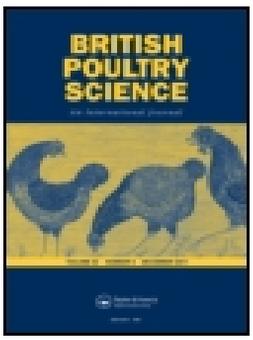

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Fat accumulation, fatty acids and melting point changes in broiler chick abdominal fat as affected by time of dietary fat feeding and slaughter age

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Running title: Supplementation time and dietary fat

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Abstract. 1. This work aims to quantify changes in fatty acid profile, melting point, abdominal fat accumulation and TBARS production depending on dietary fat source and age at slaughter, and to estimate the optimal date for the change from an unsaturated fat to a saturated fat diet, or vice versa.

2. Treatments established were: 1) Birds fed 8% tallow from 21 to 49 d; 2) Birds fed 8%-tallow from 21-37 d and 8%-sunflower oil from d 38-49; 3) Birds fed 8%-sunflower oil from 21-37 d and 8%-tallow from d 38-49; 4) Birds fed 8%-sunflower oil from 21-41 d and 8%-tallow from d 42-49; 5) Birds fed 8%-sunflower oil from 21-49 d. Birds from each group were slaughtered on d 21, 29, 38, 40, 42, 44, 46 and 49.

3. The PUFA proportion in the SSS group reached maximum values at d 40 and fitted a quadratic response. This group also showed a decrease in SAT and MUFA of lower intensity than the PUFA increase. The highest synthesis of SAT+MUFA was found in the SSS and TSS groups, whereas these had the lowest body-to-dietary PUFA ratio.

4. A high and quadratic increase in the MUFA proportion was observed during the first 10 d of feeding with the tallow-enriched diet at the expenses the proportion of PUFA that quadratically decreased (minimum values at d 38).

5. Lipogenic and desaturation capacity decreased with age.

6. The TSS group increased tissue PUFA content faster that the SST group decreased PUFA content after the change in diet which indicates that the earlier feeding has to be taken into consideration for obtaining higher or lower changes in quality parameters.

7. The melting point of the SSS group showed a lower response to the dietary treatment in the initial period when compared to the TTT treatment.

8. The TTT, STT, SST and TSS groups showed similar fat accumulation, and changes in lipid oxidation were related to the d of dietary sunflower oil supplementation.

9. Based on the results, it would be possible to determine the most appropriate dietary program and optimum slaughter age to obtain chicken meat with the desired quality characteristics.

INTRODUCTION

Control of the fatty acid profile in poultry meat is a subject of interest. Fatty acid composition is responsible for fat firmness and sensorial and technological properties and it is easily altered by the incorporation of fats or oils into diets (Hrdinka *et al*., 1996; Bavelaar and Beynen, 2003). Modification of the fatty acid profile in meat is also interesting because saturated fatty acids are negatively associated with the incidence of cardiovascular diseases and other degenerative illnesses (Kraus *et al*., 2001), whereas their substitution by monounsaturated or polyunsaturated fatty acids decreases this risk (Wood *et al*., 2004). Poultry meat is relatively high in polyunsaturated fatty acids when compared to pork or red meat (Bavelaar and Beynen, 2003), so when unsaturated fats are used in diets to reduce the proportion of saturated fatty acids, this causes an even lower melting point. This is in turn associated with a decrease in firmness (Hrdinka *et al*., 1996; Sanz *et al*., 1999) and poorer consumer acceptance. Furthermore, adding unsaturated fats to poultry diets has been associated with increased TBARS production, which affects product shelf life (Cortinas *et al*., 2005). Hence, increasing the fatty acid saturation in broiler chicken tissues by adding saturated fat to the diet during the late fattening phase is a feeding strategy that helps achieve beneficial carcass quality characteristics (Scaife *et al*., 1994, Hrdinka *et al*., 1996). Consequently, these two contrasting feeding practices, using monounsaturated and polyunsaturated fatty acids to decrease the risk of cardiovascular and other diseases and adding saturated fat in the late fattening phase to increase meat firmness and lipid stability, are frequently used in commercial poultry production, but require close dietary intervention.

Moreover, it has been reported that the replacement of saturated with unsaturated fats in the diet of broiler chickens increased abdominal fat percentages by modifying lipogenic enzymes and other enzymes involved in ^{2} -oxidation (Smink *et al*., 2010). Since excessive fat deposition is an unfavourable trait for producers and consumers, its possible modifications by dietary interventions requires more attention.

Saturated fatty acids can come from external sources (from the diet), but they also can be synthesised endogenously in the tissues, and this must be considered in broiler diet formulation. However, there is little information in the literature quantifying the contribution of the *de novo* fatty acid synthesis to tissue fatty acid composition in response to dietary fat type or the supplementation time. In a previous paper Villaverde *et al*. (2006) quantified the effect of dietary PUFA on endogenous synthesis and deposition of fatty acids in chicken. Moreover, Sanz *et al*. (2000b) found that birds fed unsaturated fat during the first stages of growth and its replacement by saturated fat for a few d before slaughter resulted in lower abdominal fat deposition and an acceptable fat fluidity compared to the use of a saturated fat source during the whole growing and finishing period. Carmona *et al*. (2008) subsequently reported that birds given a sunflower oil-enriched diet for 16 d and tallow for the last 12 d before slaughter would produce optimal meat quality characteristics. However, these studies were evaluated at 49 d of age. To our knowledge no information is available in the literature on the quantitative changes of the fatty acid profile (including the contribution of the *de novo* synthesis), melting point, abdominal fat accumulation or lipid stability at different slaughter ages, when birds are fed on a saturated fat or unsaturated fat diet, or on the effect of changing the administration time when the change is made from an unsaturated to a saturated-enriched diet or *vice versa*. This knowledge could help determine the optimum "switching time" for obtaining an adequate fatty acid profile and the resulting desired melting point, fat accumulation and other fat and meat quality characteristics.

The objectives of this work were to quantify changes in characteristics such as saturated (SAT), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, melting point, abdominal fat accumulation and TBARS production depending on the dietary fat source and age at slaughter and to estimate the optimal date for the change from an unsaturated fat to a saturated fat diet or *vice versa*.

MATERIAL AND METHODS

All the experimental procedures used in this study were in compliance with the Spanish guidelines for the care and use of animals in research (BOE, 2005).

Animals and experimental diets

The experiment was conducted using 500 one-d-old Hybro G female broiler chickens. Twenty birds of similar weight were placed in 25 wood-floor pens measuring 2 x 4 m. All chicks were fed on a commercial starter diet from 0 to 21 d and were allowed free access to water and food. At 21 d birds were fed on the experimental diets (5 pens per treatment). Feed and water were continuously available. Lights were switched off for 1 h per d throughout the experiment and room temperature ranged between 22 and 26 ºC. Diets were formulated in order to fulfil nutrient requieremnents of chicks (National Research Council, 1994). Throughout the experiment the birds were handled according to the principles of the care of experimental animals (National Research Council, 1985).

Ingredients composition and nutrient analysis for pre-experimental and experimental diets are shown in Table 1. The diets were formulated to maintain a constant ratio of energy and protein. The apparent metabolisable energy from fat values on which the experiment diets were based (tallow AME = 29.7 MJ/kg; sunflower oil AME = 36.9 MJ/kg) were calculated in a preliminary experiment (unpublished data) by estimation of the difference in fat availability between a basal diet with no added fat and an experimental diet including 8% of the test fat, as described by Blanch *et al*. (1995). Celite was used in the sunflower oil diets as inert filler.

