# METABOLISM AND NUTRITION

# Effect of dietary fat sources on fatty acid deposition and lipid metabolism in broiler chickens

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**ABSTRACT** The hypothesis tested was that dietary vegetable fats rich in saturated fatty acids, when compared with a vegetable oil rich in linoleic acid, increase fat deposition in broiler chickens and affect synthesis or oxidation, or both, of individual fatty acids. Diets with native sunflower oil (SO), a 50:50 mix of hydrogenated and native SO, palm oil, and randomized palm oil were fed to broiler chickens. Intake of digestible fat and fatty acids, whole body fatty acid deposition, hepatic fatty acid profile, and hepatic enzyme activities involved in fatty acid oxidation and synthesis were measured. The fat deposition:digestible fat intake ratio was significantly lower for the SO group in comparison with the groups fed the vegetable fats rich in saturated fatty acids. The difference between digestible intake and deposition of C18:2, reflecting its maximum disappearance rate, was highest for the SO group and lowest for the palm oil- and randomized palm oil-fed birds. The calculated minimal rate of de novo synthesis of monounsaturated fatty acids (MUFA), calculated as deposition minus digestible intake, was more than 50% lower for the SO group than for the other 3 dietary groups. Based on the fatty acid profiles in the liver, it would appear that increasing contents of C18:2 decrease the desaturation of saturated fatty acids into MUFA. It is concluded that a diet rich in C18:2 in comparison with different kinds of vegetable saturated fatty acids decreases the deposition of fat, especially of MUFA. It appears to be caused by a higher β-oxidation and a reduced de novo synthesis of MUFA, but this conclusion is not fully supported by the measured activities of enzymes involved in fatty acid synthesis and oxidation.

**Key words:** chicken, fatty acid, metabolism, fat deposition, liver

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## INTRODUCTION

The replacement of beef tallow by vegetable fats rich in polyunsaturated fatty acids (**PUFA**) like sunflower oil, soybean oil, or linseed oil resulted in a decrease of abdominal fat deposition in broilers (Sanz et al., 1999, 2000ab; Bilal et al., 2001; Crespo and Esteve-Garcia, 2002a; Newman et al., 2002; Ferrini et al., 2008; Wongsuthavas et al., 2008). Abdominal fat represents a small part of the total fat deposition in chickens. Studies of fat sources on whole-body fat content are scarce and show no statistically significant effect of PUFA source on fat deposition in broilers (Pinchasov and Nir, 1992; Crespo and Esteve-Garcia, 2002a). The saturated fatty acid-rich sources used in deposition and metabolism studies in chickens are mostly tallow or lard. These fat sources contain high levels of both palmitic (C16:0) and stearic acid (C18:0) as saturated fatty acids (**SFA**). Palm oil is often used as a vegetable fat source to replace animal fat. We are not aware of studies on deposition and metabolism of fatty acids in broiler chickens fed with palm oil (C16:0-rich) and hydrogenated sunflower oil (as a C18:0-rich source) in comparison with PUFA. A high fraction of palmitic acid in palm oil is bound at the *sn*-1 or *sn*-3 position of the glycerol molecule (Breckenridge, 1978; Mu and Høy, 2004). Long-chain SFA that are esterified on the *sn*-1 and *sn*-3 positions are absorbed less efficiently than those bound on the *sn*-2 position. From results of our previous study (Smink et al., 2008), the digestibility of the C16:0 and the proportion of C16:0 in abdominal fat and breast meat was found to be higher in broilers fed randomized palm oil in comparison with the birds fed with palm oil.


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Fat accumulation is the net result of absorption, de novo synthesis, and oxidation of fatty acids. Results of studies in rats (Shimomura et al., 1990) and in broiler chickens (Sanz et al., 2000b) indicate that dietary PUFA increase the β-oxidation and inhibit de novo fatty acid synthesis (Shimomura et al., 1990; Sanz et al., 2000b). However, in a study using linseed oil as PUFA, both an increased oxidation and de novo synthesis were found (Crespo and Esteve-Garcia, 2002b). Thus, the effect of high PUFA intake on de novo synthesis of fatty acids is not clear. In this study on the effect of native sunflower oil versus vegetable fats rich in SFA, we readdressed the issue of the relations between fatty acid deposition, synthesis, and oxidation. It is well known that PUFA versus SFA are preferentially oxidized (Beynen and Katan, 1985), which may explain the above-mentioned lowering of abdominal fat in broiler chickens fed diets rich in PUFA. Thus, in this study, we tested the hypothesis that in broilers fed various dietary vegetable fats rich in SFA, instead of a vegetable oil rich in linoleic acid, there is more fat deposition in the body, which is associated with less fatty acid oxidation and an unpredictable change in fatty acid synthesis. Fatty acid oxidation and synthesis at the level of the whole body were assessed on the basis of calculated rates of net fatty acid disappearance and appearance. The liver plays a dominant role in deposition and oxidation of fatty acids. Thus, we also measured hepatic enzyme activities, liver fat content, and hepatic fatty acid composition. The effects of the dietary treatments on fatty acid digestibility have been published elsewhere (Smink et al., 2008).

## MATERIALS AND METHODS

The experimental protocol was approved by the animal experiments committee of the Faculty of Veterinary Medicine, Utrecht University, the Netherlands.

