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Original article

Effect of genotype and overfeeding on lipid deposition in myofibres and intramuscular adipocytes of breast and thigh muscles of ducks

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Abstract – We conducted a study to evaluate the effects of genotype (Muscovy, Pekin and their crossbred, hinny and mule) and overfeeding (14 days from 12 weeks of age) on lipid deposition in myofibres and intramuscular adipocytes of breast and thigh muscles of ducks. Birds of the four genotypes were also reared contemporaneously with a growing diet distributed ad libitum. Muscle samples (*Pectoralis major* and *Sartorius*) were collected at 14 weeks of age on 8 ducks per treatment. The muscle fibre typing, the total lipid and triglyceride contents in myofibres and the relative surface occupied by adipocytes on the cross-sectional area of the muscles were determined by histological and image analysis. Overfeeding induced a marked increase of body weight but had no significant effect on the muscle weight, the cross-sectional area (CSA) of myofibres and the muscle typology. In muscles, overfeeding induced a large accumulation of lipids, mainly in adipocytes whose relative surface increased 1.5 fold in *P. major* and 2.1 fold in *Sartorius* and an increase in triglyceride content of fast twitch oxydo-glycolytic and glycolytic fibres in *P. major* only (+ 37 and + 16% respectively). Genotype had no significant effect on the muscle typology. By comparison with the other genotypes, Muscovy ducks exhibited the highest body weight, the highest muscle weight which could partly be explained by the highest fibre CSA and the lowest intramuscular fat content in adipocytes and myofibres (only fast twitch oxydo-glycolytic fibres in *P. major*). We observed the reverse situation for the Pekin ducks. The crossbred ducks always presented intermediate values except for body weight.

lipids / myofibres / adipocytes / muscles / ducks

1. INTRODUCTION

In bovines [1] and rabbits [2] it has been demonstrated that intramuscular fat (IMF) was stored both in the myofibres and between the fasciculi, in adipocytes. In bovines [3], pigs [4] and rabbits [22], the IMF increase in muscles results mainly from hypertrophy and hyperplasia of adipocytes. Lipid content in poultry meat is low, particularly in the breast meat of chicken (around 1 g per 100 g of meat, [5]) but can be manipulated

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Table I. Main characteristics of feed for rearing and overfeeding periods. Preparation for overfeeding contained corn (25%), corn meal (35%) and water (40%).

Characteristics	Starting (0–4 weeks)	Growing (4–12 weeks)	Overfeeding (12–14 weeks)
ME kcal·kg^{-1}*	2830	2850	3330
CP (%)	18.21	15.98	8.28
Lipids (%)	3.34	2.84	3.38
SFA (%)	17.17	16.10	14.52
MUFA (%)	24.98	28.36	27.44
PUFA (%)	57.85	55.54	58.02

* Calculated value for metabolisable energy (ME); CP = crude protein; SFA, MUFA, PUFA = saturated, mono-unsaturated and poly-unsatured fatty acids.

to a large extent by different factors: species, age, sex, nutrition and selection [5]. In addition, muscles which differ in their contractile and metabolic properties are not homogeneous with regards to their final lipid content [5]. However, until now, the localisation of IMF in poultry, has received little attention. By using different duck genotypes (Pekin, Muscovy and their crossbred, hinny and mule) presenting different susceptibilities for the storage of lipids in the liver or in peripheral tissues such as adipose tissues and muscles during an overfeeding period [6, 7], we were able to obtain a wide range of lipid content in meat [8]: 2.3 to 7.6 g per 100 g of breast meat (*Pectoralis major*) and 2.2 to 5.7 per 100 g of thigh meat (*Iliotibialis superficialis*). Pekin ducks showed a much marked extra-hepatic fattening (in adipose and muscle tissues) and, at the extreme, Muscovy ducks exhibited a much marked steatosis and the lowest lipid deposition in adipose tissues and muscles. In France, the most commonly used species are mule and Muscovy ducks. By using Pekin and hinny ducks, we were able to generate a higher variability in muscle lipid content. Therefore, it seemed interesting to use this model to determine the localisation of IMF in duck muscles in relationship with their final lipid content and fibre type composition: *Pectoralis major* (a breast muscle) and *Sartorius* (a thigh muscle).