Five treatments were established according to the feeding program for the period from 21 to 49 d of age: 1) Birds fed a 8%-tallow-enriched diet throughout the 28 d from d 21 to 49 (TTT); 2) Birds were fed 8% tallow for the first 16 d from d 21 to 37 d and 8% sunflower oil for the last 11 d from d 38 to 49 (TSS); 3) Birds were fed 8% sunflower oil for the first 16 d from d 21 to 37 and 8% tallow for the last 11 d from d 38 to 49 (STT); 4) Birds were fed 8% sunflower oil for the first 20 d from d 21 to

41 and 8% tallow for the last 7 d from d 42 to 49 (SST) and 5) Birds fed a 8%-sunflower oil-enriched diet for 28 d from d 21 to 49 (SSS).

Table 1 near here

Controls and sampling

In order to determine the change in the fatty acid profile and melting point of fat, a sequential slaughter was carried out. The slaughter schedule was as follows: D 21: 12 random animals prior to equalising the number of animal per pen; d 29 and 38: 6 random birds from each type of diet; d 40, 42, 44 and 46: 5 animals per treatment; d 49: 30 animals per treatment.

On d 21, 29, 38, 40, 44, 46 and 49, animals were slaughtered, bled and plucked at a local slaughter house (Lominchar, Toledo, Spain). Immediately after the slaughter, carcasses were refrigerated until reaching 4 – 5 ºC. At this moment the abdominal fat pad was sampled and stored at -20 ºC until further analysis.

Chemical analysis

Samples of each diet were analysed for nitrogen content (Kjedhal method: AOAC, 1996), crude protein (N x 6.25), dry matter (by drying in an oven at 103 °C for 4 h) and lipid content (6 h Soxhlet extract). Lipids from the abdominal fat were extracted by the procedure proposed by Bligh and Dyer (1959). Fat extracts were methylated in the presence of sulphuric acid for gas chromatographic identification of fatty acids as described elsewhere (López-Bote *et al*., 1997). This was done on a 5890 Hewlett Packard gas chromatograph equipped with a split injector and flame ionisation detector (FID). A split radio of 50:1 was used. A 30 m x 0.32 mm x 0.25 µm cross linked polyethylene glycol capillary column was used (Hewlett Packard, Avondale, PA). Analyses were performed with a temperature programme from 170 to 245 ºC. The injector and detector were maintained at 250 ºC. The carrier gas was nitrogen at a flow rate of 3 ml/min. Tricosanoic acid (Sigma, St Louis) was used as internal standard.

The melting point of the abdominal fat was measured to estimate fat firmness (AOAC, 1996).

The liability of intramuscular fat to iron-ascorbate-induced lipid oxidation was determined by a modification of the method of Kornbrust and Mavis (1980). Homogenates (approximately 1 mg protein/ml buffer) were incubated at 37°C in 40 nM Tris-maleate buffer (pH 7.4) with 1 mM $FeSO_4$ in a total volume of 10 ml. At fixed intervals (0, 30, 60, 90 and 120 min), 0.4 ml were removed for measurement of 2-thiobarbituric acid-reactive substances (TBARS). TBARS were expressed as nmol malondialdehyde (MDA)/mg protein. Protein was measured by the procedure of Bradford (1976). Immediately after homogenisation samples were taken and analysed. Bradford reagent (Biorad protein assay) was added to the sample homogenates and vigorously mixed. After 20 min, absorbance was measured at 595 nm and values compared to those obtained from a standard curve to express protein as mg/ml.

Statistical analysis

Data were analysed following a completely randomised design using the general linear model (GLM) procedure contained in SAS (version 9; SAS Inst. Inc., Cary, NC). The comparative analysis between means was conducted using the Duncan test (SAS V.9, SAS Inst. Inc., Cary, NC). Dietary treatment and age were considered fixed effects according to the following model:

$$Y_{ta} = ¼ + \pm_t + ^2{}_a + (\pm^2)_{ta}$$

where Y_{ta} is the dietary treatment or age response dependent variable, ¼the overall mean, $\pm_t$ the dietary treatment effect, $^2{}_a$ the effet of age (at which samples were performed) and the corresponding interaction $(\pm^2)_{ta}$.

Data were presented as the mean of each group and root mean square error (RMSE) together with the significance levels (*P*-value). Differences between means were considered statistically significant when $P < 0.05$.

Linear and quadratic patterns of the regression equations were carried out in order to estimate the relationship between melting point and the main fatty acid proportions in abdominal fat for each dietary treatment and slaughter age (Statgraphics Centurion XVI, v. 16.1). The Student's t-test was used to compare slopes. The lineal and quadratic responses were calculated using the following models: $y= a+bx$ and $y= a+bx+cx^2$.

RESULTS

The diet containing tallow (TTT) had the lowest concentration of PUFA, while the PUFA-enriched diet (SSS) had the highest (19.4 vs. 57.5%). The lowest concentration of SAT was detected in the sunflower oil-enriched diet (37.5 vs. 13.4% respectively). Dietary energy values (13.4 MJ/kg) were similar for the diets containing saturated or polyunsaturated fat (Table 1).

Chick performance parameters at d 49 of age are presented in Table 2. There were no statistical differences in the initial and final body weights, average daily weights and daily feed intakes ($P > 0.05$) between treatments. The group that consumed the sunflower oil-enriched diet for 11 d (from 38 to 49 d of growing: TSS) had higher food conversion ratio ($P < 0.05$) than the others that were fed with sunflower oil for 16 (STT), 20 (SST) and 28 d (SSS). Groups STT, SST and SSS did not differ in the food conversion ratio.

Tables 2 and 3 near here

The accumulation of abdominal fat according to growing stage and dietary treatment is presented in Table 3. Dietary treatment modified the abdominal fat pad. Hence, the lowest abdominal fat pad was observed in the SSS group when compared to the TTT group, whereas there were no significant differences between the STT, SST and TSS groups showing intermediate amounts of abdominal fat. In addition, the abdominal fat pad increased with age ($P = 0.0001$).

The melting point of the fat as affected by dietary treatment and age is presented in Table 3. Slip point was affected by dietary treatment ($P = 0.0001$), whereas age did not affect this fat characteristic.