### Birds and Housing

One-day-old female broilers (Ross 308) were purchased from a local hatchery. On arrival, they were wing-banded, weighed, and housed in wire-floor suspended cages. There were 4 dietary treatments each consisting of 12 replicates (cages). The experiment started with 6 birds per cage during the starter period of 2 wk. Then, up to 2 randomly chosen birds in each cage were removed, leaving 4 birds for the grower-finisher period. Continuous lighting was provided throughout the experimental period. The temperature in the cage at arrival was 32°C and was decreased gradually to ambient temperatures during the course of the experiment.

### Diets

The diets were mainly based on wheat and soybean meal and are given in detail elsewhere (Smink et al., 2008). The birds received a starter feed until d 14 and a grower-finisher feed between d 15 to 35. Four different fat sources were used in the starter and grower-finisher diets. The diets were in pelleted form (2.5 mm). The diets were fed on an ad libitum basis and the birds had free access to tap water. The inclusion level of experimental fat was 4 and 8% (wt/wt) in the starter and grower-finisher period, respectively. The 4 experimental fats consisted of sunflower oil (**SO**), a 50:50 mix of fully hydrogenated sunflower oil and sunflower oil (**HSO + SO**), palm oil (**PO**), and randomized palm oil (**RPO**). The macronutrients and the major fatty acids are summarized in Table 1.

### Sampling and Analysis

Excreta were collected in the starter period from d 10 to 14 and in the growing-finishing period during 17 to 21 and during 31 to 33 d of age. Excreta were collected quantitatively per cage, dried at 60°C, weighed,

**Table 1.** Analyzed macronutrient and fatty acid content (g/kg) of the experimental diets in the starter and grower-finisher period[1,2]

| | Starter period (d 0 to 14) | | | | Grower-finisher period (d 15 to 35) | | | |
|---|---|---|---|---|---|---|---|---|
| Item | HSO + SO | SO | PO | RPO | HSO + SO | SO | PO | RPO |
| Ash | 49 | 49 | 53 | 52 | 45 | 45 | 53 | 52 |
| CP | 216 | 214 | 217 | 213 | 204 | 201 | 203 | 197 |
| Crude fiber | 43 | 42 | 43 | 42 | 44 | 45 | 44 | 43 |
| Crude fat | 95 | 97 | 93 | 95 | 130 | 131 | 127 | 125 |
| Fatty acid | | | | | | | | |
| C14:0 | 1.5 | 1.4 | 0.51 | 0.63 | 0.19 | 0.09 | 1.01 | 1.06 |
| C16:0 | 10.0 | 9.58 | 27.3 | 28.1 | 11.9 | 11.4 | 45.8 | 44.7 |
| C16:1 | 0.00 | 0.13 | 0.15 | 0.15 | 0.00 | 0.00 | 0.19 | 0.20 |
| C18:0 | 23.1 | 3.14 | 3.32 | 3.43 | 42.1 | 4.52 | 4.90 | 5.01 |
| C18:1n-9 | 15.6 | 20.9 | 25.4 | 26.2 | 18.2 | 28.1 | 36.9 | 36.2 |
| C18:1n-7 | 1.10 | 1.15 | 1.16 | 1.18 | 1.25 | 1.34 | 1.34 | 1.35 |
| C18:2n-6 | 35.1 | 5.12 | 25.7 | 25.7 | 44.8 | 73.2 | 25.3 | 24.5 |
| C18:3n-3 | 2.00 | 2.09 | 2.05 | 2.10 | 1.90 | 1.97 | 1.92 | 1.91 |

[1]Values are means of duplicate analysis.

[2]Diets with hydrogenated sunflower oil mixed (50:50) with sunflower oil (HSO + SO), sunflower oil (SO), palm oil (PO), and randomized palm oil (RPO).

and ground. On d 33, two birds per cage were killed to determine body composition. Whole bodies were cut into pieces and ground and were for each replicate pooled, homogenized, sampled, and then dried to a constant weight in a forced-air oven at 60°C for a period of 3 d. The dried birds were weighed again and the percentage of water was calculated. To determine the initial body composition, 2 broilers were killed at d 1, applying procedures as described above. Also at d 35, up to 2 birds per cage were killed and used to determine enzyme activities in the liver. Livers were removed, placed in ice-cold saline, divided into portions for the different assays, snap-frozen in liquid nitrogen, and stored at −70°C until analysis. Hepatic lipids were extracted according to the method of Bligh and Dyer (1959) from the homogenate used for the assay of citrate synthase (**CS**) and 3-hydroxyacyl-coenzyme A dehydrogenase (**3-HOAD**). Part of the extract was also used for the determination of triacylglycerols and part for determination of it for the fatty acid composition of total hepatic lipids.

Crude fat concentration of diets and excreta was determined after acid hydrolysis (AOAC, 1990; method 954.02). To determine the fatty acid composition of the diets, and the whole bodies, samples were extracted with a chloroform:methanol (2:1, vol/vol) mixture according to the method of Folch et al. (1957). Then, 20 to 25 mg of the extracted fat was saponified with 0.5 *M* methanolic sodium hydroxide and methylated with boron trifluoride in methanol according to the method of Metcalfe et al. (1966). The fatty acid composition, DM, ash, crude fiber, and nitrogen were analyzed as is given in the former study (Smink et al., 2008).

