# Influence of slaughtered age on fatty acid profile and amino acid composition of breast from Mos roosters breed

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## Introduction

Currently the markets are facing an increasingly demanding consumer, concerned about maintaining a healthy and well-balanced diet, in which there is no room for excess fat or cholesterol, as is well known for its association with the appearance of cardiovascular disease. Meat and meat products are important sources for protein, fat, essential amino acids, minerals, vitamin and other nutrients (Biesalski, 2005). The "Mos" cock is an autochthonous breed located in Galicia (in the NW of Spain), which now is in recovery. Nowadays, "Mos" is the only poultry native breed of Galicia included in the Official Catalogue of Cattle's Breeds of Spain as being in danger of extinction (R.D. 2129/2008).

The Scientific evidence and nutritional guidelines recommend a reduction in total fat intake, particularly of saturated fatty acids (SFA) (World Health Organization, 2003). For these reasons, the consumer demands for healthier meat and meat products with reduced level of fat, cholesterol, decreased contents of sodium chloride and nitrite, improved composition of fatty acid profile and incorporated health enhancing ingredients are rapidly increasing worldwide (Wangang *et al.*, 2010).

Thus, the aim of this work was to study the effect of slaughtered age on fatty acid profile and amino acid composition from breast "Mos" cock breed.

## Materials and Methods

### Animals

To compare the effect of slaughtered age in the nutritional composition of breast, a total of seventy-five rooster from "Mos" breed were used. Birds were fed with a standard compound feed (ME: 13.19 MJ/kg, CP: 230 g/kg as fed basis), provided by Piensos Biona (Lalin, Spain). All birds were slaughtered in an accredited abattoir by manual exsanguination, plucked and

eviscerated. Twenty-five animals were slaughtered age at 6 months, 25 at 8 months and the other 25 at 10 months. Carcasses were refrigerated for 24 hours at 4 ºC and them the breast was excised for the analysis.

Fatty acid analysis

Before analysis, intramuscular fat was extracted from 5g of ground meat sample, according to Folch *et al.* (1957). Lipids were transesterified with a solution of boron trifluoride (14%) in methanol, as described by Carreau and Dubacq (1978). Fifty milligrams of the extracted lipids were esterified and the FAME´s were stored at -80ºC until chromatographic analysis.

Separation and quantification of the fatty acid methyl esters was carried out using a gas chromatograph (GC, Agilent 6890N) 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). The chromatographic conditions were as follows: initial column temperature 120 ºC maintaining this temperature for 5 min, programmed to increase at a rate of 5 ºC/min up to 200 ºC maintaining this temperature for 2 min, then at 1 ºC/min up to 240 ºC maintaining this temperature for 5 min. The injector and detector were maintained at 260 and 280 ºC respectively. Helium was used as carrier gas at a constant flow-rate of 1.1 mL/min, with the column head pressure set at 35.56 psi. The split ratio was 1:50, and 1 μL of solution was injected. Nonanoic acid methyl ester (C9:0 ME) at 0.3 mg/mL was used as internal standard. Individual fatty acid methyl ester, were identified by comparing their retention times with those of authenticated standards. Fatty acids were expressed as a percentage of total fatty acids identified.

Amino acid analysis

The hydrolysis of the protein was carried out on 100 mg of sample of breast sample with 5 ml of HCL (6N) in an ampoule glass sealed for 24 h at 110 ºC. After the hydrolysis, the solution was diluted with 200 ml of distilled water; it was filtered through 0.45 μm (Filter Lab, Spain). A corresponding aliquot was frozen at -20 ºC and stored until its posterior analysis. Tryptophan determination was not possible, because acidic hydrolysis transform it into ammonium.

HPLC systems used were a high performance liquid chromatograph Alliance 2695 and 2475 scanning fluorescence detector. Empower 2$^{TM}$ advanced software was used to control system operation and results management.

