Gene expression and protein content in relation to intramuscular fat content in Muscovy and Pekin ducks

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ABSTRACT Independent of their nutritional condition, Pekin ducks always exhibit higher i.m. fat content than Muscovy ducks. To understand this difference between species, the expression level of genes involved in lipid metabolism was analyzed in the pectoralis major muscle of Pekin and Muscovy ducks ad libitum-fed or overfed. The lipoprotein lipase (LPL) gene expression was not different between species and not influenced by overfeeding. The protein content for LPL was higher in Pekin ducks than in Muscovy ducks when birds were ad libitum-fed, whereas in overfed ducks, we found no difference between species. Adipocyte fatty acid-binding protein (A-FABP) gene expression and protein content were higher in Pekin ducks than in Muscovy ducks for each nutritional condition (suggesting a higher intracellular transport within i.m. adipocytes of fatty acids mainly provided by liver for this species). Overfeeding did not affect the expression of genes involved in oxidation [carnitine palmitoyl transferase 1A (CPT1A), cytochrome-c oxidase 4 (COX4), succinyl-coenzyme A:3-ketoacid coenzyme A transferase (SCOT)] but increased the expression of fatty acid synthase (FAS) involved in lipogenesis. For all nutritional conditions, Pekin duck exhibited higher expression levels of CPT1A, COX4, SCOT, and FAS than Muscovy ducks. Results for mRNA SCOT suggested that the muscles of Pekin ducks use ketone bodies as an energy source. In conclusion, i.m. lipogenesis could contribute to the i.m. fat, particularly in Pekin ducks.

Key words: duck, fatness, gene expression, lipid metabolism, pectoralis major muscle

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INTRODUCTION

Meat quality is largely influenced by i.m. fat (**IMF**) content, which affects sensory properties and nutritional values of meats (Ruiz et al., 2001). The IMF content varies between species. For example, in ducks species, Chartrin et al. (2006a) observed different IMF content in pectoralis major (**PM**) muscle between Muscovy and Pekin ducks ad libitum-fed (2.3 vs. 4.6%) or overfed (3.7 vs 7.6%). De novo lipogenesis is essentially hepatic in avian species (Pearce, 1977); therefore, the differences in IMF content observed between these ducks can result from different mechanisms. Davail et al. (2003) suggested that the degree of liver steatosis and IMF in waterfowls may partly depend on the peripheral activity of lipoprotein lipase (**LPL**). The role of fatty acid-binding protein in the intracellular trafficking of long-chain fatty acids within i.m. adipocytes has been shown to be related to IMF level in different species (Bénistant et al., 1998; Damon et al., 2006; Jurie et al., 2007). It can be also hypothesized that difference in the balance between muscular lipogenesis and oxidation of the long-chain fatty acids (precursor of triacylglycerol synthesis) may be an important factor implicated in different IMF content between species (Jeukendrup, 2002; Holloway et al., 2009) and within species (Gondret et al., 2004).

In this context, the aim of the present study was to determine the effect of species (Muscovy and Pekin ducks) and dietary level (overfeeding and ad libitum feeding) on gene expression and protein content of factors involved in lipid deposition in PM muscle in relation with muscle fat metabolism (lipogenesis and oxidation), muscle ability in lipid uptake via the LPL protein, and the intracellular transport of long-chain

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fatty acids via the adipocyte fatty acid-binding protein (**A-FABP**) protein (which is also considered as an indicator of total i.m. adipocyte number; Jurie et al., 2007). Most of these factors are known to be regulated by the nutritional status. Therefore, we analyzed their expression under different nutritional conditions: ad libitum-fed or overfed for 7 or 14 d, ducks being killed 2 to 4 h or 12 to 14 h after their last meal.

MATERIALS AND METHODS

All experimental procedures were performed in accordance with the French National Guidelines for the care and use of animals for research purposes (Certificate of Authorisation to Experiment on Living Animals no. 7740, Ministry of Agriculture and Fish Products, Paris, France).

Birds

This experiment was carried out using 1-d-old male ducks from 2 different species: Muscovy and Pekin provided by Grimaud Company (Roussay, France). The birds were distributed into 2 pens (1 per species) and reared under usual conditions of light and temperature at the Experimental Station for Waterfowl breeding (INRA, Artiguères, France). From hatching to 6 wk of age, ducks were ad libitum-fed first with a starting diet then with a growing diet (Table 1). From 6 to 12 wk of age, they were fed with the same but restricted diet (about 80% of ad libitum feeding). For the first experiment (Chartrin et al., 2006a), at 12 wk of age, 10 ducks per species were overfed with corn and corn meal at the maximum of their ingestion potential for 14 d (Table 1). During the overfeeding period, 10 ducks per species were maintained on the growing diet ad libitum-fed (controls). At 14 wk of age, birds were deprived of feed overnight (12 to 14 h) and provided with water. Ducks were then killed by sectioning of the neck.