## Determination of Enzyme Activities

For the assays of acetyl-coenzyme carboxylase (**ACC**; EC 6.4.1.2) and fatty acid synthase (**FAS**; EC 2.3.1.85) activities, fresh liver pieces were homogenized immediately with 5 strokes of a loosely fitted Dounce homogenizer in 3 volumes of ice-cold 250 m*M* mannitol, 50 m*M* HEPES, 6.2 m*M* Na-EDTA, 4 m*M* potassium citrate, and 2.5 m*M* β-mercaptoethanol, pH 7.5. The crude homogenate was centrifuged at 12,000 × *g* for 5 min. The supernatant was stored at −70°C until analyzed for the activities of ACC and FAS as described (Tijburg et al., 1988).

For the assay of CS (EC 2.3.3.1) and 3-HOAD (EC 1.1.1.35) activities, part of the frozen liver was placed in 9 volumes of 25 m*M* HEPES, 5 m*M* β-mercaptoethanol, pH 8.0, and homogenized with an IKA Ultra-Turrax T-5 tissue homogenizer (IKA, Staufen, Germany). The CS and 3-HOAD activities in the homogenate were assayed as described (Geelen et al., 2001).

Carnitine palmitoyltransferase-I (**CPT-I**; EC 2.3.1.21) activity was assayed in mitochondria isolated from a 20% homogenate in 250 m*M* sucrose, 20 m*M* Tris-HCl, and 1 m*M* EDTA (pH 7.4). The frozen liver was homogenized with 5 strokes of a glass-Teflon Potter-Elvehjem tissue homogenizer (OMNI International, Kennesaw, GA). The homogenate was centrifuged at 600 × *g* for 5 min (4°C). The supernatant was again centrifuged at 10,000 × *g* for 15 min (4°C). The pellet was resuspended in the homogenizing buffer and called mitochondrial preparation. The CPT-I activity was monitored as the incorporation of radio-labeled carnitine into acylcarnitine as reported by Guzman et al. (1994). Carnitine palmitoyltransferase-I activity that was insensitive to 100 μ*M* malonyl-coenzyme A was always subtracted from the CPT-I activity experimentally determined.

Measurement of diacylglycerol acyltransferase (EC 2.3.1.20) activity was described previously (Tijburg et al., 1988).

## Calculations

The intake of digested fat and fatty acid intake was calculated as analyzed fatty acid intake × apparent fat and fatty acid digestibility (fraction of intake). The digestibility coefficient measured during d 10 to 14 was used to determine the digestible intake during the first 14 d. The digestibility coefficients determined during d 17 to 21 and during 31 to 33 d were used for the calculation of the digestible intake during d 15 to 22 and d 29 to 33, respectively. Then, average of the determined digestibility of the 2 periods was used to calculate the intake of digestible fat and fatty acids during the period of d 22 to 29.

The deposition of fat and fatty acids was calculated by difference between the fat mass per bird in each cage minus the initial quantity present, estimated as the average body fat mass of the 1-d-old broilers.

The minimal de novo synthesis rate of individual fatty acids was calculated by difference between the quantity of a particular fatty acid deposited in the carcass and the digested quantity. For essential fatty acids, the digested minus deposition was calculated. This number represents the maximum disappearance rate.

The hepatic fatty acid content per liver was calculated using a conversion factor (Geelen and Gibson, 1976) and an assumed molecular weight of 850 g per triglyceride and 95% fatty acids per triglyceride.

## Statistical Analysis

Data from birds housed together in one cage served as the experimental unit. The effects of dietary treatment were statistically analyzed by 1-way ANOVA. The statistical differences between treatments were determined by a Tukey test. The level of statistical significance was preset at $P < 0.05$. Results are presented as least squares means and a pooled SEM. Statistical analysis was done with the SAS program (SAS, 2000).

**Table 2.** Effect of dietary fat source on fat content and fatty acid profile (% of methyl esters) of the whole body of broilers

| Item | Diet[1,2] | | | | Pooled SEM | *P*-value diet effect |
|---|---|---|---|---|---|---|
| | HSO + SO | SO | PO | RPO | | |
| Crude fat (g/kg) | 415[a] | 417[a] | 452[b] | 443[ab] | 6.9 | <0.001 |
| Fatty acids (%) | | | | | | |
| C14:0 | 0.47[b] | 0.41[a] | 0.78[c] | 0.83[d] | 0.007 | <0.001 |
| C16:0 | 18.8[b] | 15.6[a] | 27.0[c] | 28.5[d] | 0.19 | <0.001 |
| C16:1 | 3.58[b] | 1.97[a] | 4.33[c] | 4.27[c] | 0.13 | <0.001 |
| C18:0 | 8.15[c] | 6.28[b] | 5.06[a] | 5.16[a] | 0.10 | <0.001 |
| C18:1n-7 | 2.11[b] | 1.51[a] | 2.14[b] | 2.10[b] | 0.03 | <0.001 |
| C18:1n-9 | 33.9[b] | 29.6[a] | 40.5[c] | 39.6[c] | 0.30 | <0.001 |
| C18:2n-6 | 27.8[b] | 37.9[c] | 15.3[a] | 14.6[a] | 0.43 | <0.001 |
| C18:3n-3 | 0.96[b] | 0.84[a] | 0.97[b] | 0.97[b] | 0.02 | <0.001 |
| C20:1n-9 | 0.51[b] | 0.46[a] | 0.49[ab] | 0.47[ab] | 0.01 | 0.010 |
| C20:4n-6 | 0.66[b] | 0.78[c] | 0.53[a] | 0.51[a] | 0.03 | <0.001 |
| Total unsaturated (U) | 69.9[c] | 73.6[d] | 64.6[b] | 62.9[a] | 0.22 | <0.001 |
| Total saturated (S) | 27.9[b] | 23.0[a] | 33.4[c] | 35.1[d] | 0.21 | <0.001 |
| U:S ratio | 2.51[c] | 3.20[d] | 1.93[b] | 1.79[a] | 0.03 | <0.001 |