2. MATERIALS AND METHODS

2.1. Bird management

We used male ducks from four different genotypes: Pekin (*Anas platyrhynchos*), Muscovy (*Cairina moschata*) and their crossbred, mule (male Muscovy duck × female Pekin duck) and hinny (male Pekin duck × female Muscovy duck). The ducks (50 per genotype) were issued from the same sires and dams provided by the Grimaud Company (Roussay, France). They were reared under natural conditions of light and temperature at the Experimental Station for Waterfowl Breeding (INRA Artiguères, France). From hatching to 6 weeks of age, they were fed ad libitum. From 6 to 12 weeks of age, they were fed on a restricted diet at levels appropriate for each genotype (200–250 g per duck at the beginning, increasing to 360–380 g at the end of the period [8]). At 12 weeks of age, 35 ducks per genotype were overfed at the maximum of their ingestion potential for 14 days with corn and corn meal. During the overfeeding period, 12 ducks per genotype were kept ad libitum with the growing diet (controls). The main characteristics of diets (starting, growing and overfeeding) are shown in Table I.

At 14 weeks of age, 8 ducks per genotype and randomly chosen dietary treatment were

weighed and sacrificed by sectioning of the neck. Immediately after bleeding, a breast (*Pectoralis major*) and a thigh muscle (*Sartorius*) were excised and weighed. One sample of *P. major* and the whole *Sartorius* were quickly frozen in isopentane cooled with liquid nitrogen and stored at –80 °C until histochemical analysis was performed as previously described by Baéza et al. [9].

The present work was carried out in agreement with French legislation on animal experimentation and with the authorisation of the French Ministry of Agriculture (Animal Health and Protection Directorate).

2.2. Histological analysis

Serial cross-sections, 12 μm thick, were obtained at –20 °C in a cryostat (Jung Frigocut 2800N, Leica, Rueil-Malmaison, France). Six serial cross-sections per bird were prepared to evaluate the size, the typology and the lipid content of myofibres. Fibre types were determined on the basis of their ATPase activity after preincubation at pH 4.10 and 10.50 [10] and their succinate dehydrogenase (SDH) activity [11]. Myofibres were classified as type βR and α according to the terminology of Ashmore and Doerr [11]. Slow-twitch fibres (type βR) were identified as being stable after acid preincubation and labile after alkaline preincubation, while fast-twitch fibres (type α) were labile after acid preincubation and stable after alkaline preincubation. SDH staining made it possible to characterise fibres with high oxidative activity (deep blue granulation, types αr and βr) and low oxidative activity (pale blue stained fibres, type αW). The *Sartorius* muscle can be divided into a fast portion (superficial) composed of type αr and αw fibres and a mixed portion (deep) composed of type βR, αR and αW fibres [12]. We only studied the mixed portion which represents the third part of a muscle cross-section after ATPase activity determination. One other section of the muscle was stained with red azorubin, which permits clear identification of the interfibre network by staining only myofibres in red, and it was named the reference stain. Percentage and mean cross-sectional area (CSA) of each fibre type were determined using a computerised image analysis system [13]. For image acquisition and treatment, the software, called RACINE, was implemented on a UNIX workstation equipped with a graphic card linked with a CCD video camera placed on a microscope (Leica DMRB, Leica, Rueil-Malmaison, France).

Triglycerides and total lipids were differentially stained in myofibres with Red oil (RO) and Sudan black (SB) according to Koopman et al. [14] and Dubowitz [15], respectively. RO and SB stain intensities were measured on individual myofibres as the mean pixel luminance determined from 100 pixels located around the central area of the fibres, expressed on a 256 grey level scale. Triglycerides and total lipids on each sample and for one given fibre type were determined photometrically from luminance measurements according to the following formula: Σ Li Si/Σ Si, where Li and Si were the average RO or SB staining luminance and the cross sectional area of the ith fibre, respectively, and named Lc RO and Lc SB according to Fernandez et al. [16]. Luminance was negatively correlated with a lipid presence.

All these variables were determined for approximately 200 fibres for each muscle.

To evaluate the relative surface occupied by adipocytes on the cross-sectional area of each muscle sample, three serial cross-sections were prepared. Oil Red 0 was used to stain, in red/orange, the clusters of adipocytes according to Dubowitz [15]. To calculate the mean relative area of clusters of adipocytes three microscopic fields (0.92 mm^2) around a blood vessel (the only localisation for lean birds) were randomly chosen for each section. The measurements were carried out on the three serial cross-sections for the same microscopic field after image digitalisation and evaluation with VISILOG software (Noesis, Courtabœuf, France). The results were expressed as the relative

Table II. Effects of genotype and overfeeding on body weight and weight of *Pectoralis major* and *Sartorius* muscles of ducks (means ± SEM, $n = 8$).

