

## Hybrid ducks overfed with boiled corn develop an acute hepatic steatosis with decreased choline and polyunsaturated fatty acid level in phospholipids

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**Summary** — Hepatic and plasma lipid changes were investigated in hybrid ducks after steatosis was induced by overfeeding. Fourteen hybrid ducks were force-fed (11 kg corn in 13 days). The liver of the overfed ducks weighed ten times more than that of controls (695 vs 69 g). The phospholipid, ash, water and protein content of the liver were only slightly higher (2.6-, 3.6-, 4.4- and 3.2-fold, respectively) but the liver lipid concentration was 16.5 times higher after than before overfeeding (56.1 vs 3.4 g/100 g liver, respectively). Liver phosphatidylcholine concentration was higher in the control group (32.4 vs 22.9 mol/100 mol phospholipid) revealing a choline deficiency that could contribute to the development of liver steatosis. In liver phospholipid, the level of linoleic and arachidonic acids were markedly decreased in the overfed ducks, from 5 and 18.1 to 3.7 and 7.1 mol/100 mol fatty acids, respectively, and were mainly replaced by oleic acid. Plasma triacylglycerols increased 5.9-fold, cholesterol 2.2-fold and phospholipids 1.6-fold. Heat-induced (50 min at 105 °C) fat release, an index of poor liver integrity, was particularly high for the large, fat livers which contained low levels of phospholipids and phosphatidylcholine.

**liver steatosis / hybrid ducks / overfeeding / phospholipids / essential fatty acids**

**Résumé** — Le gavage de canards mulards avec du maïs provoque une forte stéatose hépatique associée à une diminution du taux de choline et des acides gras polyinsaturés des phospholipides. Les modifications hépatiques et plasmatiques dues au gavage ont été étudiées chez 14 canards mulards gavés (11 kg de maïs en 13 jours). Le gavage a augmenté le poids de foie (695 versus 69 g). Alors que les phospholipides, les cendres, l'eau et les protéines du foie n'augmentent que légèrement (respectivement 2,6, 3,6, 4,4 et 3,2 fois), le pourcentage de triglycérides est multiplié par 8 (56,1 versus 3,4 g/100 g de foie). Le pourcentage de phosphatidylcholine hépatique diminue avec le

*Abbreviations:* DPG: diphosphoglyceride; LPC: lysophosphatidylcholine; PA: phosphatidic acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; SM: sphingomyeline; TLC-FID: thin layer chromatography with flame ionization detector.

*gavage (32,4 versus 22,9 mol/100 mol de phospholipides), suggérant une déficience en choline qui pourrait contribuer à la stéatose hépatique. Les taux d'acide linoléique et arachidonique des phospholipides hépatiques sont considérablement diminués (de 5,0 et 18,1 à 3,7 et 7,1 mol/100 mol acides gras) et pratiquement remplacés par de l'acide oléique chez les animaux gavés. Les triglycérides, le cholestérol et les phospholipides plasmatiques sont multipliés respectivement par 5,9, 2,2 et 1,6. Le taux de fonte (pertes de lipides après chauffage pendant 50 minutes à 105 °C) est un critère de faible intégrité cellulaire et de mauvaise qualité technologique du foie gras. Il est élevé pour les foies gros et gras contenant peu de phospholipides et de phosphatidylcholine.*

### **stéatose hépatique / canard mulard / gavage / phospholipides / acides gras essentiels**

## **INTRODUCTION**

The fatty liver or *foie gras* obtained by over-feeding geese and ducks is a unique model for nutritional steatosis. Force-feeding a hyperenergetic, hypercarbohydrate diet (99% corn + 0.5% NaCl + 0.5% goose fat) turns a normal liver (weighing less than 100 g) into a fatty liver (400 to 1 000 g) in less than 3 weeks. A few studies have been carried out on the mule hybrid duck (Auvergne, 1992; Salichon et al, 1994), which is now commonly used for *foie gras* production. Studies carried out in geese and the muscovy duck have demonstrated that most of the lipid accumulated in the liver is triacylglycerol (Leclercq et al, 1968; Blum et al, 1990; Baudonnet-Lenfant et al, 1991). Protein, water and phospholipids are proportionally reduced, but their total amounts in the liver are increased. In geese, it appears that triacylglycerols are actively synthesized from carbohydrates within the liver and partly released into blood in the form of very low density lipoproteins (VLDL). In spite of the increase in blood VLDL levels and very active triacylglycerol transport resulting in peripheral fatness (Rousselot-Paillet et al, 1992), synthesis of VLDL by the liver appears insufficient to prevent steatosis (Hermier et al, 1991; Salichon et al, 1991).