[a–d]Means in the same row with different superscripts differ significantly ($P < 0.05$).

[1]Diets with hydrogenated sunflower oil mixed (50:50) with sunflower oil (HSO + SO), sunflower oil (SO), palm oil (PO), and randomized palm oil (RPO).

[2]Numbers of observations: HSO + SO, 9; SO, 12; PO, 11; and RPO, 8.

## RESULTS

The effects of the dietary treatments on BW gain, feed intake, and feed conversion and effects of dietary fat source are presented elsewhere (Smink et al., 2008). Briefly, BW gain was not affected by the dietary treatments. Feed intake and feed conversion were significantly decreased for the SO birds. The results of body fat and fatty acid composition are presented in Table 2. The RPO- and PO-fed birds showed the highest content of body fat on d 33. The content of unsaturated and saturated fat was significantly different between the 4 diets. The RPO showed the highest content of saturated fat followed by the groups PO, HSO + SO, and SO. This difference was mainly related to differences in the concentration of C16:0. The concentration of unsaturated fatty acids was highest in SO birds, followed by HSO + SO, PO, and RPO, and was mainly related to differences in C18:2n-6.

The results on fat balance are given in Table 3. The intake of digestible fat was highest for the SO-fed broilers. The different dietary fats had a clear effect ($P = 0.02$) on fat deposition, which was highest in the PO and RPO broilers and lowest in the SO broilers. The ratio of fat deposition:digestible fat intake was significantly lower for the SO broilers in comparison with the others. Effects of the dietary treatments on the digestible intake and deposition of individual fatty acids are presented in Table 4. Both digestible intake and deposition of fatty acids were significantly affected by the dietary treatments. The PO and RPO group showed a high deposition of C16:0 and C18:1n-9. The predominant fatty acids in the HSO + SO and SO groups were C18:1n-9 and C18:2n-6. The ratio deposition:digestible intake for the essential fatty acids C18:2n-6 and C18:3n-3 was significantly higher for the PO and RPO groups. Results of the calculated minimal synthesis (deposition − digestible intake) of nonessential fatty acids and the maximum disappearance (digestible intake − deposition) of essential fatty acids are given in Table 5. The calculated minimal synthesis of monounsaturated fatty acids (**MUFA**) was significantly lower for the broilers in the SO group in comparison with the other groups. The minimal synthesis of SFA was highest in the SO and HSO + SO group. The calculated maximal disappearance of the essential fatty acids C18:2n-6 and C18:3n-3 was highest for the SO group and lowest in the broilers of treatment groups PO and RPO.

Effects of the dietary treatments on hepatic fat content and enzyme activities are shown in Table 6. There were no statistically significant effects of diet on hepatic enzyme activities. There were significant dietary effects on hepatic fatty acid content for MUFA (Table 7). The livers of the broilers in the SO group had the lowest content of MUFA, whereas the amount of PUFA was not affected. The ratios of C16:1:C16:0 and C18:1:C18:0 were lowest in the SO group and significantly different from those in the HSO + SO, PO, and RPO groups. There is a negative correlation between hepatic PUFA profile of the birds ($R^2 = 0.85$; $P < 0.001$ and $R^2 = 0.93$; $P < 0.001$ for C16:1:C16:0 and C18:1:C18:0, respectively; Figure 1).

## DISCUSSION

### *Body Deposition of Fatty Acids*

Body weight gain was similar among treatment groups. However, in SO birds, feed intake and feed conversion was lower (Smink et al., 2008). This difference was quantitatively accounted for by a difference in the digestibility of the fat source. Fat digestibility of the SO group was much higher than in the other groups (75% in SO vs. 61% as a mean in the other groups),

**Table 3.** Effect of dietary fat source on the fat balance in broilers during d 1 to 33

| Item | Diet[1,2] | | | | Pooled SEM | *P*-value diet effect |
|---|---|---|---|---|---|---|
| | HSO + SO | SO | PO | RPO | | |
| Fat intake (g/bird) | 373[b] | 343[a] | 359[ab] | 348[a] | 4.7 | <0.001 |
| Digestible fat intake (g/bird) | 200[a] | 256[c] | 226[b] | 234[b] | 5.7 | <0.001 |
| Fat deposition (g/bird) | 242[a] | 254[a] | 281[a] | 284[a] | 10.5 | 0.020 |
| Digestible intake:fat intake | 0.54[a] | 0.75[c] | 0.63[b] | 0.67[b] | 0.016 | <0.001 |
| Fat deposition:digestible fat intake | 1.22[b] | 0.99[a] | 1.24[b] | 1.21[b] | 0.040 | <0.001 |

[a–c]Means in the same row with different superscripts differ significantly ($P < 0.05$).