Genotypes	Feeding plan	Body weight (g)	*P. major* weight (g)	*Sartorius* weight (g)
	Overfed	6295 ± 369 a	303 ± 33	9.20 ± 1.00
	Control	4838 ± 380 b	297 ± 29	9.66 ± 0.90
Overfeeding effect		***	ns	ns
Muscovy		5905 ± 345 a	403 ± 43 a	11.64 ± 1.37 a
Hinny		5585 ± 436 b	293 ± 27 b	9.65 ± 0.94 b
Mule		5464 ± 359 b	294 ± 28 b	9.65 ± 0.67 b
Pekin		5311 ± 378 b	212 ± 26 c	6.78 ± 0.72 c
Genotype effect		***	***	***
Muscovy	Overfed	6393 ± 441 a	408 ± 49	10.92 ± 1.56
	Control	5418 ± 245 b	398 ± 39	12.37 ± 1.27
Hinny	Overfed	6315 ± 402 a	290 ± 25	9.68 ± 0.87
	Control	4854 ± 497 c	297 ± 29	9.63 ± 1.06
Mule	Overfed	6473 ± 351 a	309 ± 28	9.74 ± 0.69
	Control	4455 ± 392 c	278 ± 30	9.56 ± 0.69
Pekin	Overfed	5999 ± 353 a	208 ± 33	6.47 ± 0.87
	Control	4623 ± 426 c	216 ± 18	7.08 ± 0.59
Interaction effect		**	ns	ns

*, **, *** Significant effect with $P < 0.05$, $P < 0.01$ or $P < 0.001$; ns = no significant; a,b,c: significant difference between groups for one criterion.

surface occupied by adipocyte cluster per observed field (%).

2.3. Statistical analysis

Data were analysed by analysis of variance using the General Linear Model procedure of SAS [17]. The model included the main effects of genotype, feeding plan and their interaction. Among the different groups, significant differences between means were shown according to the Newman-Keul test. We also calculated coefficient correlations of Pearson and regression equations between the relative surface occupied by adipocytes in the cross-sectional area of *P. major* muscles, the average of luminance values in each fibre type for total lipid and triglycerides and the total lipid and triglyceride contents in the same samples previously determined by chemical analysis with the same experimental design [8].

3. RESULTS

Overfeeding induced a large increase in body weight (+30%) but had no significant effect on muscle weight (Tab. II). Body weight increase was about 45% for mule ducks, 30% for hinny and Pekin ducks and 18% for Muscovy ducks.

By comparison with the other genotypes, Muscovy ducks exhibited significantly higher body and muscle weights (Tab. II). Pekin ducks exhibited the lowest muscle weight. Mule and hinny ducks showed intermediate values.

Overfeeding induced a highly significant increase in the relative surface occupied by adipocytes on cross sections of *P. major* (3.74 ± 0.77 vs. 2.45 ± 0.49% in ad libitum ducks) and *Sartorius* (3.72 ± 1.08 vs.1.80 ± 0.46% in ad libitum ducks) muscles (Tab. III).

The relative surface occupied by adipocytes in the cross-sectional area of the

Table III. Effects of genotype and overfeeding on the relative surface occupied by adipocytes on cross-sections of *Sartorius* and *Pectoralis major* muscles of ducks (means ± SEM, $n = 8$).

Genotypes	Feeding plan	Surface (%) in *Sartorius*	Surface (%) in *P. major*
	Overfed	3.72 ± 1.08 a	3.74 ± 0.77 a
	Control	1.80 ± 0.46 b	2.45 ± 0.49 b
Overfeeding effect		***	***
Muscovy		1.73 ± 0.45 c	1.57 ± 0.35 c
Hinny		2.75 ± 0.56 b	3.10 ± 0.69 b
Mule		2.79 ± 0.85 b	3.06 ± 0.78 b
Pekin		3.76 ± 1.28 a	4.66 ± 0.72 a
Genotype effect		***	***
Muscovy	Overfed	2.40 ± 0.53 c	1.98 ± 0.39 c
	Control	1.07 ± 0.39 d	1.15 ± 0.34 d
Hinny	Overfed	3.63 ± 0.68 b	3.74 ± 0.95 b
	Control	1.88 ± 0.45 cd	2.47 ± 0.34 c
Mule	Overfed	3.58 ± 1.15 b	3.52 ± 0.94 b
	Control	2.00 ± 0.46 cd	2.60 ± 0.66 c
Pekin	Overfed	5.27 ± 1.77 a	5.73 ± 0.85 a
	Control	2.26 ± 0.60 c	3.59 ± 0.62 b
Interaction effect		*	*

*, **, *** Significant effect with $P < 0.05$, $P < 0.01$ or $P < 0.001$; ns = no significant; a,b,c: significant difference between groups for one criterion.

muscle was the highest in Pekin ducks (3.76 ± 1.28% and 4.66 ± 0.72% in *Sartorius* and *P. major* respectively) and the lowest in Muscovy ducks (1.73 ± 0.45% and 1.57 ± 0.35% in *Sartorius* and *P. major* respectively, Tab. III).

In control birds, the relative surface occupied by adipocytes was higher in *P. major* than in *Sartorius*: 2.45% vs. 1.80% (Tab. III). In overfed birds it was higher in *Sartorius* than in *P. major* for Muscovy ducks and equivalent in both muscles for the other genotypes.

