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Meat quality of veal: discriminatory ability of weaning status

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Abstract

The physicochemical and fatty acid compositions of meat from 81 calves were measured in order to prove whether the extension of suckling period could distinguish "Normal" from "Suprema" class calves. Three groups of animals were investigated depending on their weaning status: NW (animals were milk-fed until seven months), W1 and W2 (animals which were weaned at different ages and reared either in family farms or in feedlot, respectively). There was no difference among animals in relation to the main components (moisture, protein and ash). Significant differences ($p < 0.05$) were found regarding intramuscular fat; the contents were higher in animals weaned at early ages. Color and texture parameters were significantly affected by weaning status. The intramuscular fatty acid composition in the three types of animals showed that saturated fatty acids were the predominant ones in the three groups with values ranging between 48.71% and 49.52% of total methyl esters. Significant differences ($p < 0.001$) were found depending on weaning status in linolenic acid and in the *n-6/n-3* ratio, obtaining the highest values in NW animals. Factor analysis method using principal component was applied to the data. The data matrix constructed was subjected to a canonical discriminant analysis in order to classify the NW, W1 and W2 groups. These results showed that 96.6% for the NW group, and 66.7% and 82.4% for W1 and W2 groups respectively, were correctly classified.

Additional key words: beef; milk feeding; physicochemical composition; fatty acids; discriminant analysis.

Introduction

The unique quality and reputation of Galician veal meat led the European Union (EU) to accept, in 1996, "Ternera Gallega" (Galician veal) as the Protected Geographical Indication (PGI) of Galician Veal "Ternera Gallega". The PGI comprises the "Rubia Gallega" (RG) purebred and its crosses, and classifies the animals as calves (98%) and steers (2%), depending on whether the slaughter is earlier or later than ten months, respectively. Two types of veal are commercialized; the "Normal Ternera Gallega" (40%), where calves are weaned at different ages and mainly fed on forages and PGI authorized commercial feedings and the "Ternera Gallega Suprema" where animals suckle from their mothers for a minimum of seven months. The two classes of calves are mainly differentiated by period of time the animals have been suckling (Ternera Gallega, 2010).

Milk feeding has a market effect PGI "Ternera Gallega" veal performance and meat quality attributes (Bispo *et al.*, 2010a,b, 2011). Thereby, several physi-

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Abbreviations used: CAN (discriminant function of classification); CDA (canonical discriminant analysis); CL (cooking loss); EU (European Union); FA (fatty acid); FAME (fatty acid methyl ester); FtA (factor analysis); IA (index of atherogenicity); IMF (intramuscular fat); IT (index of thrombogenicity); LD (*m. longissimus dorsi*); LSM (least squares mean); MET (metmyoglobin); MUFA (monounsaturated fatty acids); MYO (myoglobin); NV (nutritional value); NW (animals milk-fed until seven months); OX (oximyoglobin); PCA (principal component analysis); PGI (Protected Geographical Indication); PUFA (polyunsaturated fatty acids); SFA (saturated fatty acids); TPA (texture profile analysis); WB (Warner-Bratzler); WHC (water holding capacity); W1 (animals weaned before seven months of life and reared in family farms); W2 (animals weaned before the third month of life and reared in feedlot).

cochemical factors related to meat organoleptic characteristics, such as pH, color, cooking losses and tenderness, might be affected. These parameters are considered important as quality indicators and they have an impact on consumer acceptability (Koohmaraie, 1996; Andersen *et al.*, 2005; Mennecke *et al.*, 2007; Dunne *et al.*, 2009). Furthermore, is known that suckling period may cause differences in meat fatty acid (FA) composition (Bispo *et al.*, 2011). Milk consumption has an important influence on the intramuscular fat content (IMF) (Moreno *et al.*, 2006), because of milk's high energy content, which increases fat deposition in suckling animal. Thereby, polyunsaturated/saturated fatty acids (PUFA/SFA) and sensorial quality are higher in milk-fed beef (Moreno *et al.*, 2006).

The purpose of this research was to evaluate the possibility of employing the physicochemical and nutritional characteristics of meat as discriminating factors among calves weaned at different ages.

Material and methods

Experimental design and animal management

For this study, 81 calves were obtained from different exploitations in Galicia. These cattle were classified into three groups depending on their weaning status:

— NW: 29 calves (17 males; 12 females) were raised on farms with mother dams that had a period of suckling of seven months. Then the animals were reared in family exploitations where they were fed with commercial concentrates to complete their feeding (CF-3; Table 1). Moreover, their diet was supplemented with food produced on the exploitation (grass, dry grass, silage, cereal, grain). The animals were slaughtered with a mean age of 9.2 months and a mean carcass weight of 229 kg.

— W1: 24 calves (13 males; 11 females) from farms with dairy cows. The animals were weaned at different ages but in all cases before seven months of life. The animals were reared in family exploitations where they were fed with food produced for them on the farm: grass, dry grass, grass silage, corn silage, cereal, grain. Their diet was supplemented with commercial feeding (CF-3; Table 1). The animals were slaughtered with a mean age of 9.4 months and a mean carcass weight of 227 kg.

— W2: 28 calves (19 males; 9 females) were weaned at different ages but in all cases before the third month of life. The animals were reared in feedlot. Initially at weaning, the calves were fed with a starter concentrate (CF-1; Table 1) and then based the diet on CF-2 (Table 1). At the last two months of life, the diet was changed to finishing (CF-3; Table 1). The diet was normally supplemented with barley straw. The animals were slaughtered with a mean age of 9.7 months and a mean carcass weight of 221 kg.