To quantify the relationship between the melting point of the abdominal fat and the age of

slaughter for each dietary treatment, regression equations were calculated within the groups TTT, TSS, STT, SST and SSS as shown in Figure 1 and Table 4. The closest relationship was found for the group SSS (R^2 =0.83) followed by TTT (R^2 =0.80). Both linear and quadratic terms showed good fit ($P <$ 0.05). The relationship of the melting point and age in the groups STT, SST and TSS showed linear fits of R^2 = 0.48, 0.46 and 0.46, respectively. As expected, the fat from the groups that received a SAT-enriched diet since d 21 (TTT) showed a higher melting point, while a lower melting point was observed in those fed a PUFA-enriched (SSS) diet. It is of interest to observe (Figure 1) that the SSS group had a lower response to the dietary treatment in the initial period (from d 21 to 39) when compared to the TTT group that had a higher response, as can be observed in the linear term of the regression function (0.71 vs. 1.01). However, changes in the melting point of fat were of higher magnitude in the SSS group after 39 d of age when compared to the TTT group, as shown in the quadratic term of the regression function (-0.015 vs. -0.009). Hence, the fat melting point of birds fed the PUFA-enriched diet since d 21 (SSS) did not change or only slightly changed during the first 7 d, while the fat melting point in birds fed on the SAT-enriched diet was higher by approximately 8°C. From 39 to 49 d of age, the fat melting point in birds fed SSS decreased more (7°C), while the fat melting point in the TTT group decreased less (1°C). It is also very interesting to observe that when dietary fat was changed to either SAT (STT) or PUFA (TSS) after 39 d of age, the response of the melting point either increased or decreased at similar rates (slopes: 0.45 vs 0.43). So, at d 46 of age, both groups (STT and TSS) showed a fat melting point of 24.3°C (Figure 1). Finally, when birds fed on the unsaturated-enriched diet were changed to the saturated-enriched one after d 42 of age (SST), the melting point increased less than in the STT group as observed in the statistically different slope of the regression equation (0.36 vs 0.43), reaching a final value at d 49 close to that observed in the TSS group (23.2 vs 23.4 °C).

Figure 1 near here

The relationship between the melting point and the main groups of fatty acids against age at slaughter was also quantified by regression equations (Table 4). The melting point was highly related to the SAT (R^2 = 0.86), MUFA (R^2 = 0.78) and PUFA (R^2 = 0.86) proportions.

The fatty acid profile of fat as affected by dietary fat or chicken age is presented in Table 3. SAT, MUFA and PUFA proportions were mainly affected by dietary treatment (P = 0.0001). Moreover, SAT, MUFA and PUFA were modified in a different range with birds age (interaction treatment x age). Regression equations of the SAT, MUFA and PUFA of the abdominal fat as a function of the slaughter age (21, 29, 38, 40, 42, 44, 46 and 49 d) were also calculated (Figure 2A, 2B, 2C and Table 5). For SAT, the proportion decreased at the beginning in groups SSS and TSS, while TTT, STT and SST had increased proportions with increasing age. It is interesting to note that the main decrease of SAT in the group SSS occurred from d 21 to 39 (initial stage) (quadratic response), while in the group that received a SAT-enriched diet from d 21 to 49 (TTT), the changes were linear from the beginning (Figure 2A). Also of interest is that when the birds fed on a PUFA-enriched diet were changed after 39 or 42 d to a saturated diet and received tallow for 11 or 7 d (STT and SST, respectively), the SAT proportion increased at a higher rate than that observed for the birds that received a SAT-enriched diet from d 21 to 49 (TTT) (linear slope: 0.21 and 0.17 vs 0.10). Hence, at d 46, groups TSS and STT had similar SAT proportions (26.6% vs 27.2%). Also, similar SAT was observed at d 49 for the groups TSS and SST (26.6% vs 25.6%).

The proportion of PUFA was opposite to that found for SAT. Hence, in abdominal fat, birds fed on a PUFA-enriched diet (SSS) increased PUFA content by 50% from 21 to 49 d, with the main increase being from 21 to 38 d (36%). On the other hand, the PUFA content in birds fed on a SAT-enriched diet decreased (60%) from d 21 to 38 to a minimum value. Moreover, those birds fed a SAT-enriched diet for 16 d that were changed at d 39 (TSS) to a PUFA-enriched diet increased PUFA proportion more rapidly (67% from d 38-46) when compared to those birds fed on PUFA from the beginning. Also, birds fed on a PUFA-enriched diet for 16 d and changed to a SAT-enriched diet at d 39 (STT) showed a faster decrease in the proportion of PUFA than the SST group (fed with a PUFA diet for the first 20 d of experimental period) (Table 5, regression slopes: - 0.44 vs -0.30).

Results for MUFA are presented in Table 5 and Figure 2A, 2B and 2C. The MUFA proportions

Figure 2 and Tables 4 and 5 near here

fit a quadratic response in the TTT and SSS groups. Those birds fed on a SAT-enriched diet from d 21 (TTT) had a maximum value of MUFA at d 38. Similarly, STT and SST groups showed increased MUFA content with age. At d 49, STT and TSS groups reached similar MUFA proportions. It is also of interest to note that the increase in MUFA proportion in group STT was higher than in the SST group (regression slopes: 0.21 vs 0.17). The other groups (SSS and TSS) showed a decreased in MUFA proportion with age as shown by the negative slope in the linear term of the regression equation, which was lowest for the SSS group.

The body-to-dietary fatty acid ratio of SAT, MUFA and PUFA as affected by the dietary treatment and slaughter age is presented in Table 6. As established by Crespo and Esteve-Garcia (2002), ratios above 1 indicate net fatty acid synthesis and values lower than 1 show net fatty acid catabolism. The highest synthesis of SAT+MUFA (ratio >1) was found in the SSS and TSS groups (P = 0.0001), whereas these groups had the lowest body-to-dietary PUFA fatty acid ratio. Also bird age modified the body-to-dietary fatty acid ratios. Hence, the estimated SAT and MUFA synthesis decreased with age (P = 0.0001).

Tables 6 and 7 near here

Changes in the oxidation rate of the intramuscular fat are presented in Table 7. The SSS group had the highest MDA concentration for the initial measurement and from 30 to 150 min. of incubation, whereas the TTT and TSS groups had the lowest. A change at d 38 from an unsaturated-enriched diet to a saturated-enriched one (STT group) decreased the MDA production. Moreover, lipid oxidation rate was also affected by bird age. Hence, lipid oxidation after 60 min. of incubation was higher in younger birds when compared to those of 44 or 46 d of age.

DISCUSSION

The fatty acid composition of the diets (Table 1) reflected the expected results and was in accordance with previous reports. Scaife *et al.* (1994) fed birds with fats of different degree of unsaturation, beef tallow and vegetable oil, and found similar proportions for saturated fatty acids (15.4 vs. 38.6

respectively) and polyunsaturated fatty acids (43.8 vs. 69.6 respectively).

Concerning the performance parameters at 49 d of age it of interest to observe that those chickens fed on a sunflower oil-enriched diet for a shorter period (TSS) had the worst feed convertion ratio. A lower conversion ratio with increased dietary polyunsaturated fatty acids has previously been reported by other authors (Sanz *et al*., 2000a). Lipid structure has been related to the absorption capacity. Hence, it has been described that fatty acid binding protein has higher affinity for polyunsaturated fatty acids than for saturated (Ocker and Manning, 1974). Moreover, differences in feed conversion ratio could be attribuited to the different use of the energy from sunflower oil or tallow. Hence, Sanz *et al*. (2000a) found that the intake and use of the metabolic energy for weight gain was higher in birds fed on sunflower oil than in those fed on tallow.