In *P. major* muscle, the mean percentages of type αR and αW fibres were 86 and 14%, respectively. In *Sartorius*, the mean percentages of type βR, αR and αW fibres were 12, 52 and 36% respectively. Overfeeding had no significant effect on typology (data not shown) and size (CSA) of fibres in *P. major* and *Sartorius* muscles (Tabs. IV and V).

Genotype had no significant effect on the typology of fibres (data not shown). In *P. major* muscle, the average CSA of type αR fibres was significantly higher in Muscovy ducks than in mule and Pekin ducks: 929 ± 200 vs. 720 ± 133 μm^2 and 683 ± 248 μm^2, respectively (Tab. IV). Genotype had no significant effect on the mean CSA of type αW fibres whose size was higher than that of type αR fibres (2.3 fold). In the *Sartorius* muscle, the mean CSA of type αW fibres was significantly higher in Muscovy ducks than in the other genotypes: 2193 ± 767 μm^2 vs. 1651 ± 383 μm^2 in Pekin ducks (Tab. V). Genotype had no significant effect on the mean CSA of type βR and αR fibres. The mean CSA of type αW fibres was 1.5 and 1.9 fold higher than the mean CSA of type βR and αR fibres respectively.

The mean CSA of type αR fibres was higher in *Sartorius* than in *P. major* (+ 27%). The mean CSA of type αw fibres was equivalent in both muscles.

In *P. major* muscle, overfeeding had no significant effect on the total lipid content

Table IV. Effects of genotype and overfeeding on cross-sectional area (CSA) of fibres and on luminance due to the presence of triglycerides (Lc RO) or total lipid (Lc SB) in fibres of *Pectoralis major* muscle of ducks (means ± SEM, $n = 8$).

Genotypes	Feeding plan	Fibres αR			Fibres αW		
		CSA (μm²)	LC SB	Lc RO	CSA (μm²)	LC SB	Lc RO
	Overfed	764 ± 252	97 ± 23	96 ± 22 b	1769 ± 518	128 ± 19	140 ± 18 b
	Control	811 ± 170	106 ± 21	152 ± 29 a	1885 ± 592	132 ± 26	166 ± 28 a
Overfeeding effect		ns	ns	*	ns	ns	***
Muscovy		929 ± 200 a	101 ± 22	137 ± 24 a	2028 ± 644	126 ± 25	158 ± 24
Hinny		817 ± 269 ab	101 ± 21	111 ± 29 b	1886 ± 557	132 ± 19	147 ± 25
Mule		720 ± 133 b	104 ± 25	132 ± 26 ab	1785 ± 546	132 ± 27	160 ± 22
Pekin		683 ± 248 b	99 ± 22	115 ± 26 ab	1608 ± 507	130 ± 21	146 ± 24
Genotype effect		*	ns	***	ns	ns	ns
Muscovy	Overfed	908 ± 250	94 ± 22	113 ± 26	1978 ± 660	121 ± 23	145 ± 22
	Control	951 ± 152	107 ± 23	160 ± 23	2077 ± 672	131 ± 29	172 ± 28
Hinny	Overfed	787 ± 345	100 ± 24	85 ± 20	1691 ± 448	133 ± 17	134 ± 21
	Control	848 ± 190	102 ± 19	137 ± 38	2081 ± 681	131 ± 22	159 ± 30
Mule	Overfed	707 ± 124	98 ± 27	100 ± 20	1902 ± 594	130 ± 26	147 ± 16
	Control	732 ± 151	111 ± 25	164 ± 32	1669 ± 535	134 ± 30	173 ± 28
Pekin	Overfed	653 ± 291	96 ± 25	84 ± 28	1505 ± 445	128 ± 14	134 ± 15
	Control	712 ± 216	102 ± 21	145 ± 27	1711 ± 594	131 ± 28	159 ± 31
Interaction effect		ns	ns	ns	ns	ns	ns

*,**,*** Significant effect with $P < 0.05$, $P < 0.01$ or $P < 0.001$; ns = no significant; a,b,c: significant difference between groups for one criterion.
Lc SB and Lc RO: luminances due to the staining with Sudan black (total lipids) and Red oil (triglycerides).
Fibres αR and αW: fast-twitch fibres, oxydo-glycolytic and glycolytic respectively.

of fibres (Tab. IV) but, it induced an increase in the triglyceride content of type αR and αW fibres: +37 and +16%, respectively. In *Sartorius* muscle, overfeeding induced a decrease in total lipid and triglyceride content of all fibre types: –18 and –12% in type βR fibres, –18 and –17 % in type αR fibres and –18 and –14% in type αW fibres, respectively (Tab. V).