The cattle were transported to the abattoir the day before slaughter, trying to minimize animal stress. Animals were stunned with a captive bolt, and slaughtered and dressed according to current EU regulations (Council Directive 93/119/EC; OJ, 1993), in an accredited abattoir. Carcasses were chilled for 24 h in a conventional chamber at 2°C (relative humidity: 98%). At this point, the *m. longissimus dorsi* (LD) was extracted from the left half of each carcass, between the fifth and the tenth rib. Samples were packed under vacuum conditions and stored under refrigerated conditions until analysis. The LD was cut into six steaks of 2.5 cm thickness. The first three steaks were used to determine pH, colour and proximate composition. The fourth and fifth steaks were used to determine water holding capacity and texture parameters, respectively, whereas the sixth was used for analysis of fatty acid methyl esters.

Meat quality attributes

The pH, of the samples was measured using a digital portable pH-meter (Hanna Instruments, Eibar, Spain) equipped with a penetration probe. A portable colorimeter (Konica Minolta CM-600d, Osaka, Japan) with the next settings machine (pulsed xenon arc lamp, angle of 0° viewing angle geometry, standard illuminant D65 and aperture size of 8 mm) was used to measure the meat color in the CIELAB space. Three measurements were performed for each sample in homogeneous and representative areas, free of intramuscular fat. Samples were allowed to bloom for 1 h before measuring directly in contact with air. Results were expressed as lightness (L^*), redness (a^*) and yellowness (b^*). Chroma (c*) and hue (h*) were calculated from a* and b* values according to formula

$$c^* = \sqrt{(a^*)^2 + (b^*)^2}$$

and

$$h^* = \arctan\left(\frac{b^*}{a^*}\right).$$

Table 1. Chemical composition and ingredients of commercial feeding offered to calves

	CF-1	CF-2	CF-3
Ingredients			
Corn	40.0	35.0	40.0
Barley	26.6	22.8	28.4
Extraction flour toasted soybean extraction flour toasted soybean	18.2	20.0	20.8
Calcium carbonate	1.8	2.6	2.4
Fatty acid calcium salt	0.5	1.1	2.0
Sodium chloride	0.2	0.4	0.4
Others:			
— Roasted soybean extruded	3.0	—	—
— Palmist extraction flour	—	7.0	3.5
— Palm oil	—	1.0	1.5
Chemical composition (%)			
Protein	15.2	15.3	15.0
Ash	10.1	8.7	7.5
Fat	3.4	3.9	4.8
Fiber	3.8	—	—
Cellulose	—	5.8	5.1
Mineral/vitamin mix			
Cu (mg kg^{-1})	82.8	2	2
Vitamin A (UI kg^{-1})	55,145	7,350	7,350
Vitamin D_3 (UI kg^{-1})	11,050	1,500	1,500
Vitamin E (mg kg^{-1})	13.5	15	15

CF-1: Commercial feeding for calves (Starter). CF-2: Growth commercial feeding for calves. CF-3: Commercial feeding for finishing.

The relative content of myoglobin (MYO), metmyoglobin (MET) and oximyoglobin (OX) at the surface of the loin is based on measurements of reflex attenuance of incident light at the isobestic points 572, 525, 473 and 730 nm (Krzywicki, 1979).

Moisture, intramuscular fat (IMF), protein and ash were quantified according to the ISO recommended standards 1442:1997, 1443:1973, 937:1978 and 936:1998, respectively. Briefly, moisture percentage was calculated by weight loss experiment by the sample maintained in the oven (Memmert UFP 600, Schwabach, Germany) at 105°C, until constant weight. For IMF content determination, samples were subjected to a liquid-solid extraction using petroleum ether in an extractor apparatus (Ankom^{HCI} Hydrolysis System, Macedon NY, USA) at 90°C during 60 min. The fat content was obtained based on gravimetric difference. Protein content was determined according to Kjeldahl total nitrogen method, multiplying the total nitrogen content by 6.25. Sample was subjected to reaction with sulphuric acid (cuprum sulphate was employed as a catalyst) in a digester (Gerhardt Kjeldatherm KB, Bonn, Germany). Organic nitrogen was transformed to ammonium sulphate, which was distilled in alkali conditions in a distillation apparatus (Gerhardt Vapodest 50 Carrousel, Bonn, Germany). Ash percentage was calculated by weight loss experiment by maintaining the sample in a muffle furnace (Carbolite RWF 1200, Hope Valley, England) into a porcelain capsule at 600°C until constant weight.

The water holding capacity (WHC) was measured as cooking loss (CL). Steaks were cooked placing vacuum package bags in a water bath with automatic temperature control (JP Selecta, Precisdg, Barcelona, Spain) until they reached an internal temperature of 70°C, controlled by thermocouples type K (Comark, PK23M, UK), connected to a data logger (Comark Dilligence EVG, N3014, UK). After cooking, samples were cooled in a circulatory water bath set at 18°C during a period of 30 min and the percentage cooking loss was recorded. All samples were cut or compressed perpendicular to the muscle fibre direction at a crosshead speed of 3.33 and 1 mm s^{-1} for Warner- Bratzler

(WB) and textural profile analysis (TPA) tests respectively. A texture analyzer (TA-XT2, Stable Micro Systems, Godalming, UK) was used in both tests. Seven pieces of meat of 1 × 1 × 2.5 cm (height × × width × length) were removed parallel to the muscle fibre direction. Samples were completely cut using a WB shear blade with a triangular slot cutting edge (1 mm thickness). Maximum shear force, shear firmness and total necessary work performed to cut the sample were obtained. The first one, shown by the peak higher of the force-time curve, represents the maximum resistance of the sample to the cut. Shear firmess is represented by the slope from the beginning of the cut up to the highest point of the force-time curve and total work by the area under the curve. A minimum of five pieces of meat of 1 × 1 × 1 cm (height × width × × length) parallel to the muscle fibre direction were removed for TPA test according to methodology proposed by Bourne (1978). Textural parameters were measured by compressing to 80% with probe of 19.85-cm^2 of surface contact. Between the first and second compression, the probe waited for 2 seconds. Hardness, cohesiveness, springiness, gumminess and chewiness were obtained. Hardness represents the maximal force of the first compression of the product. Cohesiveness is represented by the ratio of work done between the second and the first deformation, whereas springiness is measured at the down stroke of the second compression. Finally, gumminess and chewiness are calculated as Hardness × Cohesiveness and Gumminess × × Springiness, respectively.