It was also observed that the fat release during the exposure of the dissected liver to heat (50 min at 105 °C), an index of poor liver integrity currently used in grading *foie*

*gras*, was high for large livers. In geese, a significant negative correlation ( $r = -0.7$ ) was found between fat release and liver phospholipid contents (Blum et al, 1990).

Phospholipids of cell membranes and their fatty acid composition are related to membrane fluidity (Kinsella, 1991). Phospholipids are also needed for triacylglycerol transport in VLDL and may be involved in liver integrity. The present experiment was carried out to determine the lipid characteristics of liver steatosis in hybrid ducks, with particular emphasis on phospholipids in relation to heat-induced fat release. The fatty acid composition of total liver phospholipids was also determined.

## **MATERIALS AND METHODS**

### **Animals and diets**

One-day-old male hybrid ducks (male muscovy duck x female common duck) of commercial origin (Sepalm, 40250 Mugron, France) were raised according to Rousselot et al (1992). From day 1 to 12 weeks of age, the ducks were fed on a series of diets (table I). The implementation of the experimental protocol was registered with the French Institutional Animal Care Committee (Agricultural Ministry, Animal Health and Protection Bureau). The ducks were given free access to these diets until 6 weeks of age; thereafter, they were restricted to a meal of 250 g once a day between days 43 and 63, and of 220 g/day between days 64 and 74. This was ingested in one single meal, very quickly in the late stages (approximately

**Table I.** Composition of the experimental diets (g/kg diet).

<i>Ingredient</i>	<i>0 to 1 week (ad libitum)</i>	<i>1 to 10 weeks (AL then R)<sup>1</sup></i>	<i>10 to 12 weeks (R then AL)<sup>1</sup></i>	<i>12 to 14 weeks (Force-fed)</i>
Wheat	100	100	100	
Corn	544	403	420	990
Barley		100		
Pea		23.8	100	
Soybean meal	238	187	71	
Sunflower meal	25	30	60	
Corn distillers	22	25	50	
Wheat bran		20	60	
Alfalfa meal	5	40	59	
Meat meal	40	20	13.8	
Sugarcane molasses		20	20	
Limestone	7.7	7.7	7.2	
Dicalcium phosphate	10.7	13	12.5	
Salt	3	3	3	5
DL-methionine	1.2	1.5	1	
Premix <sup>2</sup>	3.5	3.5	2.5	
Poultry fat <sup>3</sup>		2.5	20	5
Calculated analysis				
Metabolizable energy (MJ/kg)	12.2	11.5	11.5	13.7
Protein (%)	20.5	18.2	15.5	8.9

<sup>1</sup> AL: ad libitum; R: restricted. <sup>2</sup> The composition of vitamin and trace element premix (mg/kg) was as follows: retinol, 940; cholecalciferol, 14; all-rac- $\alpha$ -tocopherol, 2 900; menadione, 600; riboflavin, 1 200; calcium pantothenate, 2 200; nicotinic acid, 6 900; vitamin B12, 3; choline chloride, 114 000; FeSO<sub>4</sub>, 35 000; CuSO<sub>4</sub>, 7 125; ZnO, 29 000; MnO<sub>2</sub>, 47 000; CoSO<sub>4</sub>, 260; Na<sub>2</sub>SeO<sub>3</sub>, 90; KI, 458. <sup>3</sup> Mixed poultry fat during the rearing period; goose fat during the final over-feeding period.

10 min on day 74). Ten days before force-feeding, the food supply was progressively increased (+17 g/day) until it finally reached 390 g/day. From 12 weeks of age, some ducks were force-fed twice a day for 12.5 days (25 meals and a total consumption of 11 kg corn). The force-feeding diet of whole yellow corn and salt (0.5%) was boiled with 30% (w/w) water and mixed with goose fat (0.5%) just before force-feeding.

The effect of overfeeding was evaluated by comparison with controls slaughtered at 12 weeks of age. This comparison is possible as adult body weight is attained at about 11 weeks of age and ducks maintained beyond 15 weeks of age with free access to food do not show significant increases in body weight and liver composition (Auvergne, 1992).