In *P. major* muscle, genotype only had a significant effect on the triglyceride content of type αR fibres which was lower in Muscovy ducks in comparison with the other genotypes (average luminance of 137 vs. 111, 132 and 115 in hinny, mule and Pekin ducks respectively). In *Sartorius* muscle, genotype had no significant effect on the triglyceride and total lipid content of the fibres.

The triglyceride and total lipid content of type αR and αW fibres was significantly lower ($P < 0.05$) in *Sartorius* than in *P. major*.

Using data reported by Chartrin et al. [8], we calculated correlations between total lipid (g per 100 g of muscle) and triglyceride content (% of total lipid) or the quantity of triglycerides in muscle (g per 100 g of muscle) and the relative surface occupied by adipocytes on muscle microscopic fields of all ducks. In *P. major*, highly significant correlations ($P < 0.001$) were observed in all cases with r values of 0.80, 0.78 and 0.83 respectively (Fig. 1). Significant correlations ($P < 0.05$) were also observed with the average luminance values for the Red oil staining procedure (triglycerides). For type αR fibres, the r values were –0.65, –0.65 and

Table V. Effects of genotype and overfeeding on cross-sectional area (CSA) of fibres and on luminance due to the presence of triglycerides (Lc RO) or total lipid (Lc SB) in fibres of *Sartorius* muscle of ducks (means ± SEM, $n = 8$).

Genotypes	Feeding plan	Fibres βR			Fibres αR			Fibres αW		
		CSA (μm^2)	LC SB	Lc RO	CSA (μm^2)	LC SB	Lc RO	CSA (μm^2)	LC SB	Lc RO
	Overfed	1232 ± 329	157 ± 28 a	177 ± 27 a	1017 ± 223	150 ± 29 a	177 ± 26 a	1980 ± 611	162 ± 30 a	182 ± 27 a
	Control	1320 ± 439	134 ± 18 b	159 ± 18 b	975 ± 180	127 ± 17 b	151 ± 17 b	1738 ± 484	137 ± 19 b	160 ± 19 b
Overfeeding effect		ns	***	**	ns	***	***	ns	***	***
Muscovy		1355 ± 357	142 ± 23	168 ± 25	1105 ± 145	137 ± 22	164 ± 26	2193 ± 760 a	147 ± 23	172 ± 27
Hinny		1340 ± 394	150 ± 21	173 ± 22	993 ± 192	144 ± 22	169 ± 20	1737 ± 560 b	149 ± 23	171 ± 23
Mule		1164 ± 411	148 ± 26	164 ± 23	891 ± 214	138 ± 29	161 ± 19	1856 ± 439 b	150 ± 26	170 ± 18
Pekin		1245 ± 413	143 ± 23	167 ± 24	996 ± 256	136 ± 24	162 ± 25	1651 ± 415 b	150 ± 28	171 ± 27
Genotype effect		ns	ns	ns	ns	ns	ns	*	ns	ns
Muscovy	Overfed	1398 ± 327	150 ± 29	178 ± 33	1134 ± 152	144 ± 28	177 ± 34	2356 ± 945	156 ± 29	183 ± 35
	Control	1311 ± 408	134 ± 18	157 ± 17	1075 ± 147	129 ± 15	152 ± 17	2029 ± 588	138 ± 18	160 ± 18
Hinny	Overfed	1282 ± 350	162 ± 25	177 ± 23	989 ± 206	159 ± 27	176 ± 24	1851 ± 657	164 ± 23	178 ± 23
	Control	1399 ± 459	138 ± 19	170 ± 21	996 ± 190	130 ± 17	161 ± 16	1622 ± 491	134 ± 25	165 ± 24
Mule	Overfed	1133 ± 273	166 ± 35	174 ± 29	923 ± 259	153 ± 39	178 ± 21	2158 ± 544	166 ± 35	183 ± 20
	Control	1194 ± 536	130 ± 17	154 ± 18	859 ± 178	123 ± 14	145 ± 18	1554 ± 341	134 ± 15	157 ± 17
Pekin	Overfed	1114 ± 419	152 ± 27	181 ± 30	1023 ± 294	146 ± 27	177 ± 29	1554 ± 187	160 ± 36	184 ± 34
	Control	1375 ± 436	134 ± 20	154 ± 19	970 ± 233	126 ± 23	146 ± 21	1748 ± 578	140 ± 20	158 ± 20
Interaction effect		ns	ns	ns	ns	ns	ns	ns	ns	ns

*,**,*** Significant effect with $P < 0.05$, $P < 0.01$ or $P < 0.001$; ns = no significant; a,b,c: significant difference between groups for one criterion.
Lc SB and Lc RO: luminances due to the staining with Sudan black (total lipids) and Red oil (triglycerides).
Fibres βR: oxydative slow-twitch fibres. Fibres αR and αW: fast-twitch fibres, oxydo-glycolytic and glycolytic respectively.