For the second experiment, at 12 wk of age, 6 ducks per species were overfed by being given 2 meals a day for 7 or 14 d with a diet rich in corn or maintained ad libitum-fed with the growing diet for 7 d (Saez et al., 2008, 2009). Ducks were killed by sectioning the neck 2 to 4 h after the last meal. In this case, birds, ad libitum-fed or overfed for 7 or 14 d, were not reared and killed at the same time; therefore, this experiment only allowed the analysis of species effect. For each rearing period, total feed consumption per duck was recorded for each species. The data are detailed for each period and each experiment on Table 2.

For the 2 experiments and immediately after bleeding, PM muscle was removed. One sample was directly frozen in liquid nitrogen. Another one was frozen in isopentane cooled with liquid nitrogen (Baéza et al., 1999). Samples were then stored at −80°C until further analysis.

RNA Isolation and Reverse Transcription

Total RNA was extracted from muscle samples using a commercial kit (RNeasy Mini Kit, Qiagen, Courtaboeuf, France) according to the instructions of the manufacturer. Ribonucleic acid concentrations were measured by spectrophotometry (optical density at 260 nm) using a NanoDrop ND-1000 (NanoDrop Technolo-

Table 1. Composition and main characteristics of diets

Item	Starting (0 to 4 wk)	Growing (4 to 12 wk)	Overfeeding (12 to 14 wk)
Composition (g/kg)			
Wheat	200.00	254.50	
Wheat starch			2.90
Corn	357.02	370.48	988.49
Sorghum	—	80.00	
Triticale	100.00	—	
Extruded soybean seeds	40.00	15.00	
Rapeseed oil meal	30.00	50.00	
Soybean meal	184.75	138.75	
Sunflower meal	29.00	44.50	
Sugarcane molasses	20.00	15.00	
Calcium carbonate	13.50	10.00	
Dicalcium phosphate	17.75	15.00	
Sodium chloride	1.00	1.75	1.45
Sodium bicarbonate	2.50	1.50	1.45
DL-Methionine	1.88	1.12	
Choline-HCl 75%	0.60	0.40	
Vitamin and mineral supplement	2.00	2.00	5.71
Characteristic (g/kg)			
ME (MJ/kg)	11.83	11.68	13.92
CP	175.10	160.00	82.46
Total lipids	30.40	27.40	37.36
Lysine	9.20	7.80	
Sulfur amino acids	7.70	7.10	
Calcium	11.00	9.00	
Available phosphorus	4.50	4.00	

Table 2. Total feed consumption (kg) per duck and per period for experiments 1 and 2

	Experiment 1		Experiment 2		
Item	0 to 12 wk	12 to 14 wk	0 to 12 wk	12 to 13 wk	13 to 14 wk
Ad libitum-fed ducks					
Muscovy	13.4	3.1	15.9	1.7	
Pekin	16.1	3.9	17.5	2.0	
Overfed ducks					
Muscovy		8.2		4.1	7.6
Pekin		9.7		4.1	7.6

gies, Wilmington, DE) and their integrity was checked by electrophoresis. After DNase treatment (using Ambion's DNA-Free 1906 kit obtained from Clinisciences, Montrouge, France), 5 μg of total RNA was reverse-transcribed using RNase H^- Moloney murine leukemia virus reverse transcriptase (Superscript II, Invitrogen, Illkirch, France) and random primers (Promega, Charbonnières les Bains, France).

Determination of mRNA Levels by Real-Time Reverse Transcription-PCR

After reverse transcription, the cDNA of LPL, A-FABP, genes involved in oxidative metabolism [succinyl-coenzyme A:3-ketoacid coenzyme A transferase (**SCOT**), cytochrome-c oxidase 4 (**COX4**), carnitine palmitoyl transferase 1A (**CPT1A**)] and fatty acid synthase (**FAS**) were amplified by real-time reverse transcription-PCR using an ABI Prism 7000 apparatus (Applied Biosystems, Courtaboeuf, France) with the SYBR Green I qPCR Master Mix Plus (Eurogentec, Angers, France). The 18S rRNA chosen as a reference was determined with the TaqMan Universal qPCR Master Mix Kit and a predeveloped TaqMan assay reagent (Applied Biosystems). For each gene, specific primers were designed from the corresponding duck (A-FABP) or chicken (LPL, SCOT, COX4, CPT1A) sequence to be intron-spanning to avoid co-amplification of genomic DNA (Table 3) and were obtained from Eurogentec. Each gene-specific primer pair was tested for PCR amplification efficiency with cDNA dilutions from 1:10 to 1:810. The cycling conditions consisted of a denaturation step at 95°C for 10 min, followed by a 2-step amplification program (15 s at 95°C, followed by 1 min at 60°C) repeated 40 times. At the end, a dissociation program consisting of 15 s at 95°C, 20 s at 60°C, and 15 s at 95°C was performed. The amplification products for all cDNA were checked by electrophoresis and exhibited the expected size (Table 3). For each individual sample (n = 6), the cDNA of genes under study were amplified in triplicate in the same run. Each PCR run included a no-template control and triplicates of control (i.e., a pool of all cDNA) and samples. The calculation of absolute mRNA levels was based on the PCR efficiency and the threshold cycle deviation of an unknown cDNA versus the control cDNA according to the equation proposed by Pfaffl (2001) and as already described in Guernec et al. (2003). Absolute mRNA levels were corrected for 18S rRNA to give relative levels.