### **Fat release**

Fat release was determined immediately after slaughter for each liver using a 60 g sample heated to 105 °C for 50 min. This release was expressed as the percent of liver sample weight. All the other analyses were performed using frozen samples (-18 °C).

### **Liver lipid analyses**

Liver lipids were extracted quantitatively by homogenizing a minced tissue specimen in chloroform/methanol 2/1 (v/v) (Folch et al, 1957). Total lipids were quantified gravimetrically.

Cholesteryl ester, triacylglycerol, free fatty acid, free cholesterol, monoglyceride and total phospholipid contents were determined by thin layer chromatography with a flame ionization detector (TLC-FID) using a Iatroskan analyzer with ten silica-gel thin layer chromatography rods (chromarods) and developing tank (Iatron Laboratories, Japan). Ten  $\mu\text{g}$  of lipid in 0.3  $\mu\text{L}$  chloroform were spotted with a syringe on each chromarod. The chromarods were developed for 30 min in the developing tank saturated with the development solvent: hexane/diethyl ether/acetic acid 85/15/1 (v/v/v) (Innis and Glaninin, 1981). After evaporation of the solvent at 50 °C, the chromarods were scanned with the Iatroskan. The hydrogen flow rate was 175 mL/min, the air flow rate 1 850 mL/min, the scanning speed 0.4 cm/s and the recorder sensitivity 50 mV. The software used (Boreal, JMBS Development, Grenoble, France) recorded chromatograms and integrated peaks with reference to an external standard (Sigma Chemical Co, St Louis, MO, USA). Due to the low concentrations of phospholipids in the fatty liver, the phospholipids had to be separated and concentrated using silica-gel Sep-Pak cartridges (Waters division of Millipore, Milford, MA, USA). Aliquots of total lipid extract were loaded onto the top of the Sep-Pak cartridge. Nonpolar lipids were removed with 30 mL chloroform and total phospholipids were eluted with 7.5 mL methanol. The methanol was evaporated and the phospholipids dissolved in chloroform (Juaneda and Rocquelin, 1985). Phosphatidic acid (PA), diphosphoglyceride (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylcholine (PC), sphingomyeline (SM) and lysophosphatidylcholine (LPC) contents were also determined by TLC-FID with the Iatroskan. Chloroform/methanol/water 80/35/5 (v/v/v) was used as the development solvent with a 35 min development time (Innis and Glaninin, 1981). Fatty acid profiles were determined from the total liver phospholipid extracts. Samples were transesterified with 10 mL methanol:  $\text{H}_2\text{SO}_4$  (5% v/v) in a flask with a reflux condenser at 80 °C for 2 h. The flask contents were transferred to a test tube containing 10 mL *n*-hexane and 10 mL distilled water. After thorough mixing, the two layers were separated. The upper layer, a solution of fatty acid methyl esters in *n*-hexane, was analyzed using a gas chromatograph (Gira Instruments, Morlaas, France) according to Slover and Lanza (1979). Samples were eluted by using a programmed column temperature increase of

3 °C/min from 150 °C to 190 °C after a 6 min stage at 150 °C, and an injector temperature of 250 °C. The FID temperature was 250 °C and a helium flow rate of 1.5 mL/min was applied in a 25 m capillary column (0.32 mm of diameter) with polyethylene glycol (0.5  $\mu\text{m}$  thickness) as stationary phase from Scientific Glass Engineering (Australia).

### **Blood plasma lipid analyses**

Blood was sampled using 0.12% EDTA as an anticoagulant after a 12 h food deprivation period, immediately before slaughter. Plasma was obtained by using low speed centrifugation of blood. Total plasma cholesterol, free cholesterol, triacylglycerol and phospholipid levels were determined by enzymatic methods using kits (refs 61236, 61491, 61225, 61105) provided by BioMérieux (Charbonnière-les-Bains, France). Cholesterol esters were estimated as the difference between total and free cholesterol. Plasma phospholipids were separated from nonpolar lipids on chromarods using a hexane/diethyl oxide/acetic acid 85/15/3 (v/v/v) solvent (Mares et al, 1983) over a 40 min period. Nonpolar lipids were burned in the Iatroskan FID and phospholipids were then developed using chloroform/ methanol/water 80/35/5 (v/v/v) solvent for 35 min before solvent evaporation and scanning.