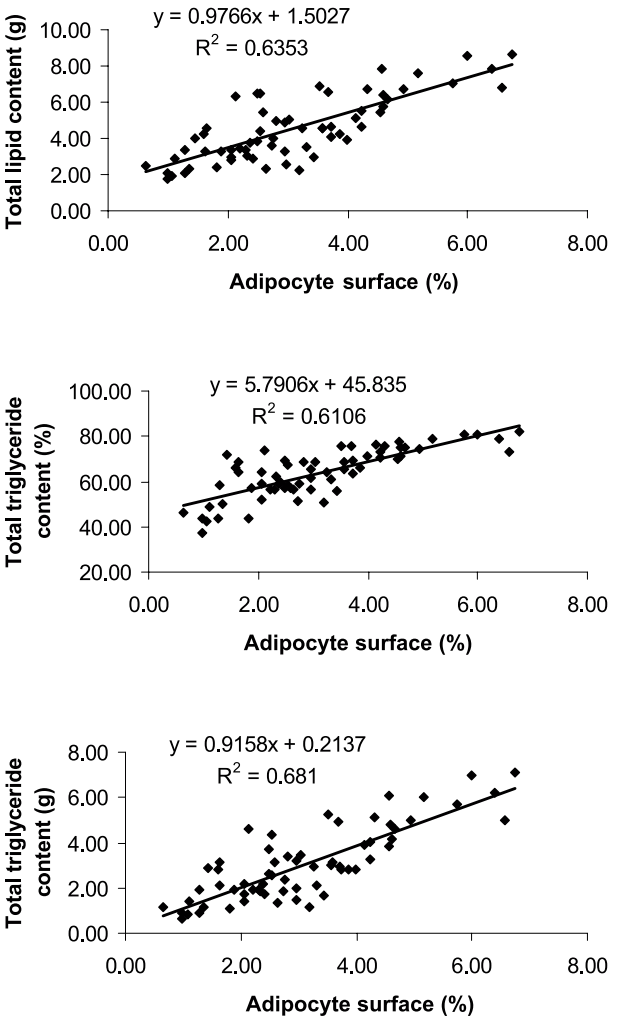

Figure 1. Relationships between the relative surface occupied by adipocytes on muscle cross-sections and the total lipid (g per 100 g of muscle) and triglyceride (expressed as % of total lipids or g per 100 g of muscle) contents in the *P. major* of ducks ($P < 0.001$).

–0.67 respectively (Fig. 2). For type αW fibres, the *r* values were lower: –0.41, –0.44 and –0.43 respectively (Fig. 3).

We also calculated these correlations for each genotype but none was significant (data not shown).

4. DISCUSSION

Overfeeding induced a large increase in body weight as previously reported [18, 19]. This resulted from a dramatic increase in the synthesis of lipids in the liver which

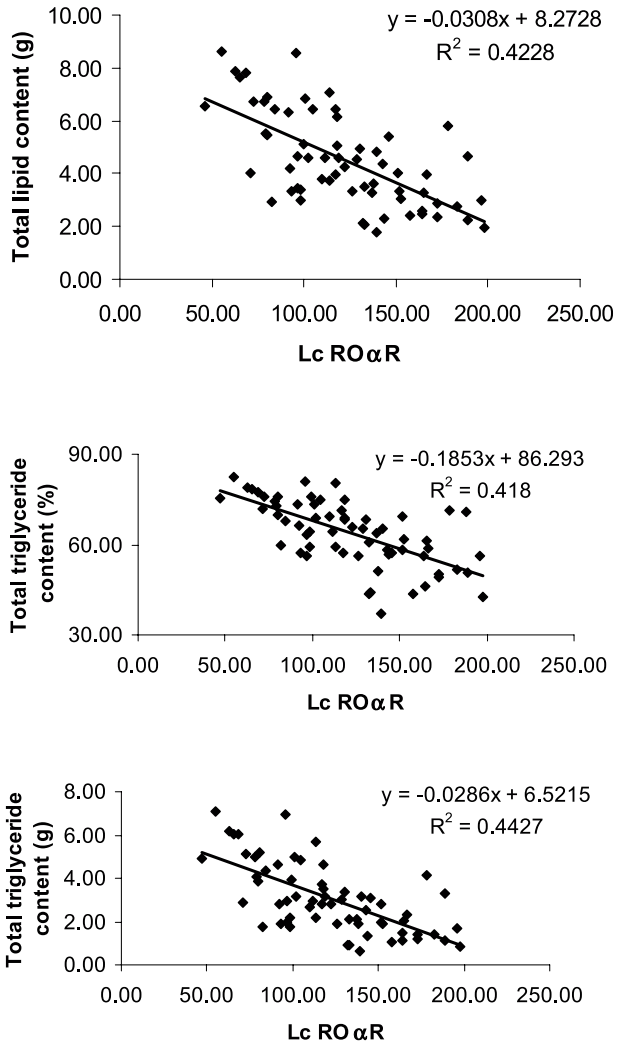

Figure 2. Relationships between the average luminance values for triglycerides in type αr fibres obtained with the Red oil staining procedure (Lc RO αR) and the total lipid (g per 100 g of muscle) and triglyceride (expressed as % of total lipids or g per 100 g of muscle) contents in the *P. major* muscle of ducks ($P < 0.05$).