[1]Diets with hydrogenated sunflower oil mixed (50:50) with sunflower oil (HSO + SO), sunflower oil (SO), palm oil (PO), and randomized palm oil (RPO).

[2]Numbers of observations: HSO + SO, 9; SO, 12; PO, 11; and RPO, 8.

which is in agreement with literature (Wiseman and Salvador, 1991; Ketels, 1994). Furthermore, the reduction in amino acid intake by SO broilers did not result in reduced BW gain, indicating that the amino acid content of all diets was not limiting growth. This was expected because optimization of the diets was based on the composition of the SO diet.

The dietary fatty acid pattern clearly affected the fatty acid pattern of the body. The relation between dietary PUFA and their proportion of fatty acids in the body agrees with literature data (Bavelaar and Beynen, 2003; Waldroup and Waldroup, 2005). The deposition of fat was higher for the PO- than for SO-fed birds. This was expected as based on other investigators finding a decrease in abdominal fat deposition using PUFA-rich sources in comparison with tallow (Sanz et al., 1999, 2000a; Newman et al., 2002). However, the total concentration of fat in broilers found in other studies was not different between birds fed either saturated or unsaturated fat (Crespo and Esteve-Garcia, 2002a). There was no difference in fat deposition between the HSO + SO and the SO group. When expressed relative to the digestible fat intake, fat deposition rates were markedly lower for the SO group when compared with all other treatments. These results indicate that both dietary C16:0 and C18:0 versus C18:2 increase fat deposition, implying that our hypothesis is confirmed.

Fatty acid deposition is the result of absorption, de novo synthesis, and β-oxidation of fatty acids. The deposition:digestible intake ratio reflects the net amount of synthesized or the proportion not oxidized, or both. A deposition:digestible intake >1 of a certain

**Table 4.** Effect of dietary fat source on the digestible fatty acid intake and deposition in broiler chickens

| Item | Diet[1,2] | | | | Pooled SEM | *P*-value diet effect |
|---|---|---|---|---|---|---|
| | HSO + SO | SO | PO | RPO | | |
| Digestible fatty acid intake (g/bird) | | | | | | |
| C16:0 | 16.0[a] | 18.6[a] | 63.3[b] | 70.0[c] | 1.66 | <0.001 |
| C16:1 | 0.00[a] | 0.02[a] | 0.33[b] | 0.34[b] | 0.007 | <0.001 |
| C18:0 | 17.8[b] | 4.81[a] | 4.62[a] | 5.94[a] | 2.14 | <0.001 |
| C18:1n-7 | 1.91[b] | 1.55[a] | 2.13[b] | 2.15[b] | 0.097 | <0.001 |
| C18:1n-9 | 40.5[a] | 56.4[b] | 78.1[c] | 77.2[c] | 1.38 | <0.001 |
| C18:2n-6 | 104.9[b] | 155.6[c] | 56.3[a] | 54.7[a] | 2.03 | <0.001 |
| C18:3n-3 | 4.51[b] | 4.18[a] | 4.30[ab] | 4.32[ab] | 0.078 | 0.044 |
| Fat deposition (g/bird) | | | | | | |
| C14:0 | 1.07[a] | 0.97[a] | 2.08[b] | 2.21[b] | 0.060 | <0.001 |
| C16:0 | 42.6[a] | 37.4[a] | 71.6[b] | 76.3[b] | 2.34 | <0.001 |
| C16:1 | 8.2[b] | 4.8[a] | 11.6[c] | 11.5[c] | 0.51 | <0.001 |
| C18:0 | 18.5[b] | 14.8[a] | 13.3[a] | 13.8[a] | 0.65 | <0.001 |
| C18:1n-7 | 4.8[b] | 3.6[a] | 5.7[c] | 5.6[bc] | 0.201 | <0.001 |
| C18:1n-9 | 77.2[a] | 70.7[a] | 107.3[b] | 106.1[b] | 3.96 | <0.001 |
| C18:2n-6 | 63.6[b] | 91.0[c] | 40.4[a] | 38.9[a] | 2.51 | <0.001 |
| C18:3n-3 | 2.18[a] | 2.01[a] | 2.58[b] | 2.59[b] | 0.085 | <0.001 |
| C20:1n-9 | 1.16[ab] | 1.10[a] | 1.29[b] | 1.26[ab] | 0.049 | 0.020 |
| C20:4n-6 | 1.43[a] | 1.78[b] | 1.33[a] | 1.29[a] | 0.055 | <0.001 |
| Deposition:digestible intake | | | | | | |
| C16:0 | 2.68[c] | 2.01[b] | 1.14[a] | 1.09[a] | 0.087 | <0.001 |
| C18:0 | 1.50[a] | 3.23[b] | 2.94[b] | 2.38[ab] | 0.251 | <0.001 |
| C18:1n-9 | 1.91[b] | 1.25[a] | 1.37[a] | 1.37[a] | 0.064 | <0.001 |
| C18:2n-6 | 0.61[a] | 0.58[a] | 0.72[b] | 0.71[b] | 0.020 | <0.001 |
| C18:3n-3 | 0.48[a] | 0.48[a] | 0.60[b] | 0.60[b] | 0.016 | <0.001 |

[a–c]Means in the same row with different superscripts differ significantly ($P < 0.05$).