The derivatization of standards and samples and chromatographic analysis conditions were as follow: 10 µl of sample was buffered to pH 8.8 (AccQ.Flour 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 acetonitrile). 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 ml/min and performed at 37 ºC. The gradient profile and composition of the mobile phase was adapted from methodology developed by Van 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.

Statistical analysis

Mean as well as standard deviation and standard error were calculated for all quantified variables. For the statistical analysis of the results of fatty acid profile and amino acid composition a one-way analysis of variance was performed. The least squares means (LSM) were separated using Duncan's range test. All statistical tests of LSM were performed for a significance level $P<0.05$. All the statistical analyses were carried out using the SPSS 19.0 for Windows (SPSS, Chicago, IL, USA) software package.

**Results and discussion**

The fatty acid (FA) composition of breast from "Mos" cocks breed is shown in Table 1. Significant difference in the fatty acid profile was observed respect to slaughtered age (6 vs. 8 vs. 10 months). Breast samples from 6 to 8 months produced an increase of total saturated (36.17 vs. 38.90%, $P<0.001$) and polyunsaturated fatty acids (25.00 vs. 27.55%, $P<0.05$), and a decrease of total monounsaturated (38.83 vs. 33.55%, $P<0.001$). However, when compared our results obtained to breast samples from 8 and 10 months did not find significant difference ($P>0.05$).

In all breast samples studied oleic acid was the most abundant fatty acid (35.67, 29.93 and 31.82%, for breast from cock slaughtered at 6, 8 and 10 months, respectively), followed by palmitic acid (25.51, 26.47 and 25.81% for breast from cock slaughtered at 6, 8 and 10 months, respectively) and linoleic acid (19.09, 19.69 and 19.22% for breast from cock slaughtered at 6, 8 and 10 months, respectively). Our results are in agree with those reported

by De Marchi *et al.* (2005). However, these results are not in agreement with those reported by Jaturasitha *et al.* (2008) in breast raw meat of Thai chicken since palmitic was the most abundant FA, followed by linolenic acid. In contrast, other studies reported linolenic as the predominat FA in Castellana Negra cocks (Miguel *et al.,* 2008). Hence, it has been established that FA composition of chicken fat varies with breed, sex and diet (Edwards and Denman, 1975).

To assess the nutritional index of breast meat fat, the PUFA/SFA ratio (P/S) were determined. In this study, breast from cocks slaughtered age at 6 and 10 months showed a P/S ratio of 0.69, while breast from cocks slaughtered at 8 months presented a P/S ratio of 0.71. This P/S ratio was higher within the range values (0.5-0.7) reported as being typical of the Mediterranean diet (Ulbrich and Southgate, 1991) but it was higher than the recommended ratio of 0.45 by the British Department of Health (1994). The P/S ratio for Mos breed was greater than those reported for broilers (0.19) and Thai indigenous chicken (0.06) (Wattanachant *et al.,* 2004). On the contrary, Jaturasitha *et al.* (2008) found P/S ratio of 0.80 and 0.85 for broiler and Thai indigenous chicken due to strong relationship between dietary fat source and adipose tissue content (Lopez Ferrer *et al.,* 1999).

The hydrolizated amino acid profiles (g/100 g protein) are shown in Table 2. The main essential amino acids present in all breast were lysine and leucine, followed by valine, threonine, phenylalanine and isoleucine, whereas aspartic acid and glutamic being the most important in the non-essential fraction. In other red meats; such as lamb, goat and camel (Elgasim and Alkanhal, 1991) and in white meats such as hen (Elgasim and Alkanhal, 1991) and ostrich (Sales and Hayes, 1996) glutamic and aspartic acid in the non-essential fraction and lysine and leucine in the essential fraction, were also the major amino acids.

Poultry meat contains high quality protein. It is easy to digest and contains all the essential amino acids presently known to be required in human diets. Since poultry contains a higher proportion of proteins than other meats, it also contains more amino acids (Mountney and Parkhurst, 1995).

## Acknowledgements

Authors are grateful to Xunta de Galicia (the Regional Government) for its financial support (PGIDIT09MRU001CT). Special thanks to Centro de Recursos Zooxeneticos de Galicia (CRZG, Fontefiz, Ourense) for samples supplied for this research.

