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Original Article

Effect of Weather Conditions on the Fatty Acid Composition of Medium-Growth Chicken Reared in Organic Production System

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■Keywords

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ABSTRACT

A total of 160 medium-sized one-day-old male chicks reared in organic conditions were studied individually from the first day of their life until slaughter (120 days). Two weather periods were considered, being period C colder than period H. A total of 24 chickens per period were randomly selected, then the breast muscle (m. Pectoralis major) was extracted for analysis. Individual fatty acids were measured by gas chromatography and expressed in grams per 100 g of fat. From the values obtained, total lipid fractions were calculated. Near infrared spectroscopy spectra (NIRS) were recorded on the surface of the breast without manipulating. Breast from chicken reared in H period had significantly lower (p<0.05) saturated fatty acids / polyunsaturated fatty acids (SFA / PUFA) ratio, and increased (p<0.05) content in PUFA and n-6. However, no significant differences were observed on the content of individual fatty acid. NIR system was not able to correctly classify the samples according to the breeding period.

INTRODUCTION

As temperatures around the globe have increased substantially during the last decades and are predicted to increase even more, the ability of organisms to deal with these high temperatures will be decisive for maintaining their production performance (Nilsson *et al.*, 2016). Poultry are particularly endangered to climate change due to the range of thermal conditions which affects the animals' behavioral and physiological activities. Hence, birds can only tolerate low temperature ranges to sustain the peak of their production for human consumption. Expected thermal variations as a consequence of climate change represent a challenge for alternative poultry production because animals graze abroad for long periods of time, where small variations in ambient temperature may cause negative effects on their productive development. Changes in temperature have been described as factors capable of influencing not only the development of animals but also the quality of meat (Akşit *et al.*, 2006), which could accelerate postmortem glycolytic metabolism, and result in pale and exudative meat. In addition, environment had been considered an important factor influencing fat deposition in birds (Zhang *et al.*, 2012).

The effects of temperature conditions on broiler performance and meat quality have been extensively investigated in intensive production under controlled humidity and temperature conditions (Akşit *et al.*, 2008, 2006; Blahová *et al.*, 2007; Qureshi *et al.*, 2018). However, these studies are more limited when we look for the effect of environmental conditions when it comes to outdoor production systems. The environmental conditions affecting the performance of chicken include temperature, relative humidity and light at a given time (Ahaotu *et*

al., 2019). Previous research on organic systems (Sarmiento *et al.*, 2020) has shown that the breeding period significantly influenced the moisture content that were lower, while pH and red colour were higher in the warmer period of the year. However, until this moment it is unknown whether these uncontrolled environmental conditions could influence the lipid composition of the meat.

Changes in the lipid profile could affect the healthiness and quality of the poultry meat. Fatty acid composition is responsible for fat firmness and sensorial and technological properties (Carmona *et al.*, 2019). Moreover, the poultry meat is characterized by its relatively high content of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) when compared to pork or red meat (Carmona *et al.*, 2019). The intake of PUFA versus saturated fatty acids (SFA) causes a lowering of serum cholesterol concentrations. However, a high PUFA content, although associated with superior health properties, is often associated with a higher incidence of oxidation and rancidity that negatively affects the shelf life of the product. Compared with mammals, the n-3 PUFA content of skeletal muscle in birds is lower and of n-6 is higher, and when birds are fed typical diets, the concentrations of n-3 PUFA in their edible tissues are relatively low (Rymer & Givens, 2005).

Near infrared spectroscopy (NIR) has been used as a successful technique in agricultural products and the food industry for chemical analysis due to its advantages as a suitable and rapid tool. As far as meat and meat products are concerned, spectroscopic techniques are used for the quantitative determination of major constituents, such as moisture, fat and protein, for estimating organoleptic quality for detecting fraud and for meat speciation (Fumière *et al.*, 2000). Previous studies (Sun *et al.*, 2012; Zuo *et al.*, 2018) have evaluated the ability of the NIRS to predict the geographical origin of certain products due to the influence of these parameters on the quality of the meat. The bibliography consulted has not shown studies evaluating the capacity of the NIRS to discriminate according to the breeding season.