[1]Diets with hydrogenated sunflower oil mixed (50:50) with sunflower oil (HSO + SO), sunflower oil (SO), palm oil (PO), and randomized palm oil (RPO).

[2]Numbers of observations: HSO + SO, 9; SO, 12; PO, 11; and RPO, 8.

**Table 5.** Effect of dietary fat source on the minimal synthesis (deposition − digestible intake) of nonessential and maximal disappearance rate (digestible intake − deposition) of essential fatty acids

| Item | Diet[1,2] | | | | Pooled SEM | *P*-value diet effect |
|---|---|---|---|---|---|---|
| | HSO + SO | SO | PO | RPO | | |
| Minimal synthesis (g/bird) | | | | | | |
| C16:0 | 26.6[c] | 18.8[b] | 8.29[a] | 6.24[a] | 2.22 | <0.001 |
| C16:1 | 8.20[b] | 4.74[a] | 11.2[c] | 11.1[c] | 0.51 | <0.001 |
| C18:0 | 0.70[a] | 10.0[b] | 8.65[b] | 7.84[b] | 2.23 | 0.021 |
| C18:1n-9 | 36.7[b] | 14.3[a] | 29.2[b] | 28.9[b] | 3.59 | <0.001 |
| C18:1n-7 | 2.87[b] | 2.05[a] | 3.53[c] | 3.45[bc] | 0.20 | <0.001 |
| MUFA[3] | 47.8[b] | 21.1[a] | 43.9[b] | 43.5[b] | 3.48 | <0.001 |
| SFA[3] | 27.3[b] | 28.8[b] | 16.9[ab] | 14.1[a] | 4.19 | 0.010 |
| Maximum disappearance rate (g/bird) | | | | | | |
| C18:2n-6 | 41.3[b] | 64.7[a] | 15.8[c] | 15.7[c] | 2.30 | <0.001 |
| C18:3n-3 | 2.35[a] | 2.17[a] | 1.74[b] | 1.71[b] | 0.073 | <0.001 |

[a–c]Means in the same row with different superscripts differ significantly ($P < 0.05$).

[1]Diets with hydrogenated sunflower oil mixed (50:50) with sunflower oil (HSO + SO), sunflower oil (SO), palm oil (PO), and randomized palm oil (RPO).

[2]Numbers of observations: HSO + SO, 9; SO, 12; PO, 11; and RPO, 8.

[3]MUFA = monounsaturated fatty acids; SFA = saturated fatty acids.

fatty acid means a net de novo synthesis, whereas the ratio of <1 indicates a net oxidation or change, or both, due to elongation and desaturation. All main nonessential fatty acids C16:0, C18:0, and C18:1 show net synthesis, whereas the ratio is <1 for the essential fatty acids C18:2, C18:3, and total PUFA, pointing at net oxidation. However, the ratios of deposition:digestible intake for C18:2 and C18:3 in the PO and RPO groups were significantly higher than in the HSO + SO and the SO-fed birds. The reason for this is not clear, but it is probably due to the lower intake of C18:2 in PO and RPO birds. Therefore, the relative amount needed for synthesizing essential fatty acids from C18:2 was higher. The maximum disappearance rate of PUFA for the SO group was higher in comparison to the other groups, indicating more oxidation of PUFA. Studies with rats indicated that C18:2 and C18:1 have higher oxidation or elongation-desaturation, or both, rates than C16:0 and C18:0 (Leyton et al., 1987).

The calculated minimal synthesis of MUFA + SFA was highest for the HSO + SO group followed by RPO, PO, and SO. The feed intake of the treatments with saturated fat is higher in comparison with the SO group (Smink et al., 2008). This means that the birds in the latter group had a lower intake of carbohydrates to be used as substrate for the de novo synthesis of fatty acids. In addition, it should be noted also that the higher (calculated) rate of fatty acid synthesis of the HSO + SO group in comparison with the PO and RPO groups might be due to the relatively high oxidation or elongation-desaturation, or both, rates of PUFA. The results of the SO group showed a lower calculated synthesis of MUFA in comparison with the broilers in the other groups. This agrees with a lower MUFA synthe-

**Table 6.** Effect of dietary fat source on hepatic fat content and hepatic enzyme activities in broiler chickens

| Item | Diet[1,2] | | | | Pooled SEM | *P*-value diet effect |
|---|---|---|---|---|---|---|
| | HSO + SO | SO | PO | RPO | | |
| BW (g/bird) | 1,776 | 1,746 | 1,767 | 1,760 | 38.0 | 0.98 |
| Liver weight (g/bird) | 56.0 | 51.2 | 52.5 | 56.8 | 2.32 | 0.27 |
| Liver fat (mg/g of liver) | 19.9 | 9.68 | 16.5 | 20.3 | 3.02 | 0.053 |
| Enzyme activities[3] | | | | | | |
| ACC[4] | 3.51 | 2.41 | 2.75 | 3.16 | 0.33 | 0.12 |
| FAS[4] | 2.60 | 1.90 | 2.17 | 2.29 | 0.23 | 0.21 |
| CPT-I[5] | 12.7 | 15.5 | 15.2 | 12.9 | 1.36 | 0.32 |
| 3-HOAD[6] | 547 | 567 | 510 | 475 | 28.2 | 0.11 |
| Citrate synthase[5] | 72.2 | 64.3 | 73.8 | 73.8 | 5.0 | 0.49 |
| DGAT[6] | 0.64 | 0.61 | 0.60 | 0.63 | 0.056 | 0.96 |

[1]Diets with hydrogenated sunflower oil mixed (50:50) with sunflower oil (HSO + SO), sunflower oil (SO), palm oil (PO), and randomized palm oil (RPO).