accumulated first in the liver but also in peripheral tissues such as adipose tissues and muscles [6–8]. Accordingly, the lipid content of muscles increased [8, 20]: 1.3 to 2.1 fold depending on muscle type and genotype. We observed a large increase in the relative surface occupied by adipocytes on muscle sections: 1.5 fold in *P. major* and 2.1 fold in *Sartorius* across genotypes. This confirmed the previous observations of Zanusso et al. [20] obtained in Muscovy ducks. The availability of data across a wide range of muscle lipid concentrations in *P. major* allowed obtaining high correlations between the relative surface occupied by adipocytes in muscle sections and the total lipid (g per

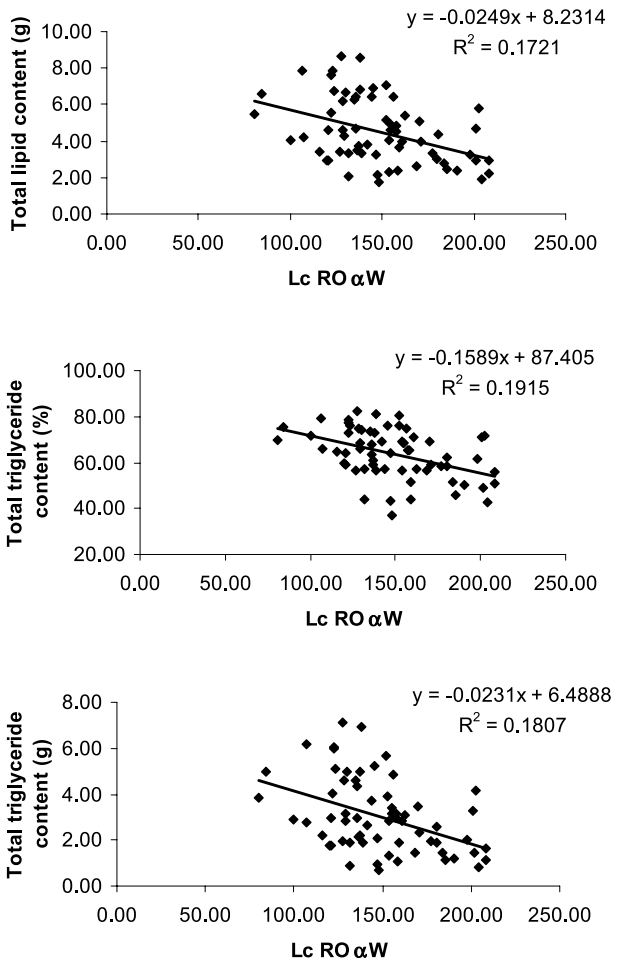

Figure 3. Relationships between the average luminance values for triglycerides in type αw fibres obtained with the Red oil staining procedure (Lc RO αW) and the total lipid (g per 100 g of muscle) and triglyceride (expressed as % of total lipids or g per 100 g of muscle) contents in the *P. major* muscle of ducks ($P < 0.05$).

100 g of muscle) and triglyceride contents (% of total lipids) or the quantity of triglycerides in this same muscle (g per 100 g of muscle). In control birds, the relative surface occupied by adipocytes was higher in the *Sartorius* than in *P. major*. In overfed birds, we observed the reverse in Muscovy ducks or we had equivalent values between both muscles in the other genotypes. Therefore, the effect of overfeeding on the increase of the relative surface occupied by adipocytes was higher in the *Sartorius* muscle. In *P. major* muscle, overfeeding had no significant effect on the total lipid content of muscle fibres (luminance of Sudan black B staining). Zanusso et al. [20] with the same staining procedure also found no significant difference of luminance between control and overfed Muscovy ducks. Inside the fibres, the proportion of phospholipids is

quite important and stable and could have masked the effect of overfeeding on triglyceride deposition. Actually, in *P. major* muscle, overfeeding induced an increase in triglyceride (depot lipids) content of type αr muscle fibres which have an oxydo-glycolytic metabolism. Moreover, the average of luminance values in type αr fibres for triglycerides obtained with the Red oil staining procedure and the total lipid (g per 100 g of muscle) and triglyceride content (% of total lipids) or the quantity of triglycerides in this same muscle (g per 100 g of muscle) were highly correlated. In *Sartorius* muscle, overfeeding induced a decrease in total lipid and triglyceride content of muscle fibres. This result was quite surprising and before trying to find an explanation we would like to confirm this observation. The total lipid and triglyceride content of type αR and αW muscle fibres was higher in *P. major* than in *Sartorius.*