Immunohistochemical and Western Blot Detection of LPL Protein

For both immunohistochemical and Western blot experiments, an anti-chicken LPL polyclonal antibody (pAb_1) produced in goat was used (gift from A. Bensadoun, Cornell University, Ithaca, NY). The specificity of this antibody was previously tested in chickens (Strieleman and Bensadoun, 1987) and duck and chicken LPL protein sequences presented more than 90% of homology.

The following immunohistochemical protocol has been retained. Frozen sections of PM muscle were incubated 5 min in absolute ethanol and H_2O_2 to en-

Table 3. Primer sequences used for reverse transcription-PCR

Gene[1]		Primer sequences (5′-3′)	Accession number	Product size (bp)
LPL	Sense	CAAAGAGAAACACCAAAATGTACTTGA	NM_205282	150
	Antisense	TCGTCTAGAGTGCCATACAGAGAGAT		
A-FABP	Sense	CAAATCAGAAAGTACCTTCAAAAATACAG	DQ358123	150
	Antisense	CCCTTTGCCATCCCACTTC		
CPT1A	Sense	GCCCTGATGCCTTCATTCAA	AY675193	60
	Antisense	ATTTTCCCATGTCTCGGTGA		
COX4	Sense	CGGGAGAAGAGCCCTTTCC	NM_001030577	92
	Antisense	ATTTCAGCCATCCAGCATACG		
SCOT	Sense	CGCCAGTATTTATCTGGTGAGCTT	BM426221	81
	Antisense	CTGCTCCTCCTGCTCTAATACGTT		
FAS	Sense	AGGAAATGGATATTGTCGCTCTGA	NM_205155	69
	Antisense	AGAAATCCATGGCTAAACGCA		

[1]LPL = lipoprotein lipase; A-FABP = adipocyte fatty acid-binding protein; CPT1A = carnitine palmitoyl transferase 1A; COX4 = cytochrome-c oxidase 4; SCOT = succinyl-coenzyme A:3-ketoacid coenzyme A transferase; FAS = fatty acid synthase.

hance immunohistochemical staining. To saturate the antigen sites, slides were incubated 20 min with PBS (Sigma, St Louis, MO) containing 15% rabbit serum (Sigma). Sections were then incubated 1 h at ambient temperature in the pAb_1 solution (1:2,000 in PBS). After 3 washes in PBS, they were incubated for 1 h at ambient temperature with a rabbit anti-goat IgG peroxidase conjugate (Ab_2, 1:500; Sigma) diluted in PBS. After rinsing in PBS, sections were incubated 6 min in diaminobenzidine (Sigma) and rinsed with H_2O. Finally, sections were mounted in an Enthelan medium (Merck, Darmstadt, Germany) and observed under a microscope (Leica, Rueil-Malmaison, France). Negative controls were performed by incubating the sections in PBS without primary antibody.

For Western blot analysis, ground PM muscle (1 g) was homogenized on ice with an Ultra-Turax homogenizer (IKA T25, Janke & Kunkd, Staufen, Germany) in a buffer containing 25 m*M* ammoniac, 0.01% SDS, 5 m*M* EDTA, 0,8% Triton X-100, protease inhibitors (10 μg/mL of leupeptin, 1 μg/L of pepstatin, 25 KIU/mL of aprotinin, Sigma), and 5,000 IU/L of Choay heparin (Sanofi, Synthelabo, France). Homogenates were centrifuged at 20,000 × *g* for 20 min at 4°C and supernatants were aliquoted and stored at −80°C. Protein concentrations were determined using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA; Smith et al., 1985). Muscle lysates (40 μg of protein) were subjected to 10% (wt/vol) SDS-PAGE under reducing conditions and electrotransferred as described previously (Duchêne et al., 2008). The membranes were then incubated overnight at 4°C with goat antibodies anti-LPL at a final dilution 1:5,000 in PBS containing 0.2% Tween-20 and 5% nonfat dry milk powder. Mouse monoclonal anti-human vinculin was purchased from Sigma. After washing, the membranes were incubated with an Alexa Fluor secondary antibody (Molecular Probes, Interchim, Montluçon, France). Bands were visualized by infrared fluorescence by the Odyssey Imaging System (LI-COR Inc. Biotechnology, Lincoln, NE) and quantified by Odyssey infrared imaging system software (Application Software, Version 1.2) related to vinculin concentration (Duchêne et al., 2008).