To our knowledge, no information is available in the literature on the influence of climatic conditions on the fat quality of chickens raised in organic production systems. This study proposes to evaluate the effect of the climatic period in the geographical area studied on the fatty acid profile of the organically raised chickens and analyze the ability of the NIR system to classify samples according to these conditions.

MATERIAL AND METHODS

Experimental birds, rearing and feeding

A total of 160 1-day-old medium-growth male chicks (*Gallus gallus domesticus*) were raised for 120 days in a certified organic farming system. RedBro lineage (Hubbard, 2019) was selected for the study. This is an intermediate-slow-growing hybrid (REDJA Ki). The birds were raised in accordance with the organic farming standard for Spain (CE - no 2018/848). Eighty chicks were assigned to each period (C - Cold, H - Hot). Each batch of chicks consisted of 10 animals with each batch being considered as a repetition. This means a total of 8 repetitions for each period.

The weather period (C and H) was determined based on the conditions that affected the chickens from the second month of life (which is when the animals had access to the outside) until their sacrifice (120 days). The study lasted a year. C period corresponds to the months of December to April while H period corresponds to the months of May to November. Climatologic values were obtained from the database of the network of agrometeorological stations of the Agroclimatic Information System for Irrigation (SiAR). The registered data correspond to the climatic conditions of Venialbo (Zamora, Spain) which is located in the northwest region of Spain. Venialbo is situated within the longitude -5.54 and 41.39 latitude. The climate is continental, it is characterized by hot-dry summer and wet and cool winter. The average temperature of period C was lower than that recorded in period H (9.32 *vs.* 16.86 °C). While the values of humidity (75.14 *vs.* 60.59%) and rainfall (1.64 vs. 0.87 mm) were higher in period C. This is justified by the climatic conditions of the geographical area where the experiment was carried out.

The breeding conditions of the chickens are described in the experiment carried out by Sarmiento *et al.* (2020). During the first month of life, the chickens were kept under controlled breeding conditions without access to the outside. Once the animals reached the age of 30 days, the floodgates were opened to allow access to the outdoor parks during day light hours, which varied with the natural time of the year, without being supplemented with artificial light at any time. Chickens had no contact with other animals in the parks. Chickens had access inside the barn throughout the day for both breeding periods to guarantee their comfort. The chickens had access to different vegetables and shrubs that grew in the parks.

Throughout the experiment, the chickens received an identical feed and water *ad libitum*. The feed was formulated according to the regulations for organic production (CE - no 2018/848). In the first month of life, the chickens were fed a starter feed (Metabolizable Energy (ME) 2461.87 kcal/kg; Crude Protein (CP):21.45%), while from day 30 the chickens received a grower-finisher-feed (ME 2179 kcal/kg; CP:15.94%). This feed fully complies with the requirements for the production of broilers (NRC, 1994). The complete composition of the diet is shown in Table 1

Table 1 – Composition and nutrient content of the two organic diets (starter and grower-finisher)

Ingredient (%)	Starter	Grower-finisher
Soybean meal	35.20	
Corn	30.00	
Wheat	12.87	30.00
Barley	9.84	30.00
Spring peas	8.00	30.00
Bicalcium Phosphate	1.93	
Calcium carbonate	0.82	
Premix[1]	0.50	2.50
Acidifier	0.30	
Common Salt	0.28	
Sodium bicarbonate	0.16	
Enzymatic complex	0.10	
Sunflower seeds		7.50
Composition	Starter	Growth-finished
Metabolizable Energy(kcal/kg)	2461.87	2179
Moisture 105 °C	9.24	10.16
Crude Protein	21.45	15.94
Fiber	3.68	5.74
Fat	5.69	5.04
Ash	7.09	4.89