[2]Numbers of observations: HSO + SO, 12; SO, 12; PO, 12; and RPO, 12.

[3]ACC: = acetyl-coenzyme A carboxylase; FAS = fatty acid synthase; CPT-I = carnitin palmitoyltransferase-I; 3-HOAD = 3-hydroxyacyl-coenzyme A dehydrogenase; DGAT = diacylglycerol acyltransferase. Enzyme activities are expressed as nanomoles per minute per milligram of protein.

[4]Measured in homogenate protein.

[5]Measured in mitochondrial protein.

[6]Measured in microsomal protein.

**Table 7.** Effect of dietary fat source on hepatic fatty acids content (mg/bird) in broiler chickens

| Fatty acid | Diet[1,2] | | | | Pooled SEM | *P*-value diet effect |
|---|---|---|---|---|---|---|
| | HSO + SO | SO | PO | RPO | | |
| C14:0 | 4.55 | 1.34 | 3.08 | 4.41 | 0.916 | 0.053 |
| C16:0 | 249[a] | 83.0[a] | 182[a] | 243[a] | 45.4 | 0.040 |
| C16:1 | 29.2[a] | 4.82[a] | 21.2[a] | 29.6[a] | 6.94 | 0.046 |
| C18:0 | 196 | 98.8 | 149 | 184 | 27.7 | 0.067 |
| C18:1n-7 | 20.4[ab] | 5.72[a] | 18.2[ab] | 23.6[b] | 4.05 | 0.016 |
| C18:1n-9 | 325[ab] | 81.5[a] | 241[ab] | 346[b] | 67.4 | 0.030 |
| C18:2n-6 | 177 | 112 | 123 | 146 | 25.5 | 0.22 |
| C18:3n-3 | 1.86[ab] | 0.982[a] | 1.44[ab] | 2.53[b] | 0.395 | 0.048 |
| C20:1n-9 | 3.88[ab] | 1.61[a] | 4.54[ab] | 5.22[b] | 0.753 | 0.009 |
| C20:2n-6 | 5.41 | 5.16 | 3.93 | 3.80 | 0.693 | 0.24 |
| C20:3n-6 | 9.52[ab] | 4.39[a] | 8.27[ab] | 9.37[b] | 1.36 | 0.032 |
| C20:4n-6 | 68.1 | 53.9 | 54.5 | 57.7 | 7.64 | 0.52 |
| C22:4n-6 | 5.91[a] | 7.02[a] | 4.34[a] | 4.36[a] | 0.757 | 0.044 |
| C22:3 | 4.97[ab] | 5.61[b] | 3.35[ab] | 3.13[a] | 0.606 | 0.013 |
| C22:6n-3 | 6.44[ab] | 3.51[a] | 8.72[b] | 8.64[b] | 0.799 | <0.001 |
| SFA[3] | 450[a] | 184[a] | 335[a] | 433[a] | 73.3 | 0.047 |
| USFA[3] | 680 | 296 | 510 | 662 | 112 | 0.063 |
| MUFA[3] | 378[ab] | 94.1[a] | 285[ab] | 405[b] | 80.7 | 0.028 |
| PUFA[3] | 302 | 202 | 225 | 257 | 37.7 | 0.27 |
| C16:1:C16:0 | 0.099[b] | 0.043[a] | 0.092[b] | 0.108[b] | 0.0119 | 0.001 |
| C18:1:C18:0 | 1.39[b] | 0.67[a] | 1.37[b] | 1.68[b] | 0.175 | 0.002 |

[a,b]Means in the same row with different superscript differ significantly ($P < 0.05$).

[1]Diets with hydrogenated sunflower oil mixed (50:50) with sunflower oil (HSO + SO), sunflower oil (SO), palm oil (PO), and randomized palm oil (RPO).

[2]Numbers of observations: HSO + SO, 12; SO, 11; PO, 11; and RPO, 11.

[3]MUFA = monounsaturated fatty acids; SFA = saturated fatty acids; USFA = unsaturated fatty acids; PUFA = polyunsaturated fatty acids.

sis recently found in pigs fed with a SO diet in comparison with a diet with 5% beef tallow (Mitchaothai et al., 2008). Villaverde et al. (2006) suggested that in chickens a mechanism exists to change the ratio of de novo synthesis of SFA:(MUFA + SFA) to keep a specific range of these fatty acids in the membranes. Monounsaturated fatty acid synthesis is regulated in the liver. Polyunsaturated fatty acids in the diet will inhibit the activity of the enzyme $\Delta^9$-desaturase in the liver (Kouba and Mourot, 1998), resulting in a reduced conversion of SFA into MUFA. This agrees with the lower ratio of C16:1:C16:0 and C18:1:C18:0 in the liver in the SO group in comparison with the other groups and appeared to have a strong negative correlation between hepatic PUFA of all birds. Such a negative correlation agrees with earlier work of Infield and Annison (1973).