Analysis of fatty acid methyl esters

Before analysis, intramuscular fat was extracted from 5 g of ground meat sample according to Folch *et al.* (1957). Lipid extracts were evaporated to dryness under vacuum at 35°C and stored at –80°C until analysis by preparation of fatty acid methyl esters (FAMEs). Lipids were transesterified with a solution of boron trifluoride (14%) in methanol, as described by Carreau & Dubacq (1978). Fifty milligrams of the extracted lipids were esterified and the FAMEs were stored at –80°C until chromatographic analysis.

Separation and quantification of the FAMEs was carried out using a gas chromatograph (GC, Agilent 6890N, Agilent Technologies Spain S.L., Madrid, Spain) equipped with a flame ionization detector and an automatic sample injector HP 7683, and using a Supelco SPTM-2560 fused silica capillary column (100 m, 0.25 mm i.d., 0.2 μm film thickness, Supelco Inc, Bellefonte, PA, USA). The chromatographic conditions were as follows: initial column temperature 120°C maintaining this temperature for 5 min, programmed to increase at a rate of 2°C min^{-1} up to 170°C maintaining this temperature for 15 min, then at 5°C min^{-1} up to 200°C maintaining this temperature for 5 min, and then increasing again at 2°C min^{-1} up to final temperature of 235°C hold 10 min. The injector and detector were maintained at 260°C and 280°C respectively. Helium was used as carrier gas at a constant flow-rate of 1.1 mL min^{-1}, with the column head pressure set at 35.56 psi. The split ratio was 1:50, and 1 μL of solution was injected. Nonadecanoic acid methyl ester (C19:0 ME) at 0.3 mg mL^{-1} was used as internal standard. Individual FAMEs were identified by comparing their retention times with those of authenticated standards. Fatty acids (FAs) were expressed as a percentage of total FAs identified.

Statistical analysis

Results were statistically analyzed using SPSS 19.0 statistical program package (Chicago, IL, USA). Initially, the statistical model (general linear model) included livestock production system and sex as fixed factors. However sex did not have influence on any variable so it was decided to eliminate it from the initial model. For this reason, one-way analysis of variance (ANOVA) was used to estimate the significance of the physicochemical and nutritional differences depending on weaning status. The least squares means (LSM) were separated using Duncan's test for a significance level $\alpha < 0.05$. Correlations between variables were determined by Pearson's linear correlation coefficient ($p < 0.05$).

A factor analysis (FtA) was made to obtain a reduced number of principal components which would explain the variability of the selected variables. The relationship among the variables was represented in a principal component analysis (PCA), which was done using a correlation matrix. A multivariate discriminant analysis was performed between physicochemical and nutritional characteristics to differentiate between class calves. Only those significant variables ($p < 0.05$) obtained through ANOVA were included in the model. The criterion for the selection of variables was Wilk's lambda (F-to-enter and out value of 3.84 and 2.71,

respectively). A linear discriminant function containing an optimal subset of variables was done to determine the coefficients that maximize the differences between samples.

Results

Physicochemical composition

Mean values of physicochemical parameters are shown in Table 2. Significant differences ($p = 0.001$) were found among the three groups of animals in pH values ranging between 5.56 and 5.70.

No significant differences in the main components of chemical composition (moisture, protein and ash) were found by weaning status effect. Mean values of 75% were found for moisture, whereas for the protein above 22% in all cases. On the contrary, significant differences ($p = 0.013$) were found among groups in relation to IMF. In general, values were low (< 1%), being higher in W2 (0.98%) and W1 (0.76%) than in NW (0.59%).

The WHC has a great importance for meat properties since it affects the consumer acceptance. Because of water losses that occur during cooking, appearance, color tenderness and juiciness, they are affected by WHC. The obtained results measured as CL were above 20%.

Color variables were significantly affected by weaning status. L* values ranged between 41.83 and 43.78, being NW samples which showed the highest values. Significant results ($p = 0.004$) were found for redness