Fatty acid composition of abdominal fat is significantly affected by the dietary fat as reported by different authors (Pinchasov and Nir, 1992, Scaife *et al*., 1994 and Hrdinka *et al*., 1996; Villaverde *et al*., 2006; Smink *et al*., 2010). Hence, the dietary fatty acid pattern affected the fatty acid composition of the body. The group fed on a sunflower oil-enriched diet for the whole period (SSS) showed the highest increase in PUFA with feeding time followed by the TSS group. However, the PUFA proportion decreased in the other groups. Moreover, birds fed on sunflower oil during the last 11 d of life (TSS) and for 28 d (SSS) showed a high decrease in the MUFA and SAT content. This inverse relationship between the PUFA and MUFA and SAT accumulation has already been described in the literature (Ajuyah *et al*., 1991; Villaverde *et al*., 2006). However, there is no information on how experimental diets affect the evolution of the different fatty acids over time. The PUFA proportion in SSS group reached maximum values at d 40 and fitted a quadratic response that suggests the preferential metabolic utilisation of PUFA. Moreover, in the present study the body-to-dietary fatty acid ratio of PUFA was calculated and the SSS group showed lower PUFA accumulation than the TTT group (0.60 vs. 0.90). Oxidation studies using labeled fats in animals (Leyton *et al*., 1987) and humans (Watkins *et al*., 1982) suggest that dietary polyunsaturated fatty acids are oxidised as fuel sources more rapidly than saturated

long-chain fatty acids. This SSS group also showed a decrease in SAT and MUFA of lower intensity than the PUFA increase as observed in the slopes of the regression equations. The body-to-dietary fatty acid ratio of SAT and MUFA was higher for the SSS group than for groups receiving a SAT-enriched diet (TTT). Other authors have reported that diets with a high PUFA content exhibit a lower inhibition effect upon hepatic lipogenesis than SAT- and MUFA-enriched diets (Crespo and Esteve-Garcia, 2002; Villaverde *et al*., 2006). Besides, the SSS group showed higher synthesis of SAT than MUFA (1.76 vs 1.33) that would indicate a higher desaturase inhibition. It has been reported that delta-9-desaturase is inhibited by PUFA (Kouba and Mourot, 1998; Hodson and Fielding, 2013). Moreover, other authors indicated that unsaturated fat reduces endogenous synthesis and thus SAT and MUFA levels (Pinchasov and Nir, 1992; Scaife *et al*., 1994; Schneiderová *et al*., 2007). Hence, the *de novo* synthesis could explain why the SAT and MUFA decrease in SSS group was not as low as expected, and this effect may also explain other quality characteristics.

It is also of interest to note in the present work that the TTT group fed on tallow for the whole experimental period showed a slightly and linear increase in the saturated fatty acids. Moreover, a high and quadratic increase in the MUFA proportion was observed during the first 10 d of feeding with the tallow-enriched diet at the expense of a low proportion of PUFA that quadratically decreased. The minimum values for PUFA in TTT group were reached at d 38 after two weeks of receiving the tallow-enriched diet and this level was maintained over time. This response may indicate that a regulation mechanism exists to avoid essential fatty acids deficiency (below 14% PUFA). By contrast, those groups fed on tallow for a shorter period (11 d in STT and 7 d in SST) showed a higher increase in the MUFA and SAT proportions with age, this increase being for MUFA higher than for SAT. This fact may indicate a fast desaturation activity from the start, which might explain in part the low SAT proportion after the first d of tallow supplementation. MUFA and SAT not only come from feeding but are also synthetised endogenously. It has been reported that the main fatty acids resulting from the lipogenesis are C16:0, C18:0 and C18:1n-9, with MUFA being obtained from SAT due to the activity of desaturase

enzymes (Crespo and Esteve-García, 2002). Stearoyl-CoA-desaturase is an integral membrane protein of the endoplasmic reticulum that catalyses the rate-limiting step in the biosynthesis of monounsaturated fatty acids from saturated fatty acids (Heineman and Ozols, 2003). These enzymes induce a cis-configuration double bond into substrates to generate palmitoleic and oleic acid (Enoch *et al*. 1976). Hence, in this research, the SAT proportion is probably regulated with an increased desaturase activity depending on the availability of PUFA for oxidisation. This is probably because PUFA (which is rapidly depleted in the TTT group) has been reported to be preferentially oxidised compared to SAT (Sanz *et al*., 1999). Dridi *et al*. (2007) reported that stearoyl-CoA-desaturase (SCD) is ubiquitously expressed in chickens and is regulated by factors such as nutritional state in a tissue-specific manner indicating a potential role for SCD in the control of body weight and energy homeostasis. Moreover, in this research, the desaturase activity and then the MUFA proportion seemed to stop after 15 d of a SAT-enriched diet as observed in treatment TTT. This result would indicate a possible loss of lipogenic capacity over time or utilisation of these MUFAs for physiological functions. This scenario agrees with the results of the present study which show that the MUFA proportion decreased with age. Moreover, Paton and Ntambi (2009) revealed that endogenously synthesised MUFAs by SCD most likely serve as the main substrates for the synthesis of hepatic triglycerides and cholesterol esters. Another possibility could be a possible inhibition of SCD by high MUFA production. However, Duran-Montgé *et al*. (2009) reported that fatty acid desaturation is not inhibited by its reaction products as shown by the fact that a diet rich in oleic acid did not reduce SCD expression.

It is also remarkable that the TSS group showed the highest body-to-dietary MUFA and SAT ratios. Intercepts of the regression equations for SAT and MUFA were also numerical higher in the TSS group than in the STT and SST groups, which would be indicators of the previous feeding. This result would indicate that lipogenesis and desaturation capacity was higher in the TSS group when compared to the STT and SST groups.When the age effect on the body-to-dietary SAT and MUFA ratios were studied, it was observed that these fatty acids decrease with bird age. This result would suggest that

lipogenesis and desaturation capacity decreased with time. Skiba *et al*. (2013) reported that activities of some elongase and desaturase enzymes decreased during the growth of pigs and estimated values of the Pearsons' correlation between body massage of pigs and activity of these enzymes an inverse relationship. Other authors also found a decrease in desaturation activity with age (Daza *et al*., 2007). Moreover, when birds were changed to either a tallow diet for the last 11 (STT) or 7 d (SST), differences in SAT, MUFA and PUFA were lower for the STT group than for the SST group. This effect could be seen in the steeper slope ($P < 0.05$) for SAT, MUFA and PUFA in the SST group (0.21, 0.30 and – 0.44 vs. 0.17, 0.26 and -0.30, respectively). Morever, the TSS group increased PUFA content faster that the SST group decreased PUFA content after the change in diet. This suggests that earlier feeding is an interesting point to consider in explaining changes in the proportion of fatty acid; the greater the initial fatty acid values the higher the decrease or *vice versa*. Morever, this would indicate that the earlier feeding might produce different responses to ^{2} -oxidation (inhibition or increase) and the de *novo* synthesis mechanism depending on the type of fatty acid available to the animal as a source of energy.

It is also of interest to observe in the present research that changes were higher for MUFA than for SAT as observed in the slopes of the regression equations for the different dietary treatments. Villaverde *et al*. (2006) reported that increasing dietary PUFA content resulted in a decrease of SAT and MUFA proportions that was more marked for MUFA. To explain this observation these authors suggested that a mechanism existed to change the ratio of *de novo* synthesis in order to maintain a relatively constant unsaturated (MUFA+PUFA) to saturated fatty acids ratio in cellular membranes. Also, Ntambi (1999) reported that the ratio of stearic acid to oleic acid is thought to be involved in the regulation of cell growth and differentiation through effects on membrane fluidity and signal transduction. Consequently, the results of the present study would indicate that there is probably a regulation in the saturation level in animals fed on SAT to avoid a decrease of PUFA below physiological levels, in which MUFA can be used as a source of energy similar to PUFA. The regulation is probably based on a higher desaturation rate that might be controlled by the level of available PUFA

for ² -oxidation.