### **Other liver analyses**

Crude liver protein ( $N \times 6.25$ ) was determined using the Kjeldhal method (AOAC, 1970). Dry matter was obtained after 12 h at 105 °C and ashes after 16 h at 550 °C.

### **Statistical analyses**

All data were expressed as means and standard error. Statistical analyses were performed using a one-way ANOVA. Pearson correlation analyses were performed to evaluate associations between fat release and biological variables. Probabilities of less than 0.05 were judged significant.

## RESULTS

### *Food intake, body and liver weights and fat release of foie gras*

During the 12.5 day overfeeding period (two meals a day) each bird was force-fed an average amount of 11 kg corn (14% moisture). As a consequence, body and liver weights were significantly higher in the overfed than in the control group, but whereas body weight was 1.6-fold higher, liver weight was 10-fold higher (table II). The liver contributed to 25% of the total body weight increase. Heat-induced fat release, negligible before over-

feeding, was 40.7% of the liver weight. Individual variability was high. Coefficients of variation were 15 and 21% for the liver weight and fat release, respectively.

### *Influence of overfeeding on liver composition*

Liver components underwent large quantitative variations during force-feeding. These variations, shown in table III, have been expressed both as a proportion of liver weight and as total amount per liver. As a proportion of liver weight, lipids were dramatically preponderant in the overfed

**Table II.** Body, liver weights and liver fat release before and after overfeeding <sup>1</sup>.

	<i>Before</i>	<i>After</i>
Body weight (g)	3 957 ± 78	6 504 ± 123 *
Liver weight (g)	69 ± 4	695 ± 29 *
Liver weight/body weight x 100	1.84 ± 0.13	10.7 ± 0.39
Fat release <sup>1</sup> (%)	0.1 ± 0.1	40.7 ± 2 *

<sup>1</sup> Values are means ± SEM for data obtained from 14 animals both controls at 12 weeks (before) and overfed 11 kg corn/bird in 13 days (after). Fat release, used as an index of liver integrity, was expressed as the mass of fat released after heating a sample of liver for 50 min to 105 °C/mass of the liver before heating. \* Significantly different from measurement before overfeeding,  $P < 0.001$ .

**Table III.** Chemical composition of liver in ducks before and after overfeeding <sup>a</sup>.

<i>Overfeeding</i>	<i>g/100 g of liver</i>		<i>g/liver</i>	
	<i>Before</i>	<i>After</i>	<i>Before</i>	<i>After</i>
Water	69.9 ± 0.4	30.7 ± 1.0 *	47.8 ± 2.5	210.2 ± 5.4 *
Ash	1.4 ± 0.1	0.5 ± 0.1 *	0.92 ± 0.1	3.35 ± 0.1 *
Crude protein	21.5 ± 0.2	6.9 ± 0.2 *	14.8 ± 0.8	47.2 ± 1.5 *
Lipids	3.4 ± 0.1	56.1 ± 1.5 *	3.1 ± 0.2	429.3 ± 2.7 *

<sup>a</sup> Values are means ± SEM for data obtained from 14 animals both controls at 12 weeks (before) and overfed group (after). \* Significantly different from measurement before overfeeding,  $P < 0.001$ .

(56.1%) than in the control group (3.4%), while the other components were unequally reduced. In absolute terms, every component was increased with large variations between each component. Whereas overfeeding induced a slight accumulation of water, protein and ash (3.4-, 2.2- and 2.6-fold, respectively), the total lipids were 138-fold higher in the overfed than in the control group. Table IV shows the changes in the composition of the liver lipids. Before overfeeding, the phospholipids accounted for 71.6% of the total lipids and for only 1.8% after, showing the lowest accumulation (2.6-fold) in the total amount per liver. Over the same period, triacylglycerols and cholesterol esters were higher in the overfed than in the control group (946- and 77-fold, respectively).

#### **Liver phospholipid composition**

The liver composition observed in hybrid ducks (table V) before force-feeding differed noticeably from that of mammals (Lucas and Ridout, 1967) or that of geese (Leclercq et al, 1968). PE was the most abundant phospholipid form, followed by PC. After force-feeding, PC was further reduced (32.4 to

22.9%,  $P = 0.06$ ). In the overfed group, PC was partly replaced by minor phospholipids such as PS, PA and DPG. Whereas the PE level was unchanged, the total amount of each class of phospholipid per liver was higher after overfeeding. The lowest increment was observed in PC (2.4-fold) compared to DPG (8-fold) or PA (4.9-fold).