## Hepatic Fatty Acids and Enzyme Activities

The enzymes ACC and FAS are involved in fatty acid synthesis in the liver. In literature, a depressed activity of these enzymes after feeding C18:2 was found in studies with mice (Javadi et al., 2007) and chickens (Sanz et al., 2000b). The negative correlation ($R^2 = 0.25$; $P < 0.001$) that we found between PUFA concentration in the liver and enzyme activities of both ACC and FAS (results not shown) is in line with these observations, but the dietary effect did not reach statistical significance.

The activity of 3-HOAD, a key enzyme for fatty acid oxidation, was similar for the SO and the HSO + SO treatments. It was expected that the high dietary PUFA and low SFA in the SO group had increased β-oxidation in comparison with the HSO + SO group (Sanz et al.,

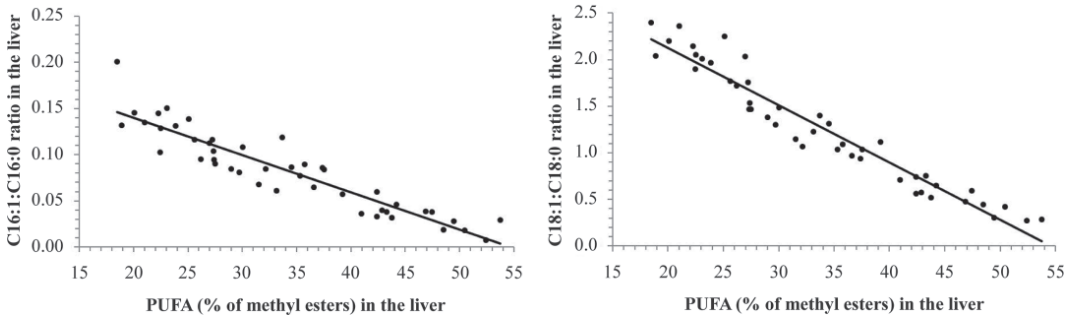


**Figure 1.** Effect of polyunsaturated fatty acids (PUFA; in % of methyl esters) in the liver on the ratio of C16:1:C16:0 and C18:1:C18:0 in the liver of broiler chickens fed with different fat sources (values are means of 2 chickens). C16:1:C16:0 = 0.22 − 0.00405 PUFA ($R^2 = 0.85$; intercept: $P < 0.0001$); PUFA: $P < 0.0001$; C18:1:C18:0 = 3.36 − 0.0616 PUFA ($R^2 = 0.93$; intercept: $P < 0.0001$); PUFA: $P < 0.0001$.

2000b). However, due to the very low digestibility of C18:0 in the HSO + SO group, the difference in the fatty acid pattern of digested fatty acids was limited between the 2 groups.

Liver fat mass tended ($P = 0.053$) to be lower in SO birds. This agrees with Pinchasov and Nir (1992), who demonstrated a reduced liver fat content at a high inclusion level of PUFA in the diet. The reduction of fatty acids in the liver of the broilers in our study was significant for MUFA. This agrees well with others (Dänicke et al., 1999; Crespo and Esteve-Garcia, 2002b). The reduced C16:1:C16:0 and C18:1:C18:0 in the SO group indicates a lower $\Delta^9$-desaturase activity. The inhibition of this enzyme will impair triacylglycerol secretion in hepatocytes (Legrand et al., 1997).

Palm oil and RPO mainly differ with regard to the position of C16:0 and C18:1 on the glycerol molecule. The change from C16:0 from the *sn*-1,3 position to the *sn*-2 position increases its digestibility in chickens as was shown previously (Smink et al., 2008). In Table 4, a significantly higher digestible intake of C16:0 in the RPO group is shown compared with PO. Contrary to C16:0, the quantitative effect of the position of C18:1 on the glycerol molecule on digestibility is small. The effect on digestibility agrees well with studies in rats (Renaud et al., 1995). Apart from an increased digestibility, there were no other effects of randomization of palm oil.

The deposition:digestible intake ratios for fatty acids and the calculated de novo synthesis and maximum disappearance rates of fatty acids were clearly affected by dietary treatment. Based on these results, it is likely that the SO diet resulted in higher oxidation or desaturation-elongation, or both, rates of fatty acids. This is confirmed by the maximum disappearance rate of fatty acids, which was highest for the SO group and lowest for the RPO and PO groups. The combination of a decreased synthesis of MUFA in the liver and the calculated reduction in the minimal synthesis of MUFA in the whole body of the broilers in the SO group indicate a reduction of de novo fatty acid synthesis. However, the group mean activities of FAS and ACC were not significantly different from the other treatments.

In conclusion, the present data show that, like tallow, vegetable saturated fat sources increase body fat deposition in comparison with PUFA. A combination of a higher de novo synthesis of MUFA and a lower fatty acid oxidation rate seems to be responsible.

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