from 2 and 2 from 3. Cluster 1 and 3 did not differ for these variables. The three clusters significantly differentiated breeds, with L in the 3rd, F in the 1st and P in the 2nd cluster.

Table 1 Least square means of the considered parameters.

	P	L	F
EE	1.10a	1.70b	2.29c
SFA	469a	766b	1112b
MUFA	337a	585b	891c
PUFA	200	203	192
n-3	9	11	11
n-6	188	188	174
PUFA/SFA	0.49b	0.32ab	0.24a
n-6/n-3	22.90b	17.07a	15.79a
h/H	2.09	1.85	1.71
AI	0.55	0.61	0.63
TI	1.44	1.65	1.59

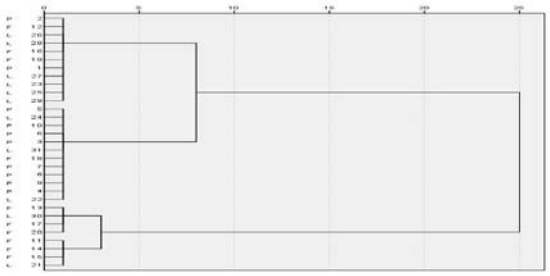

Figure 1 HCA among the three group of animals.

The results of PCA are presented in Table 2, which shows the most significant principal components generated from fatty acid data matrix. The first two PCs accounted for 82.45% of total variance explained by all the generated principal components. The PC1 had the highest eigenvalue (6.00) and described 54.52% of the total variance.

Most variables were highly correlated with PC1 and poorly correlated with PC2 (Table 2).

Table 2 Results of PCA: principal component loadings.

Components	PC 1	PC 2
EE	0.823	0.492
SFA	0.885	0.397
MUFA	0.792	0.454
PUFA	-0.459	0.818
n-6	-0.526	0.764
n-3	0.096	0.945
PUFA/SFA	-0.930	0.057
n-6/n-3	-0.503	-0.403
AI	0.890	-0.202
TI	0.815	-0.287
h/H	-0.916	0.180

PC1 had high positive loadings for EE, SFA, MUFA, AI, TI, and negative loadings for PUFA/SFA, n-6/n-3 and h/H ratio. The loadings plot displays that these parameters were placed far from the origin of PC1 (Fig. 2). The PC2 included 27.93% of the variance in the data set and showed a high positive loadings for PUFA, n-6 and n-3 content.

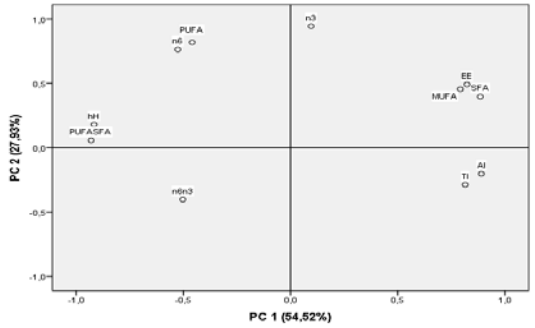

Figure 2 Plot of the first two PC loading vectors.

High positive correlation could be observed between EE, SFA, MUFA content, AI and TI index. On the contrary, PUFA/SFA, n-6/n-3, h/H ratios, being placed 180° from the previous variables, indicate a negative correlation among these traits.

Fig. 3 displays the projection of the three breeds in the first two PCs. Although no defined sets of points were created, Friesian beef was predominantly located in the right area of the figure. This means that the first PC differentiated fatty meat from lean meat. Friesian also had a higher proportion of SFA and MUFA and unfavourable AI and TI index.

The double muscled Piemontese beef was situated at the left side in the region of PUFA/SFA, h/H and n-6/n-3 ratios.

Therefore, Piemontese breed showed better PUFA/SFA and h/H ratios and unfavourable n-6/n-3 ratio, compared with the other two breeds. In general, in double muscled animals the concentration of PUFA increases, while the concentration of SFA decreases, resulting in a higher PUFA/SFA ratio.