Organic Premix (Nutega Coslada, Madrid) Values given (g) per kg of feed: 1.23 Calcium; Dry matter 4.87; Values given (mg) per kg of feed. E5 Manganese (manganese oxide): mg / kg 65.0; E6 Zinc (zinc oxide) 37.0; E4 Copper (cupric sulfate pentahydrate) 4.0; 3b202 Anhydrous calcium iodate: 1.90; E8 Selenium (sodium selenite) 0.10; E1 Iron (ferrous carbonate) 18.0; 3rd 711 Vitamin K3 1.50; Vitamin B2 3.00; 3,115 Niacinamide 15.0; 3a841 Calcium D-pantothenate 6.44; 3a890 Choline chloride 245.00. Values given (IU) per kg of feed 3a672a Vitamin at 7500.00; E671 Vitamin D3 150,000; Vitamin B12 (mcg / kg) 10.

Physico-chemical analysis

On day 120, 48 chickens were randomly selected and slaughtered in accordance with the regulations for the slaughter of organic production animals (CE - no 2018/848). After 15 minutes post-mortem, the breast muscle with skin (m. Pectoralis major) was removed and sent refrigerated to the laboratory where the samples remained frozen at -18 °C. Prior to the analysis, the breast skin was removed and the breast were thawed for 24 h at 4 °C. Afterwards, the breast was divided longitudinally into two subsamples (A_1, A_2).

Fat were determined by AOAC-approved methods (AOAC, 1990) and was reported as a percentage of dry matter (DM). Total lipid extraction from breast was performed according to (Folch *et al.*, 1987) method. The extracted lipids were utilized for the analysis of fatty acid (FA) profile. The fatty acid profile was performed by gas chromatography according to the method described by (Lurueña-Martínez *et al.*, 2010). Fatty acids were identified by comparing the FAME retention time with the standard Supelco 37 component FAME mix (Supelco, Bellefonte, PA, USA). The fatty acids concentrations were expressed as g/100 g of fat. Moreover, the ratios n-6/n-3 and Polyunsaturated fatty acids (PUFA) / Saturated fatty acids (SFA) were calculated.

Malondialdehyde concentration (MDA) was determinated using thiobarbituric acid reactive substances (TBARS) method (Buege & Aust, 1978). This method is based on the reaction between thiobarbituric acid and the aldehydes that derive from secondary oxidation of the lipids present in the sample, resulting in a coloured complex that can be measured at 530 nm. The TBARs values were expressed as mg of malonylaldehyde (MDA) per kg of sample. Three replicates were run per sample.

NIR Spectra

The NIR spectra was recorded on subsample A2, that were thawed at 4 °C for 24 hours and subsequently the recording was carried out. A Foss NIRSystem 5000 with a standard 1.5 m 210/210 bundle fibre-optic probe, Ref. nº R6539-A (Foss A/S, Hillerød, Denmark, 2000) was used for NIR spectroscopy. The probe used a remote reflectance system and a ceramic plate as a reference. The window was made of quartz with a 5 cm × 5 cm surface area. The remote reflectance fibre-optic probe was directly applied to the meat samples without any preparation. The spectral range was set at 1100-2000 nm and the spectra were recorded at 2 nm intervals and 32 scans were taken for both the reference and the samples. All samples were analyzed in triplicate in order to minimize sampling error. The NIR spectrum was obtained by applying the window directly onto the surface of the sample.

Statistical analysis

The significance of the effect was obtained by using the general linear model procedure. Means and standard deviations were calculated for all variables. The significance level at which differences were considered

was $p<0.05$. All statistical analyses were carried out using the SPSS Package 23 (IBM SPSS Statistic, 2017).