Table 2. Effect of weaning status (NW, W1, W2) on meat quality of veal

	NW	W1	W2	SD	SIG
Chemical composition					
pH	5.56[b]	5.70[a]	5.62[b]	0.02	0.001
Moisture (%)	75.0[4]	75.23	75.10	0.11	0.807
Protein (%)	22.56[a]	22.16[b]	22.36[ab]	0.07	0.089
Intramuscular fat (%)	0.59[a]	0.76[ab]	0.98[b]	0.06	0.013
Ashes (%)	1.32	1.29	1.32	0.01	0.272
Color parameters					
Lightness (L*)	43.78[a]	43.20[ab]	41.83[b]	0.35	0.037
Redness (a*)	15.61[a]	13.39[b]	15.02[a]	0.26	0.004
Yellowness (b*)	11.66[a]	10.38[b]	10.39[b]	0.19	0.004
Chroma (c*)	19.52[a]	16.96[b]	18.29[ab]	0.29	0.004
Hue (h*)	36.84[a]	37.96[a]	34.69[b]	0.39	0.002
Myoglobin (%)	36.74[b]	48.82[a]	45.95[a]	1.42	0.001
Metmyoglobin (%)	6.98[a]	4.41[b]	8.06[a]	0.47	0.010
Oxymioglobin (%)	56.28[a]	46.77[b]	45.99[b]	1.32	0.001
WHC					
Cooking loss (%)	22.41	21.50	22.03	0.39	0.688
Texture parameters					
Shear force (kg cm^{-2})	4.62[b]	5.82[a]	5.57[a]	0.18	0.020
Firmness (kg s^{-1})	1.22[b]	1.48[a]	1.46[a]	0.04	0.009
Total work (kg mm)	20.54[b]	29.54[a]	25.13[ab]	1.04	0.004
TPA-test					
Hardness (kg)	4.01[b]	5.01[a]	5.17[a]	0.14	0.001
Springiness (mm)	0.48	0.47	0.47	0.001	0.485
Gumminess (kg)	2.26[b]	2.91[a]	2.96[a]	0.08	0.001
Chewiness (kg mm)	1.08[b]	1.37[a]	1.40[a]	0.04	0.001
Cohesiveness	0.57	0.58	0.57	0.001	0.648

[a-c] Means in the same row with different letters differ significantly ($p < 0.05$). SD: standard deviation of the mean. SIG: significance value.

(a*), where the highest values were obtained in unweaned animals. Regarding yellowness (b*), significantly ($p = 0.004$) higher values were obtained in NW groups than in weaned animals (W1 and W2 groups). The values ranged between 10.38 and 11.66.

Significant differences ($p \leq 0.01$) were found between myoglobin relative content (MYO) on the loin surface and animal group, since W1 and W2 showed the highest values, 48.82% and 45.95%, respectively.

WB parameters (shear force, firmness and total work) were significantly affected by weaning status ($p < 0.05$). Shear force values showed that NW samples were the tenderest, reaching 4.62 kg cm^{-2} *vs* 5.82 kg cm^{-2} and 5.57 kg cm^{-2} forW1 and W2, respectively.

Regarding TPA, hardness and chewiness showed significant differences ($p \leq 0.001$) depending on weaning status. The lowest values were found in NW group (4.01 kg and 1.08 kg mm, respectively). On the other hand, within weaning group, W2 showed higher results than W1 (5.17 kg *vs.* 5.01 kg; 1.40 kg mm *vs.* 1.37 kg mm, respectively).

Fatty acid composition

The intramuscular FA composition of the LD muscle in the three groups is shown in Table 3. The predominant FAs were the saturated FAs (SFA), with values that ranged between 48.71% and 49.52% of total methyl esters. Monounsaturated FAs (MUFA), with values between 40.04% and 41.60%, and polyunsarated FAs (PUFA), with values in all cases < 11%, followed in importance.

With regard SFA, palmitic (C16:0) and stearic (C18:0) were the predominant FAs (around 55% and 35% of total intramuscular SFA, respectively). No significant differences were found depending on the weaning status. Palmitic acid highest values were detected for W2 group, while stearic acid was the highest in NW group (see Table 3).

Within MUFA, oleic acid (C18:1) was the most abundant (approximately 80% of total intramuscular MUFA). Furthermore, this FA was also the most abundant on FA profile in the three groups studied, which represented around 33% of the total methyl esters.

Concerning PUFA, the predominant FA was linoleic (C18:2) (77% of the total intramuscular PUFA, Table 3) followed by arachidonic acid (C20:4), which represented 15%.

To assess the nutritional properties of IMF, the ratios PUFA/SFA, n-6/n-3 and hypocholesterolemic/hypercholesterolemic FAs (h/H) ratios, the nutritional value (NV), and the indexes of atherogenicity (IA) and thrombogenicity (IT), were determined (Table 3). Only significant differences ($p = 0.001$) were found for n-6/n-3 ratio among groups. The values obtained ranged between 13.29 and 31.49, being NW the lowest. The PUFA/SFA ratio showed mean values of 0.19, being NW group the one which showed the highest values (0.21; $p = 0.183$). Regarding IA and IT indexes, mean values around 0.75 and 1.80 were obtained, respectively. The NV and h/H ratio presented mean values of 0.75 and 1.46, respectively. The higher results were found for NW group (0.78 and 1.50, respectively).

Principal components and discriminant analysis

Factor analysis using principal component

FtA aims to obtain a small number of principal components which would explain the variability of the selected compounds. When this data set was used, the first six principal components were chosen (97.69% of total variance) because the eigenvalues were > 1, and therefore, they explained higher percentage of variance than each original variable. Communality was > 0.910 for all variables, which indicates that they are well represented by the six factors. A varimax rotation was carried out to minimise the number of variables that influence each factor, and then, to facilitate the interpretation and discussion of the results. The first principal component (PC1), which explained the higher percentage of variance (27.59%), was mainly associated with $C_{18:2n-6,}$ PUFA, PUFA/SFA ratio and n-6 content. PC2, which explained the 16.50% of the total variance, was positively related to $C_{18:1n-9}$, MUFA and UFA content and negatively correlated with SFA content. PC3 explained 15.62% of total variance and was correlated to TPA parameters (hardness, gumminess and chewiness). PC4 (14.80% of total variance) was related with WB traits (firmness, total work and shear force). The remaining selected factors, with 23.08% of the total variance explained, are related to $C_{18:3n-3}$, n-3 and n-6/n-3 ratio (PC5) and colour parameters (redness and chroma) (PC6). Fig. 1 shows the projection of the variables in the rotated space defined by the first three principal components.