In order to study the effects of fatty acid change with time, other quality characteristics were studied. The melting point is a characteristic of fats and depends on the fatty acid composition. Melting and slip point are used as indicators of fat consistency. In pigs (Wood *et al*., 1978) it is directly related to the proportion of stearic acid. In poultry there is evidence that SAT and (MUFA+PUFA)/SAT are good predictors of the melting point (Hrdinka *et al*., 1996). In the present study the melting point was related to different fatty acid classes. Hence, the slope of the regression equation was positive for SAT and MUFA and negative for PUFA and the adjustment was similar for the different fatty acid classes with the melting point being highly related to the SAT and PUFA content ($R^2 = 0.86$). Ortiz *et al*. (2006) reported that the linear regression between fatty acid content and the melting point of abdominal fat gave the highest coefficient of determination for the saturated fatty acid content. Hrdinka *et al*. (1996) reported that fats composed of high amounts of double bound unsaturated fatty acids would be expected to have lower melting points and so lower firmness. Other authors have also related slip point to linoleic acid (Carmona *et al*., 2006). Similarly, Bavelaar and Beynen (2003) reported that the slip point was related to the unsaturated/saturated ratio.

When the evolution of the melting point was studied with the time of feeding the experimental diets (fig. 1), it followed a direct relationship to that found in the evolution of the SAT proportion (fig. 2A) while the evolution of PUFA proportions (fig. 2C) was the inverse. In all the studies found in the literature there is no information on the evolution of fatty acids accumulation in different tissues and the changes that take place in broilers over time. Sanz *et al*. (2000b) reported no differences in melting points at 49 d of age between groups fed on tallow for the last 8 or 12 d and Carmona *et al*. (2006) also found similar results at d 49 of slaughter. Valencia *et al*. (1993), Hrdinka *et al*. (1996) and Rebolé *et al*. (2006) reported the effects of different fats and oils at 42 d of age, Pinchasov and Nir (1992) and Villaverde *et al*. (2010) at 40 d, and Scaife *et al*. (1994) at 54 d of age. In the present study it is interesting to observe that in SSS group there was no relationship between the decrease or increase of the

melting point with the slaughter age and the evolution of the groups of fatty acids separately. Hence, for instance, while melting point decreased faster in the SSS group from 39 to 49 d (fig. 1), the main changes in MUFA and PUFA occurred from 21 to 39 d. This effect would mean that maintaining at least 7 d of sunflower supplementation can avoid the decrease in melting point. This result is in part probably explained by the higher SAT and MUFA lipogenesis observed in SSS group (Table 5), which decrease with birds' age or by the different use of unsaturated fatty acids for metabolic functions and, thus, longer administration times are needed for its effects on fat quality to be noticeable.

Concerning abdominal fat accumulation, previous studies report the beneficial effect of sunflower oil in reducing abdominal fat (Sanz *et al*., 2000; Crespo y Esteve-García, 2002). Also, Smink *et al*. (2010) found that vegetable saturated fat sources increased body fat deposition in comparison to dietary PUFA fed between d 15 and d 35 and reported that a combination of a higher *de novo* synthesis of MUFA and a lower fatty acid oxidation rate may be responsible. The results of this study support those findings. The SSS group had the lowest abdominal fat content while the TTT group had the highest. However, the results of this research provide further information on changes in abdominal fat in relation to the duration of the dietary program and the slaughter age. Hence, groups STT, SST and TSS showed similar abdominal fat accumulation than TTT group. Results concerning the effect of dietary fatty acids on the inhibition or activation of ² -oxidation and *de novo* synthesis are still controversial (Smink *et al*, 2010). Some authors have reported that dietary PUFA not only increases ² -oxidation but also lipogenesis (Crespo and Esteve-Garcia, 2002), while others found an increase in ² -oxidation and an inhibition of fatty acid synthesis (Sanz *et al*., 2000a).

When changes in lipid oxidation of intramuscular lipids were studied, interesting results were observed. As expected, the substitution of dietary unsaturated fat by saturated fat increased the resistance to oxidation (Cortinas *et al*., 2005). However, other authors did not find large differences in tissue oxidation when dietary sunflower oil was replaced by tallow (Sanz *et al*., 1999). In the present study, the SSS group showed a two-fold higher oxidation than the other groups and changes in lipid oxidation were

directly related to the age of dietary sunflower oil supplementation. However, the oxidation rates were lower as expected, which is probably explained by the higher SAT and MUFA synthesis in those birds fed on an unsaturated diet for 28 d observed in this study and to a lower n-3 proportion as reported by other authors (Rey *et al.*, 2001). Moreover, oxidation values decreased with age. To our knowledge there is no information on how lipid oxidation is related to age. In the present research it was observed that the PUFA proportion and n6/n3 fatty acid ratio increased with age and this lower proportion of n-3 fatty acids could be responsible of the lower oxidation rates.

In conclusion, the results of this research can be used to determinate the most appropriate dietary program and optimum slaughter age to obtain chicken meat with a desired fat melting point, fatty acid profile, fat accumulation and lipid stability. These feeding strategies have to take into account that high melting points as well as high proportions of MUFA and PUFA and lipid stability increase meat quality and ensure healthy products. The use of high proportions of tallow for a short period increases the MUFA proportion, probably in order to provide a substrate used by the animal to obtain energy, but it also results in an optimal melting point and product stability. To obtain a high proportion of fat PUFA the sunflower oil diet should be combined with tallow for a short period and preferably at the beginning of the growing stage in order to maintain the quality of the product. This dietary fat combination also results in lower lipid oxidation values; however it does not modify fat accumulation when compared to a long-term sunflower oil administration. Furthermore, the regression formulas presented in this study could also help in estimating quality parameters such as the main groups of fatty acids and melting points in relation to the slaughter age of the birds and the dietary fat used.

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Figure 1. *Changes in abdominal fat melting point (°C) with bird age according to the dietary fat used. TTT: Birds were fed tallow throughout the experimental period from 21 to 49 d of age; TSS: Birds were fed tallow from 21 to 37 d and sunflower oil from d 38 to 49; STT: Birds were fed sunflower oil from 21 to 37 d and tallow from d 38 to 49; SST: Birds were fed sunflower oil from 21 to 41 d and tallow from d 42 to 49; SSS: Birds were fed sunflower oil throughout the experimental period from 21 to 49 d of age.*