Determination of A-FABP Protein by ELISA

To quantitatively measure the A-FABP protein, cytosolic protein extracts were prepared as described previously (Paulussen et al., 1989; Jurie et al., 2007) and diluted at a concentration of 0.015 ng/L in buffer B (50 mmol/L of sodium carbonate, pH 9.6), and 50-μL aliquots were coated in triplicate onto the wells of microtiter plates (Polylabo, Strasbourg, France) by overnight incubation at 4°C. For calibration, amounts of 0 to 2 ng of human A-FABP (gift from H. T. B. Van Moekerk, Universitair Medisch Centrum St Radboud, Nijmegen, the Netherlands) were coated in the same way. The wells were washed 4 times with PBS-Tween (5.4 mmol/L of sodium phosphate, 1.3 mmol/L of potassium phosphate, 150 mmol/L of NaCl pH 7.4, 0.5 g/L of Tween-20) and 100 μL of diluted antiserum against human A-FABP (gift from H. T. B. Van Moekerk, Universitair Medisch Centrum St Radboud), diluted at 1:1,000 in PBS-Tween, was added. After incubation for 1 h at 20°C, the wells were washed 4 times with PBS-Tween and 100 μL of diluted horseradish peroxidase-conjugated swine anti-rabbit IgG (1:500 in buffer C, Dako A/S, Glostrup, Denmark) was added and incubated for 1 h at 20°C. The wells were washed 4 times with PBS-Tween and bound peroxidase was assessed with 100 μL of a freshly prepared solution of 2.2 mmol/L of *o*-phenylene diamine (Merck). The incubation occurred in the dark for 30 min at 20°C and was stopped by addition of 50 μL of 12.5% H_2SO_4. The product of the peroxidase reaction was determined at 492 nm using an ELISA plate reader (Labsystems Multiscan, Helsinki, Finland). The amount of immunoreactive protein (μg of A-FABP/g of tissue wet weight) was determined from calibration curves. The specificity of antiserum against human A-FABP was previously tested for duck muscle extracts by Western blot.

Histological Staining of Adipocytes

Serial cross sections, 12-μm thick, were obtained at −20°C in a cryostat (Jung Frigocut 2800N, Leica). The staining of triglycerides localized into adipocytes and muscle fibers was achieved with red oil as described previously by Chartrin et al. (2005).

Malonyl Coenzyme A Content

A 150-mg sample of frozen PM was homogenized with 0.5 mL of a chilled 6% $HClO_4$-2 mmol/L of dithiothreitol solution (Sigma). Two hundred microliters of the clarified supernatant was immediately mixed with 10 μL of an internal standard solution [6% $HClO_4$-200 μmol/L of propionyl-coenzyme A (CoA)] and 20 μL of the mixture was injected into a (C18) reverse-phase column (Merck Lichrocart, Superspher; 4 × 250 mm) as in King et al. (1988). Identification of eluted malonyl-CoA was made at 254 nm by reference to standard solutions and PM contents calculated relative to internal standard.

Statistical Analysis

Values are expressed as the means ± SE of 10 or 6 birds per species for experiments 1 and 2, respectively. The effects of species (Pekin vs. Muscovy ducks) or nutritional condition (ad libitum-fed vs. overfeeding, only for experiment 1) were analyzed by ANOVA. For the first experiment, we also calculated Pearson correlation coefficient between A-FABP protein content and total lipid content in the same samples previously determined by analysis with the same experimental design (Chartrin et al., 2006a).

RESULTS

Gene Expression in PM Muscle

First Experiment. The LPL mRNA was similar between species independent of their nutritional conditions and we did not observe any effect of overfeeding (Table 4). The A-FABP mRNA level was higher in Pekin duck than in Muscovy duck, independent of their nutritional condition. Overfeeding increased the expression of A-FABP, particularly in Pekin duck (more than 2-fold). The CPT1A mRNA levels were 2.5- and 2-fold higher in PM muscle of Pekin duck than in that of Muscovy duck ad libitum-fed and overfed, respectively. The COX4 mRNA levels were 29.9- and 4.1-fold higher in PM of Pekin duck than in that of Muscovy duck ad libitum-fed and overfed, respectively. The SCOT mRNA levels were 5.5- and 4.6-fold higher in PM muscle of Pekin duck than in that of Muscovy duck ad libitum-fed and overfed, respectively. Overfeeding did not affect the expression of CPT1A, COX4, and SCOT genes. The FAS mRNA was 10.5- and 8.9-fold higher in PM muscle of Pekin duck than in that of Muscovy duck ad libitum-fed and overfed, respectively. Overfed ducks exhibited a higher FAS mRNA level than ad libitum-fed ducks.

Second Experiment. The LPL mRNA was similar between species but the quantity of LPL mRNA seemed to increase with overfeeding (Figure 1A). The A-FABP mRNA level was 1.7-fold higher in PM muscle of Pekin duck than in that of Muscovy duck after 2 wk of overfeeding (Figure 1B). The quantity of CPT1A mRNA was not different between the 2 species of duck independent of their nutritional conditions (Figure 1C). The COX4 mRNA (more than 5-fold) and SCOT mRNA (more than 2-fold) levels were significantly higher in PM muscle of Pekin duck than in that of Muscovy duck for all nutritional conditions (Figures 1D and 1E). The FAS mRNA was more than 6-fold higher in PM muscle of Pekin duck than in that of Muscovy duck ad libitum-fed or overfed for 7 d and 2-fold higher in PM muscle of Pekin duck overfed for 14 d (Figure 1F). The FAS mRNA seemed to increase with overfeeding.