Table 3. Effect of weaning status (NW, W1, W2) on fatty acid profile of veal intramuscular fat. Results expressed as fatty acid percentage composition (percent by weight of total fatty acids)

	NW	W1	W2	SD	SIG
C14:0	2.64	2.66	2.62	0.06	0.972
C15:0	1.37[a]	0.79[b]	1.06[ab]	0.09	0.043
C16:0	26.45	26.36	27.44	0.27	0.171
C16:1	3.15	3.27	3.28	0.07	0.681
C17:0	1.18[b]	1.40[a]	1.11[b]	0.03	0.000
C17:1	0.80	0.62	0.75	0.04	0.184
C18:0	17.44	17.37	17.09	0.31	0.877
TVA	3.17[b]	4.64[a]	3.43[b]	0.15	0.001
C18:1n9c	32.83	32.64	34.04	0.60	0.578
C18:2n6c	7.73	7.37	6.62	0.31	0.271
C20:1	0.09	0.11	0.09	0.01	0.468
C18:3n3	0.71[a]	0.34[b]	0.27[b]	0.03	0.001
C21:0	0.35[a]	0.13[b]	0.20[b]	0.02	0.001
C20:3n6	0.33	0.35	0.34	0.02	0.907
C20:4n6	1.41	1.52	1.36	0.07	0.633
SFA	49.42	48.71	49.52	0.41	0.734
MUFA	40.04	41.28	41.60	0.59	0.498
PUFA	10.18	9.58	8.58	0.39	0.193
UFA	50.22	50.86	50.18	0.40	0.796
Σn-6	9.47	9.24	8.32	0.38	0.373
Σn-3	0.71[a]	0.34[b]	0.27[b]	0.03	0.001
n-6/n-3	13.29[c]	27.89[b]	31.49[a]	1.09	0.001
PUFA/SFA	0.21	0.20	0.17	0.01	0.183
NV	0.78	0.73	0.74	0.03	0.811
h/H	1.50	1.47	1.43	0.02	0.415
IA	0.76	0.73	0.76	0.02	0.797
IT	1.77	1.77	1.84	0.04	0.705

[a-c] Means in the same row with different letters differ significantly ($p < 0.05$). SD: standard deviation of the mean. SIG: significance value. TVA = *trans*-11-vaccenic methyl ester. SFA = Σ(C14:0 + + C15:0 + C16:0 + C17:0 + C18:0+C21:0). MUFA = Σ(C16:1 + C17:1 + C18:1 + TVA + C20:1). PUFA = = Σ(C18:2n6 + C18:3n3 + C20:4n6 + C20:3n6). UFA = ΣMUFA + PUFA. NV: nutritional value = = Σ(C14:0 + C16:0)/Σ(C18:1n9c + C18:2n6c) (Estévez *et al.,* 2004). h/H: hypocholesterolemic/ hypercholesterolemic ratio = [Σ(C18:1n9c, C18:2n6c, C18:3n3, C20:3n6, C20:4n6)/Σ(C14:0 and C16:0)] (Fernández *et al.,* 2007). IA: index of atherogenicity = (uS' + vS")/(xP + yM + zM'), where S' = C14:0, S" = C16:0; P = Σ(n-6 + n-3 PUFAs); and M = C18:1n9c + M'= Σ(other MUFAs). Empirical constants *v, x,* y and *z* have been provisionally set at unity, whereas *u* has been set as 4 (Ulbricht & Southgate, 1991). IT: index of thrombogenicity = $mS^{iv}/\Sigma[nM + oM + p*\Sigma(n\text{-}6)] + [q*\Sigma(n\text{-}3)] + + [\Sigma(n\text{-}3)/\Sigma(n\text{-}6)]$, where S^{iv} = Σ(C14:0 + C16:0 + C18:0); Σ(n-6) = Σn–6 PUFAs; Σ(n-3) = Σn-3 PUFAs; M = C18:1n9c; and M' = Σ (of other MUFAs). Empirical constant *m* has been set at unity; *n, o* and *p* have been assigned the value 0.5; *q* has been assigned the value 3 (Fehily *et al.,* 1994).

Discriminant analysis

The procedure adds functions until reaching the maximum number of functions as determined by the number of predictors and categories in the dependent variable. Using new matrix of data integrated by the standardised reduced original variables, which were selected from the previous FtA, a canonical discriminant analysis (CDA) was developed. This data set was subjected to the CDA according to the finishing diet and livestock production system (NW, W1 and W2). The leave-on-out cross validation was used to validate the results. Table 4 shows classification results of predicted group membership. These classification results revealed a correct aggregation for the first two discriminant factors defined.

From the data set (19 chosen parameters after FtA) subjected to discriminant analysis, only four variables (a*, PUFA, $C_{18:3n\text{-}3}$ and n-3/n-6 ratio) were retained at the end of the stepwise discriminant analysis and were linearly combined to form canonical discriminant