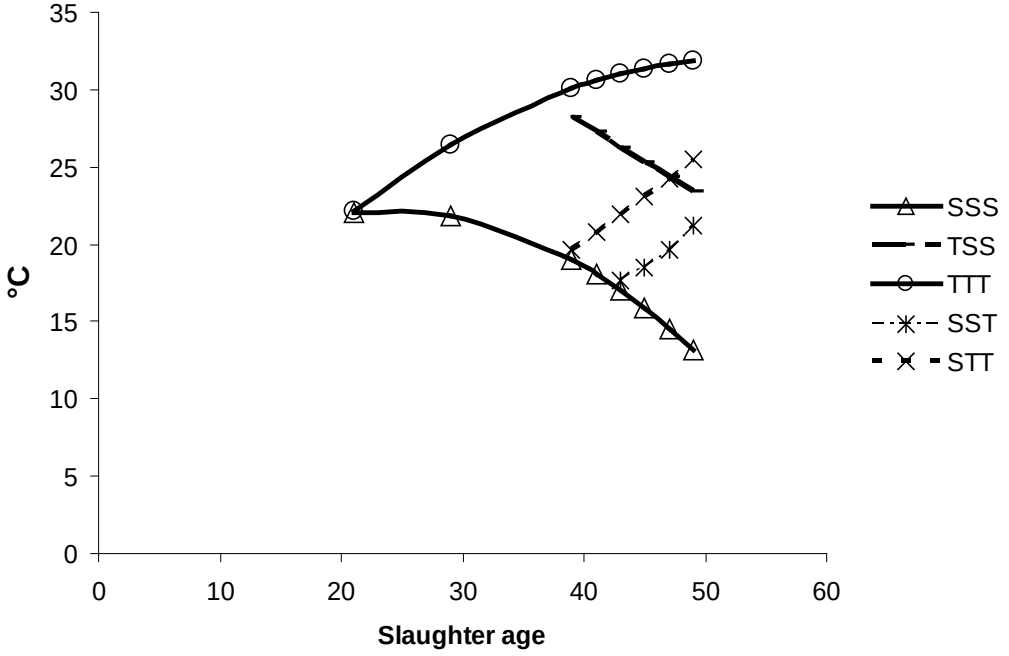
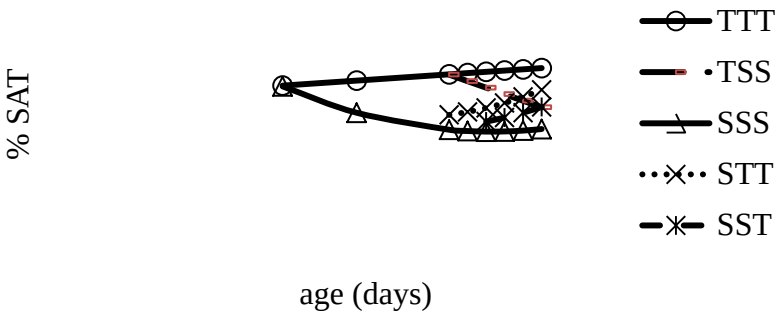


Figure 2. *Changes in abdominal fat fatty acid profile (SAT: 2A; MUFA: 2B and PUFA: 3B) with bird age according to the dietary fat used. TTT: Birds were fed tallow throughout the experimental period from 21 to 49 d of age; TSS: Birds were fed tallow from 21 to 37 d and sunflower oil from d 38 to 49; STT: Birds were fed sunflower oil from 21 to 37 d and tallow from d 38 to 49; SST: Birds were fed sunflower oil from 21 to 41 d and tallow from d 42 to 49; SSS: Birds were fed sunflower oil throughout the experimental period from 21 to 49 d of age.*

2A



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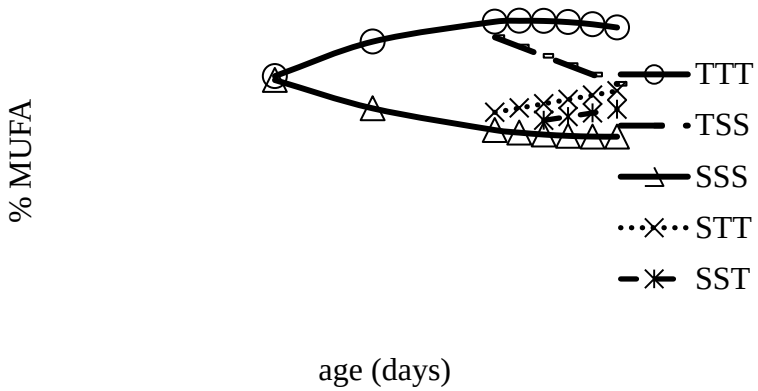

2C

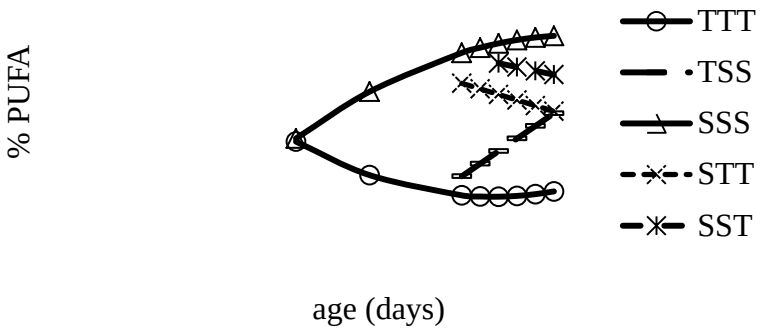

Table 1. *Ingredients, nutrient content and major fatty acid composition of pre-experimental and experimental diets*

	Pre-experimental diet	Tallow diet	Sunflower oil diet
Raw material composition (g/kg diet)			
Wheat	110	106	106
Maize	375	448	400
Soybean meal (47% CP)	300	298	307
Sunflower meal (33% CP)	50.0	...	...
Full-fat soybean	67.3	30.0	30.0
Tallow	30.0	80.0	...
Sunflower oil	29.2	...	80.0
Sodium chloride	3.00	3.00	3.00
Calcium carbonate	5.97	6.20	6.20
Dicalcium phosphate	21.4	22.0	22.0
Celite	...	...	40.0
L-Lysine	0.98	...	...
DL-Methionine	2.48	1.76	1.73
Mineral-vitamin premix[1]	5.00	5.00	5.00
Calculated nutrient composition (per kg of diet)			
AME_N (kcal)	3100	3200	3200
Crude Protein (g)	227	2000	200.0
Ether extract (g)	92.6	107	105

Digestible methionine (g)	5.60	4.50	4.50
Digestible lysine (g)	11.9	10.0	10.0
Calcium (g)	9.00	9.00	9.00
Available Phosphorus (g)	4.50	4.50	4.50
Analysed nutrient composition (per kg of diet)			
Crude protein (g)	219	196	191
Ether extract g)	87.0	100	101
Fatty acid profile (g/100 g fatty acids)			
C16:0	16.1	21.4	8.8
C18:0	11.7	16.2	4.6
C18:1(n-9)	32.7	35.9	28.2
C18:2(n-6)	34.1	18.4	56.3
C20:4(n-6)	1.00	1.00	1.20
Σ Saturated	27.8	37.6	13.4
Σ Monounsaturated	34.2	38.5	28.5
Σ Polyunsaturated	35.1	19.4	57.5
Σ (n-6)	35.1	19.4	57.5
UI[2]	1.06	0.79	1.45
U:S[3]	2.49	1.54	6.42
ACL[4]	11.3	10.3	12.7
C18:1/C18:2	0.96	1.95	0.50

[1] Vitamin mineral premix provided (per kg of diet): Vitamin A, 7500 IU; cholecalciferol, 1500IU; vitamin E (dl-alpha-tocopheryl acetate), 7.52 IU;

vitamin B2, 5.28 mg; pantothenic acid, 8 mg; vitamin B6, 1.84 mg; folic acid, 0.5 mg; vitamin B12, 12.5 mg; choline, 350 mg; Se, 0.15 mg; I, 1.9 mg; Co, 0.2 mg; Cu, 6 mg; Fe, 30.8 mg; Zn, 50 mg; Mn 80 mg; S, 232 mg.