As shown in table VI, the concentrations of C14:0, C16:0, C16:1 fatty acids were unchanged after overfeeding, while concentrations of fatty acids with longer chains were greatly modified. The concentration of stearic acid decreased, whereas that of oleic acid increased markedly from 10 to 26.3%. The concentrations of (n-6) polyunsaturated fatty acid concentration decreased from 5 to 3.7% for linoleic acid and, more dramatically, from 18.1 to 7.1% for arachidonic acid. The total level of every fatty acid occurring in phospholipid form was higher after overfeeding except arachidonic acid which remained unchanged. The largest recorded increase concerned oleic acid (2.6-fold).

#### **Plasma lipid composition**

Plasma lipid components were unequally modified by overfeeding (table VII). The

**Table IV.** Liver lipid composition before and after overfeeding <sup>a</sup>.

Overfeeding	Before	After	P <	Before	After	P <
	mol/100 mol of lipids			mmol/liver		
Cholesteryl ester	2.3 ± 0.2	1.8 ± 0.2	NS	0.11 ± 0.02	8.57 ± 1.06	0.001
Triacylglycerol	10.5 ± 1.5	94.1 ± 0.4	0.001	0.47 ± 0.1	444.5 ± 29.1	0.001
Free fatty acid	6.5 ± 1.1	1.0 ± 0.1	0.001	0.3 ± 0.07	4.55 ± 0.84	0.001
Free cholesterol	7.8 ± 0.4	0.8 ± 0.1	0.001	0.34 ± 0.03	3.85 ± 0.26	0.001
Monoglyceride	1.2 ± 0.1	0.6 ± 0.1	0.001	0.05 ± 0.03	2.43 ± 0.26	0.001
Phospholipid	71.6 ± 2.1	1.8 ± 0.2	0.001	3.13 ± 0.1	8.20 ± 0.8	0.001

<sup>1</sup> Values are means ± SEM for data obtained from 14 animals both controls at 12 weeks (before) and overfed group (after). Differences between groups were considered statistically significant when  $P < 0.05$ ; NS: not significant.

**Table V.** Liver phospholipid composition before and after overfeeding <sup>a</sup>.

<i>Overfeeding</i>	<i>Before</i>	<i>After</i>	<i>P &lt;</i>	<i>Before</i>	<i>After</i>	<i>P &lt;</i>
	<i>mol/100 mol of phospholipids</i>			<i>mmol/liver</i>		
Phosphatidic acid	3.2 ± 0.6	6.9 ± 1.1	0.01	0.10 ± 0.01	0.49 ± 0.08	0.001
Disphosphoglycerides	0.8 ± 0.1	3.2 ± 0.3	0.001	0.03 ± 0.01	0.25 ± 0.03	0.001
Phosphatidylethanolamine	60.5 ± 3.3	61.9 ± 2.8	NS	1.89 ± 0.15	4.83 ± 0.39	0.001
Phosphatidylinositol	1.0 ± 0.2	1.5 ± 0.5	NS	0.03 ± 0.01	0.13 ± 0.05	0.05
Phosphatidylserine	0.6 ± 0.1	1.2 ± 0.2	0.05	0.03 ± 0.01	0.09 ± 0.03	0.001
Phosphatidylcholine	32.4 ± 3.4	22.9 ± 3.4	(0.06)	1.00 ± 0.10	2.35 ± 0.61	0.05
Sphingomyelin	0.8 ± 0.2	1.2 ± 0.2	NS	0.03 ± 0.01	0.10 ± 0.03	0.01
Lysophosphatidylcholine	0.7 ± 0.3	1.2 ± 0.4	NS	0.02 ± 0.02	0.10 ± 0.02	0.05

<sup>a</sup> Values are means ± SEM for data obtained from 14 animals both controls at 12 weeks (before) and overfed group (after). Differences between groups were considered statistically significant when  $P < 0.05$ ; NS: not significant.

phospholipid concentration (mmol/L) was only 1.6-fold higher than in the control group whereas the triacylglycerol concentration was increased 5.9-fold. There was always

less free than esterified cholesterol: 1.3 versus 4.7 mmol/L before and 2.8 versus 7.5 mmol/L after force-feeding. The major phospholipid was always PC (table VIII). Its