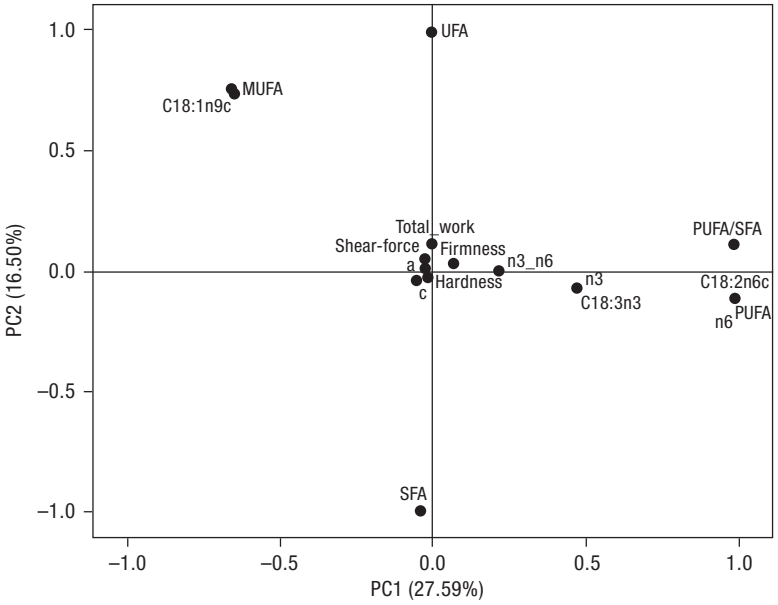

Figure 1. Projection of the variables in the rotated space defined by the two first principle components.

functions. The following two discriminant functions of classification (CAN) were obtained:

$$CAN1 = 0.189[a^*] - 0.219[PUFA] + + 0.847[C_{18:3n-3}] - 0.566[n\text{-}6/n\text{-}3 \text{ ratio}];$$

$$CAN2 = 0.845[a^*] - 2.270[PUFA] + + 1.860[C_{18:3n-3}] + 1.551[n\text{-}6/n\text{-}3 \text{ ratio}].$$

When results for function CAN1 were plotted against results obtained from function CAN2 on coordinate axes for each meat sample, a good discrimination among groups according to the finishing diet and livestock production system was observed (see Table 4). The first two CAN accounted together for 100% of total variance, the first CAN (CAN1) explaining 93.6% of total variability and the second CAN (CAN2) explaining the remaining 6.4%. CAN1 variable allowed segregation of the NW animals from the W1 and the W2 ones, while CAN2 allowed segregation

Table 4. CAN classification parameters and results according to finishing diet and livestock production system (NW, W1, W2)

Data set (19 parameters chosen after factor analysis)				
Function	**Eigenvalue**	**Variance explained (%)**	**Accumulative variance explained (%)**	**Canonical correlation**
1	4.628[a]	93.6	93.6	0.907
2	0.316[a]	6.4	100.0	0.490

Classification results[b]					
Finishing diet and livestock production system		**Predicted group membership (%)**			
		NW	**W1**	**W2**	**Total**
Original	NW	96.6	3.4	0.0	100.0
	W1	5.6	66.7	27.8	100.0
	W2	0.0	17.6	82.4	100.0

[a] First two canonical discriminant functions were used in the analysis. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case. [b] 84.0% of original grouped cases correctly classified.

of the W1 from the W2 animals (Fig. 2). The discriminant analysis correctly attributed each animal to its original group with an accuracy of 96.6% for the NW group and of 66.7% and 82.4% for W1 and W2 groups, respectively.

According to these coefficients, the parameters which mostly accounted for the segregation of CAN1 were $C_{18:3n-3}$ and n-6/n-3 ratio, while the variables accounting for group segregation of CAN2 were PUFA and a*. As reported in Table 3, n-6/n-3 ratio was significantly ($p = 0.000$) lower in NW animals compared to W1 and W2 ones, while $C_{18:3n-3}$ content was significantly ($p = 0.000$) higher in NW animals compared to W1 and W2, which may suggest a pasture feeding. In the present study, it was found that a* tended to be slightly lower in W1 animal compared to W2 animal and this difference could be responsible for the distance between the two groups described by CAN2. Finally, the following discriminant classification equations were obtained:

$$NW = -99.453 + 5.395[a^*] + 198.701[C_{18:3n-3}] - 7.763[PUFA] + 3.754[\text{n-6/n-3 ratio}];$$

$$W1 = -80.411 + 4.635[a^*] + 161.093[C_{18:3n-3}] - 6.821[PUFA] + 3.869[\text{n-6/n-3 ratio}];$$

$$W2 = -98.649 + 5.141[a^*] + 176.302[C_{18:3n-3}] - 7.717[PUFA] + 4.361[\text{n-6/n-3 ratio}].$$

Discussion

Physicochemical composition

In all cases, pH values were below 6, falling being within the acceptable range for beef (Renerre, 1986). Our results were similar to those reported by Oliete *et al.* (2006) and Bispo *et al.* (2010b) in "Rubia Gallega" breed. The lowest values were found in NW groups that were milk-fed until seven months. This outcome is in agreement with many studies confirming that animal nutrition influences ultimate meat pH (Albertí & Sañudo, 1987; Albertí *et al.*, 1988; Bispo *et al.*, 2010b).

Similar chemical composition (moisture, protein and ash) results was found by other authors for weaned and unweaned calves (*e.g.,* Moreno *et al.*, 2006), and for other endangered Galician cattle breeds (Franco *et al.*, 2010; González *et al.*, 2011). However, IMF contents of the present study were lower than those obtained by other authors (Moreno *et al.*, 2006; Blanco *et al.*, 2009; Bispo *et al.*, 2010b). The aforementioned authors also obtained higher values for weaned than for not weaned animals. This could be due to the early weaning and feeding change that W1 and W2 cattle suffered compared to milk-fed NW.

A significant correlation was found between fat and moisture ($r = -0.32$, $p < 0.01$), the main chemical composition components that have an influence on consumer acceptability.