Overfeeding had no significant effect on the weight of muscles and the CSA of muscle fibres. During overfeeding, the growth of *P. major* is reduced or stopped [18, 19]. In the Muscovy duck, Zanusso et al. [20] also showed that the CSA of muscle fibres is not significantly influenced by overfeeding. As Zanusso et al. [20], we found that the composition of fibre type of muscles was not modified by overfeeding. Actually, it is quite difficult to modulate the fibre type composition of muscles. In chickens, a selection for rapid growth does not change muscle fibre typing [21]. In Muscovy ducks, a selection for improved body weight and higher meat yield only modifies the typology of *Sartorius* muscle which exhibits a higher percentage of type αW fibres at the expense of type αR fibres [22] while the typology of *P. major* remains unchanged. In Muscovy ducks, there is a marked dimorphism on body weight which significantly influences muscle weight. Males have much heavier muscles than females but the muscle typology is the same in both sexes [9]. Concerning the effect of nutrition management, many studies have also reported that feed restriction has either no effect on the muscle typology in rats [23], in cattle [24], in pigs [25], in rabbits [26] or increases the percentage of oxidative myofibres in cattle [27], in lambs [28] and in pigs [29].

The original part of this study was the analysis of the genotype effect. Muscovy ducks exhibit the highest body weight, the highest muscle weight and the lowest fattiness in comparison with the other genotypes [6–8]. For these reasons, this species has been chosen in France for the production of duck meat [30]. The difference in muscle weight between the Muscovy duck and the other genotypes (+72% and +90% for *Sartorius* and *P. major*, respectively by comparison with the Pekin duck) could be explained partly by a higher CSA of muscle fibres (+36% for type αW fibres in *Sartorius* and +33% for type αR fibres in *P. major*, by comparison with the Pekin duck) but also by a higher number and/or length of muscle fibres. Genotype had no significant effect on the fibre type composition in muscles. Concerning muscle typology, we confirmed previous results obtained by Torrella et al. [12] in mallard ducks, Gille et al. [31] in Pekin ducks, Baéza et al. [9] in Muscovy ducks and Baéza et al. [32] in mule ducks.

Pekin ducks exhibited a higher lipid content in muscles in comparison with the other genotypes (+105 and +120% in *P. major* and *Iliotibialis superficialis*, respectively in comparison with the Muscovy duck, [8]). This data was confirmed by the highest relative surface occupied by adipocytes in *P. major* and *Sartorius* for Pekin ducks (+117 and +197% in *Sartorius* and *P. major*, respectively by comparison with the Muscovy duck) and the highest triglyceride content in type αR fibres in *P. major* of Pekin ducks (+16% in comparison with the Muscovy duck). According to Guy et al. [19] and Hermier et al. [21] the four duck genotypes (Pekin, Muscovy and their crossbred, hinny and mule) used in this study presented different susceptibilities for storage of lipids in the liver or in peripheral tissues during an overfeeding period. Pekin ducks showed a much marked extrahepatic fattening and

Muscovy ducks at the extreme exhibited the reverse situation.

5. CONCLUSION

The muscle typology was neither influenced by duck genotypes nor by overfeeding. By combining genotype and overfeeding effects, we were able to obtain a wide range of lipid content in muscles and to demonstrate that intramuscular fat was mainly stored in adipocytes since 63% of the variability in total lipid content of *P. major* muscle was explained by the variability in the relative surface occupied by adipocytes on muscle cross-sections. Overfeeding induced a large increase in muscle fat content in adipocytes and in type αR fibres, only in *P. major*. Muscovy ducks displayed the lowest intramuscular fat depot and Pekin ducks the highest. For all the criteria measured in this study, except the body weight, the crossbred, hinny and mule ducks always had intermediate values to those of the parental genotypes. Intramuscular fat can influence the flavour, colour, juiciness and tenderness of meat. By comparing overfed ducks from the four genotypes, Larzul et al. [33] showed that Muscovy ducks displayed significantly higher shear force values than Pekin ducks in raw and cooked breast meat that could be related to higher cooking loss, lower lipid content, higher collagen content, lower collagen solubility and higher fibre CSA. Therefore, by using the interaction between genotype and overfeeding, it will be interesting to analyse the relationships between intramuscular fat content and the sensorial quality of meat.

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