Protein Levels of A-FABP in PM Muscle

First Experiment. The A-FABP protein content was higher in PM muscle of Pekin duck than in that of Muscovy duck ad libitum-fed or overfed (Table 5). For experiment 1, correlation coefficient between A-FABP protein level and lipid muscle content was R = 0.55 ($P < 0.05$). There was no significant effect of dietary level on the A-FABP protein content in PM muscle of these ducks.

Second Experiment. The A-FABP protein content was more than 1.2-fold higher in PM muscle of Pekin duck than in that of Muscovy duck for each nutritional condition (Figure 2). We observed that A-FABP protein content in PM muscle seemed to increase with overfeeding, particularly for Muscovy duck.

Triglyceride Localization in Adipocytes and Muscle Fibers

In the PM muscle, triglycerides were primarily stored in i.m. adipocytes mainly localized around the fiber bundles but also in fast-twitch oxido-glycolytic type αR fibers (red fibers; Figure 3). The surface occupied by adipocytes was higher in overfed ducks.

Levels and Localization of LPL Protein in PM Muscle

For the second experiment, Western blot analysis showed that Pekin ducks exhibited a higher LPL protein content in PM muscle than Muscovy ducks when birds were ad libitum-fed ($P < 0.05$; Figure 4). No statistically significant differences were found between overfed Pekin and Muscovy ducks.

Figure 5 shows an intense LPL-related immunoreactivity at the level of endomysium (connective tissue around the fibers), epimysium (connective tissue around the bundles of fibers), the endothelial lining of blood vessel, and in the capillary lumen.

Table 4. Expression level of genes involved in lipid metabolism of pectoralis major muscle from Muscovy and Pekin ducks ad libitum-fed or overfed for 2 wk and killed 12 to 14 h after the last meal[1,2] (n = 10)

Species	Dietary level	LPL	A-FABP	CPT1A	COX4	SCOT	FAS
Muscovy	Ad libitum-fed	0.75 ± 0.46	0.42 ± 0.37^{b}	0.65 ± 0.55^{b}	0.15 ± 0.11^{b}	0.43 ± 0.40^{b}	0.10 ± 0.09^{b}
Pekin	Ad libitum-fed	1.39 ± 1.27	0.70 ± 0.50^{ab}	1.61 ± 0.63^{a}	4.36 ± 2.55^{a}	2.38 ± 1.97^{a}	0.10 ± 0.56^{b}
Muscovy	Overfed	1.15 ± 1.18	0.41 ± 0.25^{b}	0.60 ± 0.24^{b}	0.69 ± 0.59^{b}	0.41 ± 0.33^{b}	0.19 ± 0.17^{b}
Pekin	Overfed	0.91 ± 0.76	1.65 ± 1.09^{a}	1.18 ± 0.97^{ab}	3.18 ± 2.74^{ab}	1.69 ± 1.59^{ab}	1.73 ± 0.79^{a}
Species (S) effect		NS	***	**	***	**	***
Dietary level (DL) effect		NS	*	NS	NS	NS	*
S × DL effect		NS	*	NS	NS	NS	NS

[a,b]Significant differences between groups.

[1]Data presented as means ± SE.

[2]LPL = lipoprotein lipase; A-FABP = adipocyte fatty acid-binding protein; CPT1A = carnitine palmitoyl transferase 1A; COX4 = cytochrome-c oxidase 4; SCOT = succinyl-coenzyme A:3-ketoacid coenzyme A transferase; FAS = fatty acid synthase.

*,**,***Differences between groups with $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 5. Adipocyte fatty acid-binding protein (A-FABP) protein content in pectoralis major muscle from Muscovy and Pekin ducks ad libitum-fed or overfed and killed 12 to 14 h after the last meal[1]

Species	Dietary level	A-FABP concentration (log_{10} ng/mg of muscle)
Muscovy	Ad libitum-fed	0.87 ± 0.24^{b}
Pekin	Ad libitum-fed	1.38 ± 0.32^{ab}
Muscovy	Overfed	1.05 ± 0.45^{ab}
Pekin	Overfed	1.54 ± 0.25^{a}
Species (S) effect		**
Dietary level (DL) effect		NS
S × DL effect		NS

[a,b]Significant differences between groups.

[1]Data presented as means ± SE, n = 10.

**Differences between groups with $P \leq 0.01$.

Malonyl CoA Content in PM Muscle

For the second experiment, Pekin ducks had a higher malonyl CoA content in PM muscle than Muscovy ducks when birds were ad libitum-fed. In overfed ducks, the differences were not statistically significant (Figure 6).