[2] Unsaturation Index (average number of double bonds per fatty acid residue).

[3] Unsaturated to saturated.

[4]ACL (Average chain length of the fatty acid residue): Sum of the chain length per fatty acid per number of total fatty acids.

Table 2. *Chicken performance as affected by dietary treatment at 49 d of age*

	TTT[1]	TSS[2]	STT[3]	SST[4]	SSS[5]		
Time feeding sunflower oil diet (d)	**0 d**	**11 d**	**16 d**	**20 d**	**28 d**	**RMSE[6]**	***P* < F[7]**
Initial weight (g)	692.5	692.3	691.5	691.5	692.7	1.60	ns[1]
Final weight (g)	2699.3	2701.2	2721.6	2779.3	2738.8	61.59	ns
Average daily weight (g)	71.7	69.25	72.5	74.6	73.1	4.11	ns
Daily feed intake (g)	145.1	145.3	144.7	147.9	144.1	2.17	ns
Feed conversation ratio	2.02 ab	2.09 a	1.99 b	1.98 b	1.97 b	0.03	0.0219

[1]TTT: Birds were fed tallow throughout the experimental period (from 21 to 49 d of age).

[2]TSS: Birds were fed tallow from 21 to 37 d and sunflower oil from d 38 to 49.

[3] STT: Birds were fed sunflower oil from 21 to 37 d and tallow from d 38 to 49.

[4] SST: Birds were fed sunflower oil from 21 to 41 d and tallow from d 42 to 49.

[5]SSS: Birds were fed sunflower oil throughout the experimental period (from 21 to 49 d of age.

[6] RMSE = Root mean square error of the treatment, [7]P = probability of treatment effect.

a, b, c, d: Mean values in a row with no common superscript letter differ significantly ($P < 0.05$).

Table 3. *Abdominal fat pad slip point (°C) and fatty acid profile (%) as affected by dietary treatment and slaughter age*

	Abdominal fat pad (g)		Slip point (°C)		SAT		MUFA		PUFA		N6/N3		C18:0/C18:1	
TTT[1]	62.4	a	28.9	a	30.4	a	47.9	a	17.5	d	12.4	d	0.16	bc
TSS[2]	61.9	ba	25.8	b	28.0	b	45.9	b	22.5	c	22.3	c	0.15	c
STT[3]	59.8	ba	21.1	c	26.2	c	40.1	c	29.8	b	30.0	b	0.19	a
SST[4]	60.6	ba	19.0	d	24.2	d	36.9	d	35.1	a	35.3	a	0.18	ba
SSS[5]	56.1	c	17.4	e	23.6	d	37.8	d	34.5	a	34.1	a	0.18	ba
d 21	56.1	d	22.0	ba	29.0	a	43.5	a	22.9	b	11.9	c	0.18	a
d 29	56.5	d	21.7	a	25.0	c	41.1	bc	29.5	a	23.4	b	0.18	a
d 38	56.9	cd	22.5	ba	27.3	b	44.1	a	26.4	ba	25.1	ba	0.16	ba
d 40	59.0	cbd	22.7	ba	26.1	cb	41.2	bc	28.1	ba	32.8	ba	0.19	a
d 42	59.6	cbd	22.9	ba	25.8	c	41.9	bac	28.6	ba	32.5	ba	0.17	ba
d 44	60.0	b	22.6	ba	25.5	c	40.1	c	30.3	a	28.8	ba	0.19	a
d 46	60.8	cb	23.7	a	26.3	cb	41.4	bc	27.9	ba	28.6	ba	0.14	b
d 49	64.0	a	22.2	ba	26.3	cb	40.9	bc	29.8	a	31.9	ba	0.16	ba
RMSE[6]	4.92		2.27		1.90		3.41		2.64		3.16		0.04	
RMSE[7]	5.25		5.85		3.58		5.93		8.33		5.72		0.04	
***P* treatment**[8]	0.0001		0.0001		0.0001		0.0001		0.0001		0.0001		0.0017	
***P* age**[9]	0.0001		0.0949		0.0011		ns		0.0023		0.0001		0.0041	
***P* treatment x age**[10]	0.0225		0.0001		0.0001		0.0001		0.0001		0.0001		ns	

[1]TTT: Birds were fed tallow throughout the experimental period (from 21 to 49 d of age).

[2]TSS: Birds were fed tallow from 21 to 37 d and sunflower oil from d 38 to 49.

[3] STT: Birds were fed sunflower oil from 21 to 37 d and tallow from d 38 to 49.

[4] SST: Birds were fed sunflower oil from 21 to 41 d and tallow from d 42 to 49.

[5]SSS: Birds were fed sunflower oil throughout the experimental period (from 21 to 49 d of age.

[6] RMSE = Root mean square error of the treatment.

[7] RMSE = Root mean square error of the age effect.

[8] P = probability of treatment effect. [9] P = probability of age effect.

[10] P = probability of interaction treatment x age.

a, b, c, d: Mean values in a row with no common superscript letter differ significantly ($P < 0.05$).

Table 4. *Evolution of the melting point (°C) as affected by dietary treatment and slaughter age; and relationship between the melting point and the main groups of fatty acids*

	n	Intercept		x (age)			x² (age²)			R^2	RSD	*P*-linear	*P*-quadratic
TTT[1]	41	5.17	± 4.31	1.01	[a]	± 0.27	– 0.009	±	0.004	0.80	1.820	0.001	0.019
SSS[2]	40	47.0	± 8.0	0.71	[b]	± 0.23	– 0.015	±	0.003	0.83	3.660	0.038	0.0001
TSS[3]	30	13.5	± 3.6	– 0.48	[a]	± 0.18				0.21	1.460	0.013	
STT[4]	28	2.67	± 3.79	0.43	[a]	± 0.08				0.48	2.310	0.0001	
SST[5]	26	–1.49	± 4.44	0.36	[b]	± 0.03				0.45	1.980	0.038	0.0001

	Intercept		Melting point (x)		R^2	RSD	*P*-linear
SAT[6]	10.1	± 1.2	0.74	± 0.05	85.8	1.311	0.0001
MUFA[7]	25.2	± 1.5	0.70	± 0.06	78.1	1.638	0.0001
PUFA[8]	58.7	± 2.1	-1.34	± 0.09	86.0	2.177	0.0001

[1]TTT: Birds were fed tallow throughout the experimental period (from 21 to 49 d of age).

[2]SSS: Birds were fed sunflower oil throughout the experimental period (from 21 to 49 d of age).

[3]TSS: Birds were fed tallow from 21 to 37 d and sunflower oil from d 38 to 49.

[4]STT: Birds were fed sunflower oil from 21 to 37 d and tallow from d 38 to 49.

[5]SST: Birds were fed sunflower oil from 21 to 41 d and tallow from d 42 to 49.

[6]SAT: Total of saturated fatty acids.

[7]MUFA: Total of monounsaturated fatty acids.

[8]PUFA: Total of polyunsaturated fatty acids.