Regarding WHC, no significant differences were found between animals depending on weaning status. The same pattern was found by other authors (Oliete *et al.*, 2006; Bispo *et al.*, 2010b). The results obtained to CL (> 20%) were lower than those (> 30%) obtained by the aforementioned authors. Significant correlations were found when CL were correlated with color parameters L* and b* ($r = 0.26$, $r = 0.23$, $p < 0.05$, respectively), and with cohesiveness ($r = 0.26$, $p < 0.05$).

Color variables revealed that unlike other authors (Vieira *et al.*, 2005; Oliete *et al.*, 2006; Blanco *et al.*, 2009; Bispo *et al.*, 2010b), lightness (L*) values differed significantly ($p = 0.037$) among the three groups of animals. These results are in agreement with other studies where different weaning statuses were evaluated (Barker-Neef *et al.*, 2001; Blanco *et al.*, 2008). Barker-Neef *et al.* (2001) reported similar results, showing greater L* in later weaning animals. Furthermore, lightness values were similar to those obtained in other Galician cattle breeds (Franco *et al.*, 2010; González *et al.*, 2011). In contrast, Pateiro *et al.* (2012a) found meats darker (lower L* values) in cull cows, obtaining values of 35.62 *vs.* mean values of 43.00 obtained in the present study.

Concerning redness index values, they were also affected by weaning status in agreement with other authors (Oliete *et al.*, 2006; Bispo *et al.*, 2010b) who found similar values in other calves, although the a* values were lower than those obtained by Varela *et al.* (2004) and González *et al.* (2011) in other Galician cattle breeds. On the other hand, Pateiro *et al.* (2012a) found a* values higher than 20.00 in dairy cows subjected to seven months of finishing. Redness values were related to the myoglobin and iron content (Juárez *et al.*, 2009), since an increase in the amount of pigment corresponds to an increase in red index (Renerre, 1986). This is reflected in the significant correlations found between myoglobin content on the loin surface and a* ($r = -0.72$, $p < 0.01$). Yellowness (b*) was also

significantly affected by weaning status. The same pattern was found by other authors (Oliete *et al.*, 2006; Bispo *et al.*, 2010b) in not weaned and weaned calves. Our values were higher than those reported by the aforementioned authors. As expected, a significant correlation ($p < 0.05$) was found between pH and a* and b* values ($r = –0.28$ and $r = –0.34$; $p < 0.01$, respectively).

Since the sensorial standpoint, previous studies conducted with these animals reflected the existence of significant results ($p < 0.001$) in appearance descriptors (Pateiro *et al.*, 2012b). The highest scores were obtained in the animals that were weaned at an early age, W1 and W2. With respect to red scores ranged between 5.40 and 6.29, the highest values obtained in W2. Marbling scores ranged between 1.76 and 2.75, and W1 showed the highest values.

Weaning status also had an effect on chroma (c*) and hue (h*), providing significant results ($p < 0.01$) which were higher than those found in other works with "Rubia Gallega" calves (Oliete *et al.*, 2006). In accordance with other authors (Bispo *et al.*, 2010b), c* was significantly higher in unweaned animals. Unlike other authors, h* values were not affected by the weaning status (Albertí, 2001; Bispo *et al.*, 2010b), but were rather due to *post-mortem* factors. On the contrary, Albertí (2001) reported that the differences in terms of color parameters (a*, b*, c* and h*) could be due to the influence of feeding, management and maturity levels.

Unlike other studies (Vieira *et al.*, 2005; Bispo *et al.*, 2010b), the relative content of MYO, MET and OX were affected by weaning status ($p < 0.01$). The values of myoglobin in the present study were higher than those reported by the aforementioned authors.

WB parameters, in particular shear force, showed that NW samples were the tenderest. Similar trend was observed by Bispo *et al.* (2010b) with other calves, although the obtained values were above (9.57 kg cm^{-2} and 10.11 kg cm^{-2} for unweaned and weaned animals, respectively). As in NW group, similar values were found for the others Galician cattle breeds, Frieiresa (4.55 kg cm^{-2}), Caldelá (4.13 kg cm^{-2}) and Vianesa (4.50 kg cm^{-2}) but they were smaller for Cachena (3.80 kg cm^{-2}) and Limiá (3.87 kg cm^{-2}) (Franco *et al.*, 2010; González *et al.*, 2011).

According to tenderness classification proposed by Belew *et al.* (2003), NW meat could be considered as "intermediate" (3.9 kg < WB shear force > 4.6 kg), because shear force value was on the limit of this classification. However, W1 and W2 meat samples would be classified as "tough" (WB shear force > 4.6 kg).

Texture parameters showed that meat from unweaned animals was more tender. It could be due to NW calves were weaned later and they were exclusively milk-fed until seven months which would confer better tenderness in contrast to early weaned animals. Furthermore, significant correlations were found between shear force and hardness ($r = 0.32$, $p < 0.01$), and between moisture and shear force ($r = 0.31$, $p < 0.01$).

Fatty acid composition

The intramuscular FA composition of the LD muscle in the three groups of animals showed that the predominant FAs were the SFA. Bispo *et al.* (2010b) noted the same percentage for other calves and also was reported by Blanco *et al.* (2008) and Blanco *et al.* (2009) for other Spanish cattle breeds even though the aforementioned authors pointed out lower percentages, below 48% in all cases. No significant differences were found among the three groups, finding the highest values in W2 group and the lowest in W1 group. In contrast, Moreno *et al.* (2006) showed that MUFA were the predominant FAs in RG breed, with mean values around 50%.

Regarding SFA, fewer percentages of these FAs were found by other authors (Moreno *et al.*, 2006; Bispo *et al.*, 2011) in other calves. According to the statistical analysis, significant differences ($p < 0.05$) were found among groups in the less abundant SFA (C15:0, C17:0 and C21:0) but these minority acids were in all cases < 3% of total intramuscular fat.