**Table VI.** Fatty acid composition of liver phospholipids before and after overfeeding <sup>a</sup>.

<i>Overfeeding</i>	<i>Before</i>	<i>After</i>	<i>P &lt;</i>
	<i>mol/100 mol of fatty acid</i>		
14:0	2.1 ± 0.5	2.2 ± 0.3	NS
16:0	21.1 ± 0.2	21.3 ± 1.7	NS
16:1 (n-7)	1.0 ± 0.2	0.5 ± 0.3	NS
18:0	38.8 ± 1.6	30.2 ± 0.9	0.001
18:1 (n-9)	10.0 ± 1.1	26.3 ± 1.3	0.001
18:2 (n-6)	5.0 ± 0.1	3.7 ± 0.3	0.01
18:3 (n-3)	0.2 ± 0.1	Traces	
20:0	0.6 ± 0.2	1.4 ± 0.6	NS
20:2 (n-6)	1.8 ± 0.3	3.8 ± 0.5	0.01
20:3 (n-6)	1.3 ± 0.2	3.3 ± 0.5	0.01
20:4 (n-6)	18.1 ± 0.4	7.1 ± 1.0	0.001
SFA	62.6 ± 1.1	55.2 ± 1.6	0.01
MUFA	11.0 ± 1.2	26.8 ± 1.3	0.001
PUFA	26.4 ± 0.5	18.0 ± 0.9	0.001

<sup>a</sup> Values are means ± SEM for data obtained from 14 animals both controls at 12 weeks (before) and overfed group (after). Differences between groups were considered statistically significant when  $P < 0.05$ ; NS: not significant. SFA: total saturated fatty acid; MUFA: total monounsaturated fatty acid; PUFA: total polyunsaturated fatty acid.

**Table VII.** Plasma lipid composition before and after overfeeding <sup>1</sup>.

<i>Overfeeding</i>	<i>Before</i>	<i>After</i>	<i>P &lt;</i>	<i>Before</i>	<i>After</i>	<i>P &lt;</i>
	<i>mol/100 mol of lipids</i>			<i>mmol/L of plasma</i>		
Cholesteryl ester	30.9 ± 0.7	23.0 ± 1.6	0.001	4.7 ± 0.3	7.5 ± 0.8	0.01
Triacylglycerol	11.6 ± 0.6	31.2 ± 1.8	0.001	1.1 ± 0.1	6.5 ± 0.6	0.001
Free cholesterol	15.1 ± 0.5	14.9 ± 0.4	0.01	1.3 ± 0.3	2.8 ± 0.3	0.001
Phospholipids	42.4 ± 0.5	30.9 ± 0.9	0.001	3.7 ± 0.1	5.8 ± 0.3	0.001

<sup>1</sup> Values are means ± SEM for data obtained from 14 animals both controls at 12 weeks (before) and overfed group (after). Differences between groups were considered statistically significant when  $P < 0.05$ .

**Table VIII.** Plasma phospholipid composition before and after overfeeding <sup>1</sup>.

<i>Overfeeding</i>	<i>Before</i>	<i>After</i>	<i>P &lt;</i>	<i>Before</i>	<i>After</i>	<i>P &lt;</i>
	<i>mol/100 mol of phospholipids</i>			<i>mmol/L of plasma</i>		
Phosphatidic acid + disphosphoglycerides	5.2 ± 0.3	2.8 ± 0.3	0.001	0.15 ± 0.01	0.13 ± 0.02	NS
Phosphatidylethanolamine	2.0 ± 0.2	10.2 ± 1.7	0.01	0.08 ± 0.01	0.55 ± 0.11	0.01
Phosphatidylinositol + phosphatidylserine	0.2 ± 0.1	2.0 ± 0.7	0.05	0.01 ± 0.01	0.11 ± 0.40	NS
Phosphatidylcholine	89.6 ± 0.8	83.0 ± 1.0	0.05	3.32 ± 0.11	4.79 ± 0.27	0.01
Sphingomyelin	2.2 ± 0.2	1.9 ± 0.1	NS	0.08 ± 0.01	0.11 ± 0.01	NS
Lysophosphatidylcholine	0.8 ± 0.3	0.1 ± 0.1	0.05	0.02 ± 0.02	0.02 ± 0.02	NS

<sup>1</sup> Values are means ± SEM for data obtained from six animals before overfeeding and nine animals after overfeeding. Differences between groups were considered statistically significant when  $P < 0.05$ ; NS: not significant.

concentration (mol/100 mol of phospholipid) was slightly but significantly reduced by overfeeding from 89.6 to 83.0%. The PE level was higher after overfeeding (10.2%) than before (2.0%). When phospholipids were expressed in mmol/L of plasma, only PC and PE were found to be higher after overfeeding (1.4- and 6.9-fold, respectively).