**Abstract**

The effect of slaughtered age on fatty acid composition and amino acids of breast from Mos roosters breed was studied. A total of 75 Mos cock breed were slaughtered at three different ages (6 vs. 8 vs. 10 months, respectively). The fatty acid profile of breast from Mos roosters breed was significantly affected by slaughtered age. Oleic acid was the main fatty acid, higher values found in roosters slaughtered at 6 months (35.67%, P<0.001), whereas linoleic acid showed higher values in roosters slaughtered at 8 months (19.69%, P>0.05) and also higher values of linolenic acid (0.85%, P<0.001). Respect to amino acids, lysine was the most abundant essential amino acid (1296 *vs.* 1571 *vs.* 2369 mg/100 g of breast, P<0.001, for roosters slaughtered at 6, 8 and 10 months, respectively) followed by leucine and threonine, whereas glutamic acid was the main within no essential amino acids (1193 *vs.* 1601 *vs.* 2303 mg/100 g of breast, P<0.001, for cocks slaughtered at 6, 8 and 10 months, respectively) followed by aspartic acid and arginine.

Keywords: Mos rooster breed, slaughtered age, fatty acid composition, amino acid

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Table 1. Fatty acid composition of breasts. Effect of slaughtered age

| | % of total fatty acids | | | | |
|---|---|---|---|---|---|
| | Slaughtered age (months) | | | | |
| Fatty acids | 6 | 8 | 10 | SIG | SEM |
| C14:0 myristic | 0.59±0.13[a] | 0.75±0.18[b] | 0.60±0.12[a] | *** | 0.19 |
| C16:0 palmitic | 22.51±1.53[a] | 26.47±2.84[b] | 25.81±2.33[a] | *** | 0.33 |
| C16:1ω9 palmitoleic | 1.89±0.79[a] | 2.48±0.65[b] | 1.79±0.59[a] | ** | 0.09 |
| C17:0 | 0.65±0.40[b] | 0.08±0.04[a] | 0.12±0.07[a] | *** | 0.04 |
| C17:1 | 0.11±0.12[b] | 0.03±0.03[a] | 0.19±0.09[c] | *** | 0.01 |
| C18:0 stearic | 10.9±1.44 | 11.52±1.70 | 11.85±1.80 | n.s. | 0.20 |
| C18:1ω9 oleic | 35.67±4.57[b] | 29.93±1.93[a] | 31.82±3.37[a] | *** | 0.49 |
| C18:2ω6 linoleic | 19.09±1.73 | 19.69±2.66 | 19.22±2.25 | n.s. | 0.26 |
| C20:1 | 0.35±0.09[c] | 0.05±0.02[a] | 0.24±0.04[b] | *** | 0.02 |
| C18:3ω3 α-linoleic | 0.48±0.11[a] | 0.85±0.15[b] | 0.48±0.11[a] | *** | 0.02 |
| C20:2ω6 | 0.20±0.06[b] | 0.12±0.11[a] | 0.19±0.07[b] | ** | 0.01 |
| C20:3ω6 | 0.21±0.06[a] | 0.38±0.08[c] | 0.27±0.09[b] | *** | 0.01 |
| C20:4ω6 | 4.49±1.80 | 5.78±1.26 | 5.67±4.06 | n.s. | 0.31 |
| C24:1ω9 | 0.81±0.34[a] | 1.06±0.20[b] | 0.90±0.34[a,b] | * | 0.04 |
| C22:6ω3 | 0.52±0.27 | 0.61±.24 | 0.64±0.29 | n.s. | 0.03 |
| SFA | 36.17±2.25[a] | 38.90±2.42[b] | 38.60±2.29[b] | *** | 0.30 |
| MUFA | 38.83±4.70[b] | 33.55±1.97[a] | 34.95±3.38[a] | *** | 0.49 |
| PUFA | 25.00±3.09[a] | 27.55±3.01[b] | 26.46±4.31[a,b] | * | 0.42 |
| P/S | 0.69±0.02 | 0.71±0.02 | 0.69±0.01 | n.s. | 0.02 |
| W3 | 1.00±0.25[a] | 1.46±0.31[b] | 1.12±0.33[a] | *** | 0.04 |
| W6 | 23.79±2.86 | 25.84±2.96 | 25.15±4.28 | n.s. | 0.41 |
| W6/W3 | 24.53±4.13[b] | 18.45±4.15[a] | 24.41±10.72[b] | ** | 0.88 |