DISCUSSION

Effect of Overfeeding

The LPL mRNA level in PM muscle seemed to not be influenced by dietary level when birds were killed 12 to 14 h after the last meal. On the reverse, when birds

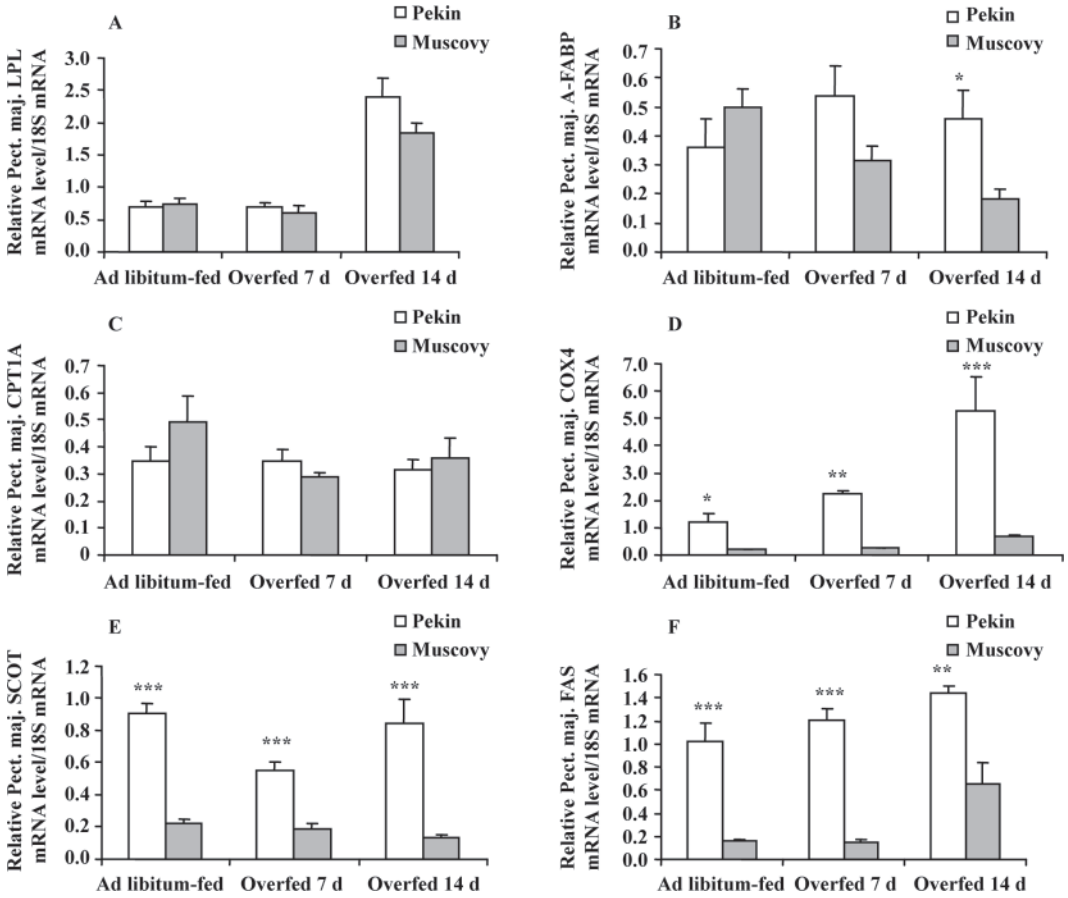

Figure 1. Expression level of genes involved in lipid metabolism of pectoralis major (Pect. maj.) muscle from Muscovy and Pekin ducks ad libitum-fed or overfed for 7 or 14 d and killed 2 to 4 h after the last meal (means ± SE, n = 6). *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$: differences between species for each dietary level. LPL = lipoprotein lipase; A-FABP = adipocyte fatty acid-binding protein; CPT1A = carnitine palmitoyl transferase 1A; COX4 = cytochrome-c oxidase 4; SCOT = succinyl-coenzyme A:3-ketoacid coenzyme A transferase; FAS = fatty acid synthase.

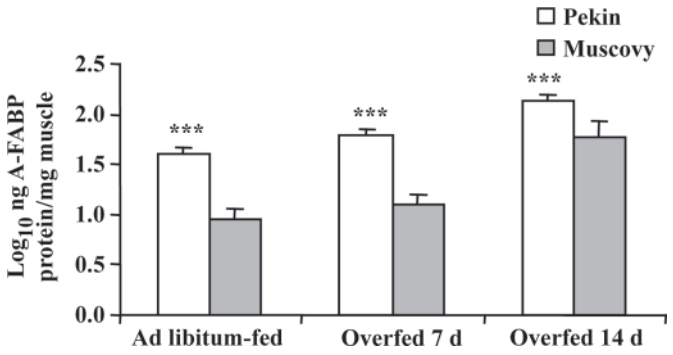

Figure 2. Adipocyte fatty acid-binding protein (A-FABP) protein content in pectoralis major muscle from Muscovy and Pekin ducks ad libitum-fed or overfed for 7 or 14 d and killed 2 to 4 h after the last meal (n = 6). The results are expressed as means ± SE. $^{***}P \leq 0.001$: differences between species for each dietary level.