#### ***Relationship between liver composition and fat release after heating of foie gras***

Significant correlations between fatty liver characteristics (weight, composition and fat release) are shown in table IX. The high correlation coefficient ( $r = +0.78$ ) between fat



Table IX. Pearson correlation coefficients between fatty liver characteristics <sup>1</sup>.

R	Fat release (%)	Liver weight (g)	Lipids (g/100 g)	Triacylglycerol (mol/100 mL)	Phospholipids ethanolamine (mol/100 mol)	Phosphatidyl- choline (mol/100 mol)	Phospholipids C18:2 (mol/100 mol)
Fat release (%)	1.00						
Liver weight (g)	0.783	1.00					
Lipids (g/100 g)	0.890	0.854	1.00				
Triacylglycerol (mol/100 mol)	0.749	0.787	0.728	1.00			
Phospholipids (mol/100 mol)	-0.856	-0.852	-0.867	-0.832	1.00		
Phosphatidylethanolamine (mol/100 mol)	0.719	0.390	0.523	0.576	-0.625	1.00	
Phosphatidylcholine (mol/100 mol)	-0.670	-0.415	-0.528	-0.567	0.648	-0.949	1.00
Phospholipids C18:2 (mol/100 mol)	-0.757	-0.851	-0.757	-0.852	-0.824	-0.569	0.611
							1.00

<sup>1</sup> The level of significance is  $P < 0.05$ ; NS: not significant,  $n = 14$  except for phospholipids C18:2 in which  $n = 8$ . Fat release, used as an index of liver integrity, was expressed as the mass of fat released after heating a sample of liver for 50 min to 105 °C/mass of the liver before heating.

release and the liver weight confirms previous results both in geese (Nir and Nitsan, 1976; Blum et al, 1990; Rousselot-Paillet et al, 1992) and ducks (Salichon et al, 1994). Concentrations of some liver components were also correlated with fat release. Of the lipids, triacylglycerols correlated positively ( $r = +0.75$ ), whereas total phospholipids and phosphatidylcholine correlated negatively ( $r = -0.86$  and  $-0.67$ , respectively). A significant correlation was also observed with the concentration of linoleic acid in the phospholipids ( $r = -0.52$ ). Nonlipid components correlated negatively ( $r = -0.82$ ,  $-0.87$  and  $-0.75$ ) with protein, water and ash concentrations, respectively. Several components were interrelated. Lipids and their major component, triacylglycerol, correlated positively with each other and with liver weight but negatively with phospholipids ( $-0.83$  to  $-0.87$ ). A high and negative correlation ( $-0.95$ ) was observed between PE and PC.

## DISCUSSION

The liver steatosis called *foie gras* has been more often described in geese than in ducks (Blum and Leclercq, 1973; Nir and Nitsan, 1976; Blum et al, 1990). In proportion to body weight, liver weight was 6.3 times higher in overfed than in control hybrid ducks. On the other hand, the proportion of body weight accounted for by liver weight in muscovy ducks is only 8.2 to 8.8% (Baudonnet-Lenfant et al, 1991) compared to geese whose livers represent 10.3% of their body weight (Bogin et al, 1984). This was mainly due to lipid accumulation which accounted for 56% of the liver weight as previously observed in geese (Blum et al, 1967; Blum and Leclercq, 1973) and in ducks (Baudonnet-Lenfant et al, 1991).

The mechanisms responsible for *foie gras* development are not yet fully understood. In birds, increased hepatic lipogenesis is a normal response to overfeeding.

Although VLDL blood concentration was found to be 13 times higher in force-fed geese (Hermier et al, 1991), lipids accumulated in the liver which could be the result of partial failure in the synthesis or secretion of VLDL compared to lipogenesis rate (Hermier et al, 1991; Salichon et al, 1991). In mule ducks, blood lipid concentrations showed similar changes. The increased plasma lipid contents (table VII) confirm that there is an active release into blood of newly synthesized lipids, especially triacylglycerol into the blood. The higher level of phospholipids in the plasma might be associated with enhanced lipoprotein secretion. It is possible that essential fatty acids are preferentially used for lipoprotein synthesis leading to lower accumulation in the liver.