Results expressed as fatty acid percentage composition (percent by weight of total fatty acids)

Significancie: *** ($p<0,001$), ** ($p<0,01$), * ($p<0,05$), n.s. = $p\geq0,05$

SEM: standard error of mean.

[a-c] Different letters in the same row show significant differences ($p<0.05$, Duncant test)

Table 2. Amino acid composition (mg/100 g meat) of "Mos" rooster breast. Effect of slaughtered age

| | Slaughtered age (months) | | | | |
|---|---|---|---|---|---|
| Amino acids | 6 | 8 | 10 | SIG | SEM |
| | | Essential | | | |
| Histidine | 868.16±59.96[a] | 898.72±198.54[a] | 1101.38±408.39[b] | ** | 32.43 |
| Isoleucine | 1063.11±41.10[a] | 888.22±227.39[b] | 1132.19±435.99[c] | * | 34.57 |
| Leucine | 1685.14±63.16[b] | 1387.17±330.01[a] | 1727.54±591.20[b] | ** | 48.06 |
| Lysine | 2213.31±116.17[b] | 1805.68±424.61[a] | 2369.94±855.83[b] | ** | 69.06 |
| Methionine | 472.36±252.51[b] | 356.19±155.07[a] | 558.60±187.11[b] | ** | 24.98 |
| Phenylalanine | 1098.61±41.57 | 978.89±250.47 | 1009.27±337.41 | ns | 28.39 |
| Threonine | 1074.30±37.79 | 956.15±241.56 | 1018.32±397.21 | ns | 31.18 |
| Valine | 1055.37±198.12[ab] | 903.62±213.49[a] | 1128.06±396.06[b] | * | 34.11 |
| Total | 20716.79±770.41[b] | 17913.83±4001.59[a] | 21394.59±7499.36[b] | * | 587.95 |
| | | Non essential | | | |
| Arginine | 2153.60±83.07 | 2215.20±779.69 | 2052.35±962.46 | ns | 82.01 |
| Alanine | 1207.18±60.49[b] | 1025.88±221.20[a] | 1264.67±451.58[b] | ** | 35.34 |
| Aspartic acid | 1908.86±135.61[b] | 1544.16±302.66[a] | 1948.43±602.36[b] | ** | 49.92 |
| Glutamic acid | 2259.45±129.29[b] | 1864.38±387.41[a] | 2303.08±763.38[b] | ** | 61.38 |
| Glycine | 936.80±45.42 | 840.27±191.933 | 964.05±341.75 | ns | 26.67 |
| Proline | 860.76±34.85[b] | 645.90±162.82[a] | 827.58±278.28[b] | *** | 23.99 |
| Serine | 867.95±28.91[ab] | 766.89±189.42[a] | 928.28±309.84[b] | * | 25.18 |
| Tyrosine | 822.84±26.26[b] | 697.27±205.66[a] | 865.94±266.11[b] | ** | 23.69 |
| Hidroxiproline | 168.98±23.92[b] | 139.22±55.78[a] | 194.91±50.07[c] | *** | 5.81 |
| Total | 9530.37±338.63[b] | 8174.65±1866.83[a] | 10045.30±3573.71[b] | * | 281.44 |

Significancie: *** ($p<0,001$), ** ($p<0,01$), * ($p<0,05$), n.s. = $p\geq0,05$

[a-c] Different letters in the same row show significant differences ($p<0.05$, Duncant test)

SEM: Standard error of mean