The percentages obtained for MUFA were similar to the values found for other Spanish cattle breeds (Blanco *et al.*, 2008, 2009). Unlike other authors, no significant differences ($p > 0.05$) were found among groups for this FA. Therefore, oleic acid was not discriminant in this case, since it was normally most abundant in milk-fed calves (Moreno *et al.*, 2006; Blanco *et al.*, 2009; Bispo *et al.*, 2010a, 2011). Higher values of this fatty acid (41.34% *vs.* mean values of 33.17% in the present work) were found in cull cows (Pateiro *et al.*, 2012a). The only significant differences ($p = 0.001$) were found for TVA.

PUFA content depends on the diet of the animals; thereby, grass feeding produced higher concentrations of n-3 PUFA and grain feeding increased *n-6* PUFA levels (Wood *et al.*, 2003, 2008). This behavior was re-

flected specially in the significant differences ($p = 0.000$) among groups depending on weaning status for linolenic acid (C18:3) (Table 3). Thus, the highest values were obtained in NW group (6.97% of total intramuscular PUFA), and similar results in weaned animals W1 and W2 (3.55% and 3.15% of total intramuscular PUFA, respectively). This could be explained as above; the highest linolenic acid levels in unweaned cattle is because these animals were reared with their mothers and were allowed to suckle freely until seven months, including grass as a part of their nutrition. However, weaned animals include a complementary concentrate diet since the first month of age and were reared in family exploitations or in feedlot therefore, the contents of linoleic acid should be higher than unweaned animals. In this case, no significant differences ($p > 0.05$) were found between animals, which were reflected in similar values for this FA. The effect of weaning status on linoleic and linolenic acids contents were also evaluated by other authors (Bispo *et al.*, 2010a, 2011) who reported that amount of linoleic acid was higher in weaned animals, while linolenic acid presented higher values in unweaned animals. The influence of diet on FA composition was also previously observed by Varela *et al.* (2004) in pasture finished animals. It was found that linolenic acid was negatively correlated to IMF ($r = –0.38$, $p < 0.01$).

Regarding nutritional properties of IMF, PUFA/SFA ratio values were lower than the typical values (0.5-0.7) of the Mediterranean diet (Ulbricht & Southgate, 1991) and the FAO (2010) recommendations for human diet (0.85). Similar values were found for this ratio by other authors (Moreno *et al.*, 2006; Blanco *et al.*, 2008, 2009; Bispo *et al.*, 2010a, 2011).

The *n*-6/n-3 ratio is highly influenced by the FA composition of the diet used to feed the animals. The n-3 sources of the commercial feedings increases the total n-3 content in the meat, which is linked to decrease deposition of intramuscular n-6 FAs, and lower n-6/n-3 ratios (Raes *et al.*, 2004). The obtained results exceed in all cases the FAO (2010) nutritional recommendations for human diet, because this ratio should not exceed 4.0. Other authors showed similar trends when studying beef although their numbers were lower than those reported in this work (Bispo *et al.*, 2010a, 2011). These papers showed higher ratios for weaned animals, ratios that also exceed the aforementioned nutritional recommendations. It was found that n-6/n-3 ratio was positively correlated to IMF ($r = 0.32$, $p < 0.01$), shear force ($r = 0.37$, $p < 0.01$) and TPA parameters hardness, gumminess and chewiness ($r = 0.42$, $r = 0.45$, $r = 0.42$, $p < 0.01$, respectively).

The IA and IT indexes were evaluated by Garaffo *et al.* (2011) in order to know the different effects that single FA might have on human health. Similar results were found among groups for both indexes. Significant correlations were found in W2 animals when IT was correlated with IMF ($r = –0.42$, $p < 0.05$). On the other hand, NV indicates the healthiness of the diet regarding its lipid content (Estévez *et al.*, 2004). NW was the group that presented the highest values. Finally, the levels obtained from h/H ratio were lower than those considered favorable (h/H ≥ 2.5; see Fernández *et al.*, 2007). The percentage of fatty acids considered as hypocholesterolemic (C18:1n9, C18:2n6, C18:3n3, C20:3n6, C20:4n6) was higher in NW (43.0%) than in weaned animals (42.4%), while the amount of hypercholesterolemic fatty acids (C14:0 and C16:0) showed the opposite behavior NW (29.1%), W1 (29.0) and W2 (30.1). As a result, the h/H ratio was more favorable in NW animals.

In summary, the length of suckling period of calves had a market effect on veal meat quality. Regarding IMF, significant differences ($p < 0.05$) were found depending on weaning status. Color parameters were significantly affected by milk feeding, NW was the sample which showed a higher value of a*. Texture parameters showed that meat from unweaned animals was tenderer. This could be due to NW animals were weaned later and had exclusive feeding with breast milk until seven months, which would confer a special tenderness against animals that were weaned after the first few months of life. Significant differences ($p < 0.001$) were found depending on weaning status for linolenic acid, obtaining the highest values in NW group because these animals were reared with their mothers and were allowed to suckle freely until seven months, including grass as part of their nutrition. The n-6/n-3 ratio is also highly influenced by the FA composition of the commercial feedings used in calves feeding. The discriminant analysis correctly attributed each animal to its original group with an accuracy of 96.6% for the NW group and of 66.7% and 82.4% for W1 and W2 groups, respectively.

This research allows obtaining more accurate results of veal meat quality regarding weaning status. The main beneficiary of the results is the consumer, eager to know objectively if there is a differentiation between weaned and unweaned calves and for which pays a different price.

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