were killed 2 to 4 h after the last meal, LPL mRNA level seemed to increase in ducks overfed for 14 d by comparison with ducks ad libitum-fed or overfed for 7 d. These contradictory observations suggest a control of LPL gene expression by nutritional status in birds as previously demonstrated in mammals. For instance, Cooper et al. (1989) demonstrated that LPL mRNA level in abdominal adipose tissue increased to a maximum of 350% of that of controls after food deprivation for 48 h and 10 h of refeeding. Bonnet et al. (2000) also showed that LPL mRNA was upregulated by refeeding in cardiac muscle of sheep. The protein level of LPL in PM muscle of Pekin and Muscovy ducks seemed not to be affected by dietary level. Sato and Akiba (2002) found no modification of LPL immunoreactive protein levels in abdominal adipose tissue of chickens fed for 7 d on diets containing 8% olive, safflower, or linseed oil (rich in C18:1, C18:2, or C18:3, respectively). Chartrin et al. (2006b) found a lower activity of LPL in PM muscle of ducks overfed for 14 d than in ad libitum-fed ducks. Dietary level should affect more LPL activity than LPL protein content and LPL gene expression. Our immunoreactivity data are in agreement with LPL location mentioned by several authors (Cryer, 1981; Bensadoun, 1991).

In ducks killed 12 to 14 h after the last meal, the A-FABP mRNA level was increased in overfed birds by comparison with ad libitum-fed birds, whereas the mRNA levels of SCOT, CPT1A, and COX4 were not affected by dietary levels. In ducks killed 2 to 4 h after the last meal, only the COX4 mRNA level in PM muscle of Pekin ducks seemed to increase with overfeeding duration. The A-FABP protein content in PM muscle was not affected by dietary level in ducks killed 12 to 14 h after the last meal, whereas it seemed to increase with overfeeding duration in ducks killed 2 to 4 h after the last meal. On the other hand, Chartrin et al. (2005) found a large increase in the relative area of adipocytes on cross sections of muscle in overfed ducks by comparison with ad libitum-fed ducks (3.74 vs. 2.45% in PM muscle). Chartrin et al. (2006b) observed that the oxidative metabolism in PM muscle, estimated by cytochrome-c oxidase and β-hydroxy-acyl CoA dehydrogenase activities, was increased in overfed ducks compared with ad libitum-fed ducks. It will therefore be interesting to study CPT1A, COX4, and SCOT protein content because it is possible that gene expression, protein content, and activity of these proteins may not evolve in the same way with overfeeding, and in our case, gene expression may not be the best indicator of muscle oxidative metabolism. However, the result for FAS, the gene involved in lipogenesis, was in agreement with the activities of other enzymes involved in lipogenesis such as acetyl CoA carboxylase or malic enzyme, which increased with overfeeding (Chartrin et al., 2006b). The FAS results suggested an ability to synthesize lipids in situ contributing partly to lipid deposition in PM muscle of overfed ducks.

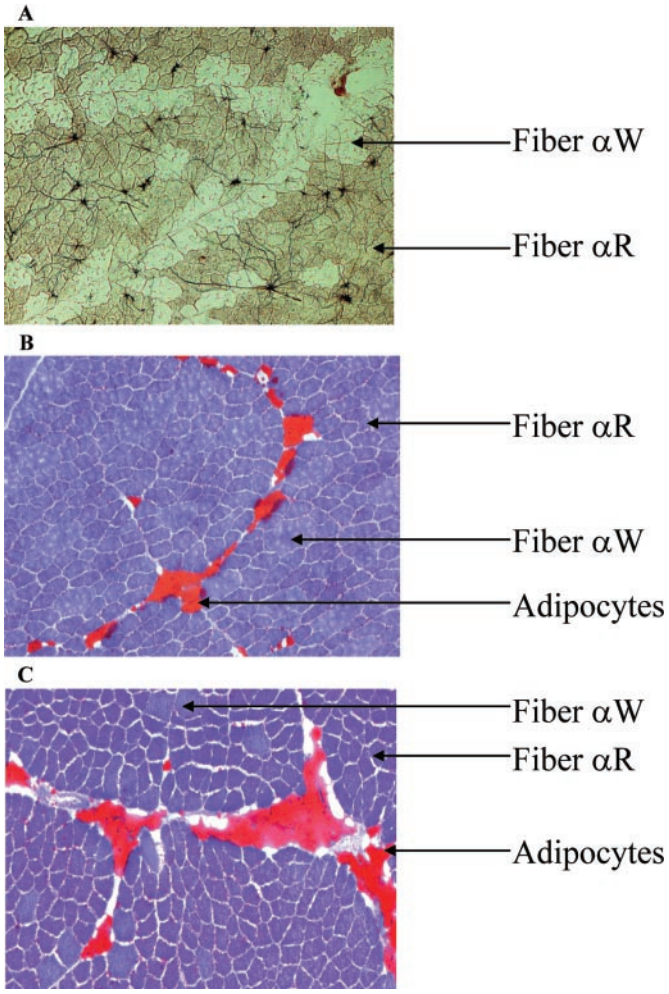

Figure 3. Cross sections of pectoralis major muscle stained with red oil to make visible triglycerides in muscle fibers of ad libitum-fed Muscovy duck (A) and with red oil and purple crystal to make visible i.m. adipocytes and muscle fibers, respectively, from Pekin ducks ad libitum-fed (B) or overfed (C). Magnification × 10. αR = red fiber or fast-twitch oxydo-glycolytic; αW = white fiber or fast-twitch glycolytic. Color version available in the online PDF.