A discriminant analysis of the samples belonging to both periods was carried out. The calibration method applied to this procedure was D-PLS as previously described (Revilla *et al.*, 2019). During the development of the model the group to which the samples belong is known, files are prepared to contain the spectra of all samples belonging to the same category; there is one file for each category. This leads to the automatic generation of a temporal matrix with samples from all categories and as many new dummy variables as categories. In each variable there is an indication of whether the sample belongs to a given group or not by means of a binary code of ones or zeros respectively. The PLS 2 regression is performed with the matrix of these new dummy variables and the spectral data of the samples. Cross-validation is used to establish the number of PLS factors and assess the model. Once the latter has been obtained, a prediction is made as to the value of each dummy variable for each sample; these predicted values are then changed by the addition of one unit. The number of samples correctly classified in the categories to which they belong indicates the acceptability of the model developed. The models obtained in each case were validated. The software used for the chemometric treatments was WinISI version 1.50 programme (Foss A/S, Hillerød, Denmark).

RESULTS

No differences ($p>0.05$) were found in total amount of fat breast from both rearing periods. In addition, no differences were found between malondialdehyde (MDA) concentration of the chicken breast from different rearing period ($p>0.05$).

For both periods, predominant fatty acids in chicken breast (Table 2) was palmitic acid (C16:0) as SFA; oleic acid (C18:1 n-9c) as MUFA and linoleic acid (C18:2 n-6c) as PUFA. Oleic and linoleic acids were the most abundant fatty acids in the various meats under analysis. On the other hand, among the SFA the fatty acid that was found in the smallest amount was arachidic acid (C20:0), while of the MUFA it was myristoleic acid (C14:1 n-5), and in the PUFA it was eicosadienoic acid (C20:2 n-6).

No significant differences ($p>0.05$) were observed between both periods when fatty acids were analyzed

Table 2 – Effect of weather conditions on the fat, TBARS and fatty acid composition of chicken breast muscle rearing in an organic system.

	C			H			*p*-value
Fat (%)	1.42	±	0.51	1.19	±	0.51	0.139
TBARS (mg MDA/ 100 g meat)	0.049	±	0.004	0.049	±	0.002	0.552
Miristic (C14:0)	0.88	±	0.40	0.69	±	0.22	0.481
Miristoleic (C14:1 n-5)	0.18	±	0.10	0.15	±	0.07	0.222
Palmitic (C16:0)	22.24	±	2.31	21.06	±	1.39	0.126
Palmitoleic (C16:1 n-9)	3.86	±	1.61	2.90	±	1.36	0.268
Heptadecanoic (C17:0)	0.15	±	0.05	0.17	±	0.03	0.853
Stearic (C18:0)	6.75	±	3.11	8.56	±	2.42	0.262
Oleic (C18:1 n-9c)	24.32	±	3.10	25.14	±	2.17	0.384
Elaidic (C18:1 n-9t)	1.94	±	0.33	2.08	±	0.28	0.831
Linoleic (C18:2 n-6c)	27.78	±	2.84	28.14	±	2.33	0.545
α-Linolenic (C18:3 n-3)	0.81	±	0.42	1.17	±	0.47	0.495
ɣ-Linolenic (C18:3 n-6)	0.22	±	0.06	0.19	±	0.06	0.409
Arachidic (C20:0)	0.09	±	0.09	0.07	±	0.03	0.857
Eicosadienoic (C20:2 n-6)	0.22	±	0.06	0.19	±	0.06	0.268
Eicosatetranoic (C20:4 n-3)	8.93	±	3.18	6.32	±	1.00	0.157
Eicosapentanoic (C20:5 n-3)	1.00	±	0.05	1.01	±	0.09	0.173
DPA (C22:5 n-3)	0.33	±	0.05	0.36	±	0.09	0.297
∑SFA	33.96	±	3.09	31.19	±	3.24	0.050
∑MUFA	29.87	±	5.52	28.64	±	3.34	0.350
∑PUFA	36.45	±	6.78	40.49	±	3.88	0.017
SFA/PUFA	1.00	±	0.38	0.78	±	0.13	0.013
n-3	1.06	±	0.69	1.12	±	0.42	0.707
n-6	35.27	±	6.52	39.32	±	3.66	0.013
n-6/n-3	43.53	±	26.51	40.30	±	16.37	0.572