Table 5. *Evolution of the main groups of fatty acids (total of saturated fatty acids: SAT; total of monounsaturated fatty acids: MUFA; and total of polyunsaturated fatty acids: PUFA) as affected by dietary treatment and slaughter age*

		N	Intercept		x (age)			x^2 (age^2)			R^2	RSD	*P*-linear	*P*-quadratic
TTT[1]	SAT	35	20.9	± 3.7	0.10		± 0.02				0.40	0.930	0.0001	
SSS[2]	SAT	40	48.2	± 3.9	– 1.21		± 0.24	0.01	±	0.003	0.78	1.045	0.0001	0.0004
TTT	MUFA	35	20.5	± 4.0	1.50	a	± 0.28	-0.02	±	0.004	0.76	1.100	0.0001	0.0001
SSS	MUFA	40	60.4	± 3.4	– 1.02	b	± 0.22	0.01	±	0.003	0.84	1.083	0.0001	0.0001
TTT	PUFA	36	48.0	± 2.8	– 1.61	b	± 0.18	0.02	±	0.003	0.93	0.726	0.0001	0.0001
SSS	PUFA	40	–6.76	± 4.6	1.78	a	± 0.29	-0.02	±	0.004	0.92	1.870	0.0001	0.0003
STT[3]	SAT	32	17.1	± 2.7	0.21	b	± 0.06		±		0.28	1.720	0.0028	
SST[4]	SAT	32	16.2	± 2.8	0.17	c	± 0.06		±		0.35	1.860	0.0008	
TSS[5]	SAT	30	50.8	± 3.1	– 0.51	a	± 0.07		±		0.68	1.380	0.0001	
STT	MUFA	32	27.3	± 3.1	0.30	b	± 0.07		±		0.40	1.940	0.0003	
SST	MUFA	32	26.7	± 3.8	0.26	c	± 0.09		±		0.25	2.400	0.0080	
TSS	MUFA	30	75.0	± 7.3	– 0.65	a	± 0.16		±		0.38	3.250	0.0006	
STT	PUFA	32	48.9	± 4.0	– 0.44	b	± 0.09		±		0.45	2.530	0.0001	
SST	PUFA	32	47.8	± 4.1	– 0.30	c	± 0.09		±		0.30	2.490	0.0038	
TSS	PUFA	30	21.5	± 4.0	0.99	a	± 0.09		±		0.83	1.690	0.0001	

[1]TTT: Birds were fed tallow throughout the experimental period (from 21 to 49 d of age).

[2]SSS: Birds were fed sunflower oil throughout the experimental period (from 21 to 49 d of age).

[3]STT: Birds were fed sunflower oil from 21 to 37 d and tallow from d 38 to 49.

[4]SST: Birds were fed sunflower oil from 21 to 41 d and tallow from d 42 to 49.

[5]TSS: Birds were fed tallow from 21 to 37 d and sunflower oil from d 38 to 49.

Table 6. *Body-to-dietary fatty acid ratio of saturated fatty acids (SAT), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) as affected by the dietary treatment and slaughter age*

	SAT		MUFA		PUFA	
TTT[1]	0.78	e	1.24	c	0.90	c
TSS[2]	1.93	a	1.56	a	0.44	e
STT[3]	0.85	d	1.09	d	1.07	a
SST[4]	0.92	c	1.10	d	1.05	b
SSS[5]	1.76	b	1.33	b	0.60	d
D 21	1.47	a	1.33	a	0.79	cb
D 29	1.34	bc	1.33	a	0.67	d
D 38	1.29	bc	1.33	a	0.71	cd
D 40	1.40	ba	1.28	ba	0.86	b
D 42	1.19	e	1.32	a	0.77	cb
D 44	1.17	fe	1.20	c	1.09	a
D 46	1.11	f	1.20	c	1.06	a
D 49	1.24	de	1.23	bc	1.01	a
RMSE[6]	0.112		0.096		0.123	
RMSE[7]	0.599		0.215		0.476	
***P*-treatment**[8]	0.0001		0.0001		0.0001	
***P*-age**[9]	0.0001		0.0001		0.0001	
***P*-treatment x age**[10]	0.0001		0.0001		0.0001	

[1]TTT: Birds were fed tallow throughout the experimental period (from 21 to 49 d of age).

[2]TSS: Birds were fed tallow from 21 to 37 d and sunflower oil from d 38 to 49.

[3] STT: Birds were fed sunflower oil from 21 to 37 d and tallow from d 38 to 49.

[4] SST: Birds were fed sunflower oil from 21 to 41 d and tallow from d 42 to 49.

[5] SSS: Birds were fed sunflower oil throughout the experimental period (from 21 to 49 d of age).

[6] RMSE = Root mean square error of the treatment.

[7] RMSE = Root mean square error of the time effect.

[8] P = probability of treatment effect.

[9]P = probability of age effect.

[10]P = probability of interaction treatment x age.

a, b, c, d: Mean values in a column with no common superscript letter differ significantly ($P < 0.05$).

Table 7. *Changes in the iron-induced lipid oxidation of muscle homogenates incubated at 37°C for 150 min according to the dietary treatment and bird age*

	MDA (nmoles MDA/mg protein)									
	0 min		30 min		60 min		90 min		120 min		150 min	
TTT[1]	0.13	b	0.27	c	0.43	d	0.47	c	0.54	c	0.54	c
TSS[2]	0.12	b	0.31	c	0.37	cd	0.51	c	0.58	c	0.58	c
STT[2]	0.14	b	0.44	b	0.57	cb	0.68	b	0.79	b	0.79	b
SST[4]	0.15	b	0.38	b	0.65	b	0.79	b	0.88	b	0.88	b
SSS[5]	0.28	a	0.63	a	0.84	a	0.98	a	1.09	a	1.09	a
D 29	0.21		0.47	a	0.69	a	0.81	a	0.95	a	0.95	a
D 38	0.13		0.42	ba	0.59	ba	0.75	ba	0.86	ba	0.86	ba
D 42	0.18		0.45	ba	0.62	ba	0.73	ba	0.82	ba	0.82	ba
D 44	0.16		0.37	ba	0.52	ba	0.61	b	0.69	b	0.69	b
D 46	0.15		0.34	ba	0.49	b	0.59	b	0.66	b	0.66	b
RMSE[6]	0.138		0.198		0.257		0.291		0.319		0.330	
RMSE[7]	0.561		0.801		0.812		0.870		0.971		1.002	
***P*-treatment**[8]	0.0030		0.0001		0.0001		0.0001		0.0001		0.0001	
***P*-age**[9]	0.4750		0.0616		0.0385		0.0141		0.0060		0.0021	
***P*-treatment x age**[10]	0.8098		0.2152		0.2240		0.1088		0.1485		0.1527	

[1]TTT: Birds were fed tallow throughout the experimental period (from 21 to 49 d of age).

[2]TSS: Birds were fed tallow from 21 to 37 d and sunflower oil from d 38 to 49.

[3] STT: Birds were fed sunflower oil from 21 to 37 d and tallow from d 38 to 49.

[4] SST: Birds were fed sunflower oil from 21 to 41 d and tallow from d 42 to 49.

[5] SSS: Birds were fed sunflower oil throughout the experimental period (from 21 to 49 d of age).

[6] RMSE = Root mean square error of the treatment.

[7] RMSE = Root mean square error of the time effect.

[8] P = probability of treatment effect. [9]P = probability of age effect.

[10] P = probability of interaction treatment x age.

a, b, c, d: Mean values in a column with no common superscript letter differ significantly ($P < 0.05$).