The huge triacylglycerol accumulation (946-fold the initial amount) was also accompanied by accumulation (2.7- to 4.4-fold) of other structural components such as moisture, ash, protein and phospholipids. A balanced increase in liver components is important both for the development of steatosis and for liver integrity. Histological observations have shown that no lesions other than enlarged cells filled with fat were observed (Blum et al, 1990). Heat-induced fat release, which is currently used in industry to evaluate the quality of *foie gras*, seems to be a better quality marker (Blum et al, 1990). It reaches maximum values when plasma membranes are broken and it correlates with various liver characteristics (positively with liver weight and lipid concentration, negatively with phospholipid titres). In muscovy ducks, fat remains in the liver but ash, protein and phospholipids are accumulated to a lesser extent, leading to the production of a smaller *foie gras* with a higher heat-induced fat release (Babilé et al, 1987; Salichon et al, 1994) which suggests lower cell integrity.

Dietary composition (99% corn) may influence the utilization of energy. Carbohydrates (80% of dried corn) stimulate fatty

acid synthesis (Tanaka et al, 1983), which explains the dominant formation of triacylglycerol (96% of liver lipids) and the relatively high concentration of cholesterol in its esterified storage form (Stone et al, 1987). The total amount of free fatty acids and monoglycerides in mmol/liver were higher because they are precursors of triacylglycerol synthesis. The diet contained only 500 mg choline/kg corn although the animals' requirements range from 1 300 to 1 900 mg choline/kg food. This deficiency has been suggested to be an inductive factor in the formation of *foie gras* (Blum et al, 1967). Even though the absolute amounts of phospholipids and therefore of choline were increased, the phospholipid composition was modified. Moreover, the lower level of choline could contribute to the development of liver steatosis by increasing the secretion of long-chain fatty acids available for lipid synthesis and decreasing lipoprotein secretion (Aarsaether et al, 1988; Zeisel and Canty, 1993). The lower concentration of phosphatidylcholine was compensated for by a higher concentration of PS which could encourage PE synthesis (Houweling et al, 1992). The higher concentration of phosphatidic acid and diphosphoglycerides could result from an increased rate of concentrated phospholipid turnover (Exton, 1994). The analysis of plasma phospholipid composition confirmed the choline deficiency. As usual in this deficiency (Lucas and Ridout, 1967), the reduced phosphatidylcholine concentration was balanced by a higher concentration of PE.

So far, only the total lipids of *foie gras* were analyzed. This revealed an increase in saturated fatty acids and a decrease in linoleic acid content (Baudonnet-Lenfant et al, 1991; Salichon et al, 1994). Our results provided evidence for an essential fatty acid deficiency concerning not only triacylglycerol but phospholipids as well. Arachidonic acid was even scarcer than linoleic acid. Of course, lipid accumulation prevented a

decrease in the total amount of essential fatty acids, but lipid composition confirmed the deficiency. It seems that phospholipids containing unsaturated fatty acids are crucial for stable lipid formation and, therefore, for triacylglycerol release into the blood (Redgrave et al, 1992). Essential fatty acids were replaced in phospholipids by minor polyunsaturated fatty acids (20:2 and 20:3) and, above all, by the monounsaturated oleic acid which is easily synthesized in poultry (Sonaiya, 1988). The higher level of n-6 polyunsaturated fatty acids (20:2 and 20:3) in phospholipids induced by force-feeding could be the result of a low activity in  $\Delta 6$  and  $\Delta 5$  desaturase. These enzymes are under insulin control and further experiments should be undertaken to understand the influence of insulin on fatty acid composition in force-fed ducks or geese.

Finally, it may be concluded that *foie gras* which is obtained in hybrid ducks does not greatly differ from that obtained in geese. Development of liver steatosis requires a failure of the fat-releasing mechanisms into the blood and also the ability of liver cells to hypertrophy and accumulate fat in association with other structural components (water, minerals, proteins, phospholipids). Choline deficiency and decreased retention of essential fatty acids were observed. Further experiments must be undertaken to determine the effects of this choline deficiency and the relation between insulin and fatty acid composition in the liver.

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