The results are presented as mean ± standard deviation. ns = Not significant; * $p<0.05$.

individually. However, there was a significant increase (p<0.05) in the total PUFA content (36.45 *vs.* 40.49) and the total n-6 content (35.27 *vs.* 39.32) and a decreasing trend (p=0.05) in content in the total SFA (33.96 *vs.* 31.19) and in the SFA/PUFA ratio (0.78 *vs.* 1.00) in period C (p<0.05). The rest of the fractions were not affected.

It was not possible to classify the samples according to the breeding period to which they belonged. Figure 1 shows the mean NIR spectrum for the samples of both periods.

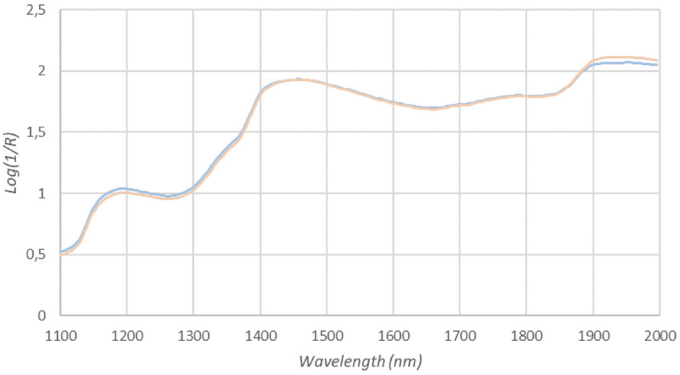

Figure 1 – Mean spectra of the samples according to weather period: cold (C: blue line) and hot (H orange line).

DISCUSSION

Chemical composition of the breast can be modified by changes in temperature (Akşit *et al.*, 2006). Seasonal fluctuations could affect meat quality by either direct effect on organ and muscle metabolism during heat exposure which can persist after slaughter. For example, seasonal fluctuations can increase the risks of pale-soft-exudative meat in turkeys, heat shortening in broilers and dehydration in most species. Also changes in poultry management practices in response to hazards that stem from seasonal fluctuations could indirectly lead to changes in meat quality (Ahaotu *et al.*, 2019). However, studies consulted have been carried out in industrial systems with controlled temperature conditions in a given period. This differs from the conditions raised in our study.

No differences were observed between both rearing periods to total fat breast and MDA concentration. TBARs values are accepted threshold of 0.8 mg MDA/kg chicken meat (O 'neill, 2015) so our results showed adequate values. Even though, a slight increase in the amount of fat was observed in the colder seasons (C) compared to the warm seasons (H) (1.42 *vs.* 1.19%) the difference was not significant. This result differs from that previous researcher (Ain Baziz *et al.*, 1990; Geraert *et al.*, 1996; Zhang *et al.*, 2012) who observed an enhanced fat deposition in breast muscle under chronic heat exposure conditions. The increase in fat content could be related to the reduction in basal metabolism and physical activity associated with higher temperatures.

Our results coincide with those shown by previous authors (Ponte *et al.*, 2008; Dal Bosco *et al.*, 2012; Bosco *et al.*, 2014; Popova *et al.*, 2018) both in the predominant fatty acids, and in their value in other medium- growing genotypes.

There was no effect of the rearing period on the individual composition of fatty acids in the breast. In contrast, (Ain Baziz *et al.*, 1990) found that exposure to heat changed the individual composition of the fatty acid profile. These authors described that birds exposed to heat exhibited higher proportions of palmitic acid and, conversely, lower levels of oleic and linoleic acid (C18:2 n-6c) in abdominal and subcutaneous fat. In our case, lower levels of oleic acid (25.61 *vs.* 24.48%) and linoleic acid (C18:2) (28.14 *vs.* 27.78%) were also observed in birds reared in the warmer period (C). Probably the absence of significant differences found in our study is due to the fact that temperatures did not remain constant as in the case of previous studies carried out in industrial production systems with controlled conditions.