The unbalanced and unfavourable n-6/n-3 ratio could be due to the method of finishing animals, based on high level of corn in concentrate diet. This situation is further worse in double muscled animals because of their high level of C18:2n-6 [8].

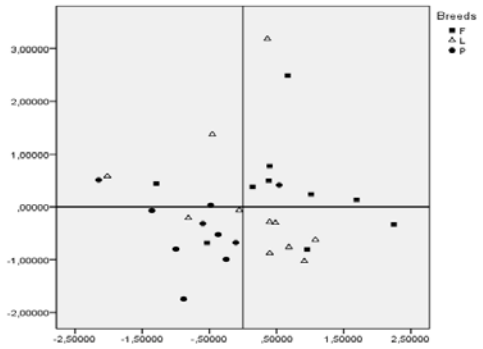

Figure 3 Plot of the first two PC score vectors.

The CDA produced two discriminant functions (Tab. 3).

Function 1 accounted for 85.50% of total variability among breeds and was mainly determined by MUFA, EE and SFA. Function 2 accounted for 14.00% of total variability and was characterized by PUFA/SFA ratio, TI, n-3, AI and h/H ratio.

Table 3 Results of CDA: loadings of correlation matrix between predictor variables and discriminant functions.

	Function 1	Function 2
MUFA	-0.732*	0.464
EE	0.697*	0.564
SFA	0.645*	0.614
PUFA/SFA	0.393	-0.546*
TI	-0.109	0.532*
n-3	-0.188	0.459*
AI	-0.203	0.398*
h/H	0.298	-0.373*

The results indicate that the 80.60% of grouped cases were correctly classified (Tab. 4). Most of the animals were in the diagonal, so this indicates that there was agreement between the assignement into groups by statistical method and the real grouping of the animals; therefore, few animals were out of the diagonal. Two P animals out of ten were included in L group, one F animal out of ten was included in P group and two and one L animals out of eleven were included in P and F groups, respectively.

Table 4 Classification matrix for the young bulls in the three breeds.

	P	F	L
Classified as P	8	1	2
Classified as F	0	9	1
Classified as L	2	0	8
Total	10	10	11

Fig. 4 reports the plot of the two discriminant functions. Function 1 indicated that the centroid of F group was located in the left side of the plot, with a high content of MUFA, EE and SFA, while the centroids of P and L groups were located in the right side.

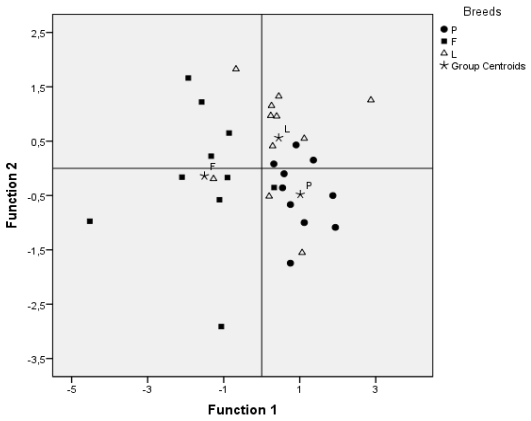

Figure 4 Plot of the two discriminant functions for classification of young bulls according to their breed.

Function 2 was able to discriminate P and L groups. In fact, P group was located in the right bottom quadrant, with a better PUFA/SFA ratio, while L had a better TI and a higher n-3 content. Finally, P and L groups showed a more homogeneous distribution than F group.

IV. CONCLUSION

Six variables out of eleven showed a major breed discrimination capacity and were considered in all three discriminant methods used. These variables were: intramuscular fat content (EE), SFA, MUFA, PUFA/SFA ratio, AI and h/H ratio. The principal component analysis and canonical discriminant analysis also included TI and n-3 variables. The PUFA and n-6 fatty acid content and n-6/n-3 ratio had a poor breed discrimination capacity.

Even if they do not provide the analytical information derived from other statistical methods, the results confirm that the multivariate procedures are a synthetic discriminating tool to highlight the relationships among variables and experimental factors.

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