However, it should be considered that, although all chickens received an identical diet based on cereals, legumes and mineral premix (Table 1), in all cases the animals had access to the outdoors and consequently to the pasture. During warm conditions (H) it is likely that the chickens would ingest a greater amount of it due to the increase in their availability. So, it is probable that the observed variations are related more with the greater availability of vegetation than with the effect of environmental conditions, that were not constant. This is in accordance with that described by Ponte *et al.* (2008) who attributed the differences found on the composition of fatty acids rather than the breeding season, to the availability of the grass. In the same way (Žlender *et al.*, 2000; Meluzzi *et al.*, 2010) considered that the differences found in their studies can be attributed to the higher intake of pastures of some groups compared to others. The linoleic acid (C18:2) present in pastures causes the decrease in MUFA content, predominantly oleic acid (C18:1) and the increase in α-linolenic acid (C18:3) (being 11% higher than in the case of chickens raised in intensive systems), and to a lesser extent, of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Žlender

et al., 2000). Although the differences were not significant, similar trends were observed for these fatty acids in our study. Then a higher value of α-linolenic and DPA were observed in the hotest period (1.17 *vs.* 0.81 and 0.36 *vs.* 0.33 respectively). In addition, the amount of PUFA n-6 and n-3 fatty acids available in grasses grows linearly, first, linoleic acid and then linolenic acid (Žlender *et al.*, 2000), which coincides with what is described in our study.

It is well known that green pastures are a good source of α-linolenic acid, and pasture consumption in ruminants leads to greater contents of this fatty acid in meat while decreasing the n-6/n-3 fatty acid ratio (Ponte *et al.*, 2008). This is in agreement with what was found in our study, so that the chickens that had been raised in the warmer climatic conditions, and therefore had greater access to the available vegetation (H), had lower levels ($p < 0.05$) of the ratio n-6 / n-3.

The n-6/n-3 ratio and SFA/PUFA is commonly used as an index to evaluate the nutritional value of dietary fat that has particular relevance on human health. Today, Western diets are rich in SFA and n-6 PUFA and relatively low in n-3 PUFA. The decrease of the ratio n6 / n3 and SFA/ PUFA consumption has been shown to have protective effects on a whole host of diverse conditions from arteriosclerosis to inflammatory and autoimmune diseases (Valencak *et al.*, 2015). As we have described, the chickens that raised in warmer climatic conditions (H), had lower levels of the ratio n6 / n3 and SFA/ PUFA, that would make this a healthier meat. Although higher amounts of PUFA were observed in H period, no significant effects of breeding period on TBARs levels of breast meat were observed which indicates a similar rate of lipid oxidation (Table 2).

Bibliography consulted has not shown data referring to the study of the NIR system to classify meat samples according to environmental conditions, but rather, to the geographical origin (Sun *et al.*, 2012). These authors evaluated lamb meat from different geographical areas, concluding that the correct classification of the samples was produced not because of the environmental conditions from the area, but because of the large differences in the food received. As shown in the previously published study (Sarmiento *et al.*, 2020) it is true that there are slight differences in the quality parameters of the meat from both periods. However, these differences are not as evident as the ones (Sun *et al.*, 2012) show. The scarce differences observed in meat composition between the C and H periods could be the responsible for the fact that the samples could not be discriminated according to the period using NIR spectroscopy.

CONCLUSION

The results shown that breast from chicken reared in heather period had significantly lower content of saturated fatty acids and ratio between SFA/ polyunsaturated fatty acids (PUFA), and increased content in PUFA and n-6. It is probable that the differences found on these parameters, due to the free-range rearing conditions of the chickens, are related with the availability of grass for the chickens' food more than to the environmental climatic conditions.

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