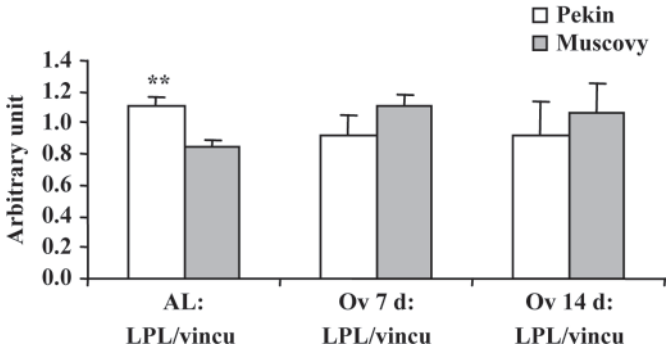

Figure 4. Concentration of lipoprotein lipase (LPL) in the pectoralis major muscle of Muscovy and Pekin ducks ad libitum-fed (AL) or overfed (Ov) for 7 or 14 d and killed 2 to 4 h after the last meal (n = 6 per nutritional condition). Protein extracts were prepared and subjected to Western blotting using anti-LPL antibodies. Vinculin (vincu) was used as loading control (n = 6). The results are expressed as means ± SE of the LPL:vincu ratios (n = 6). $^{**}P \leq 0.01$: differences between species for each dietary level.

Effect of Species

The LPL gene expression in PM muscle was similar for Muscovy and Pekin ducks independent of the dietary level and the slaughtering delay after the last meal. We also had no species effect on the LPL protein content in PM muscles of overfed ducks. On the other hand, Chartrin et al. (2006b) showed that LPL activity was higher in the PM muscle of Pekin ducks also exhibiting higher IMF content than Muscovy ducks. Moreover, Davail et al. (2003) showed a postprandial LPL activity similar in plasma of Pekin and Muscovy ducks at the beginning of the overfeeding period and then a higher LPL activity in Pekin ducks than in Muscovy ducks at the end of the overfeeding period. Therefore, LPL activity seemed to be an important factor in the control of fatty acid uptake and i.m. fattening, which was suggested by André et al. (2007), more than LPL expression or LPL protein content. On the other hand, Wu et al. (2008) showed a polymorphism in exon 7 of the LPL gene associated with fatness traits in native and Cherry Valley Peking ducks. Therefore, more investigation on LPL gene seems to be necessary to select it as a candidate gene for fatness traits and to investigate associations of gene polymorphisms with these traits in duck populations. The location of LPL in muscular tissue observed in our experiment was in agreement with the location suggested by several authors (Cryer, 1981; Braun and Severson, 1992).

In the present study, Pekin ducks exhibited a higher A-FABP gene expression and protein content in PM muscle than Muscovy ducks, in agreement with the difference of the relative area occupied by i.m. adipocytes on cross sections of muscle (Chartrin et al., 2005) and of IMF content reported by Chartrin et al. (2006a,b). In fact, these authors observed different IMF content in PM muscle between Muscovy and Pekin ducks ad libitum-fed (2.3 vs. 4.6%) or overfed (3.7 vs. 7.6%). These last results confirmed previous results in other lean and fat species such as pigs (Damon et al., 2006) or bovine (Jurie et al., 2007) and the idea that A-FABP was a marker of lipogenesis, particularly in adipocytes (Hertzel et al., 2006) because A-FABP is specifically expressed in adipocytes. Moreover, in avian species, Li et al. (2008) obtained similar results on A-FABP gene expression, which was significantly correlated with IMF content in 2 Chinese chicken breeds (Beijingyou and Jingxing).

For the expression of genes coding for enzymes involved in oxidation (CPT1A, COX4, SCOT), we found a species effect. Actually, independent of their nutritional condition and the slaughtering delay after the last meal, the tissue oxidation capacity could be higher in PM muscle of Pekin duck than in that of Muscovy duck (except for CPT1A in ducks slaughtered 2 to 4 h after the last meal). In overfed ducks, this oxidation capacity seemed not to be inhibited by malonyl CoA in which content was similar for both species. However, these data should be completed by analyzing CPT1B gene expression, which is normally the main form in muscle and for which we did not succeed to find appropriated primers. The difference observed for SCOT gene expression in ducks did not confirm previous measure-

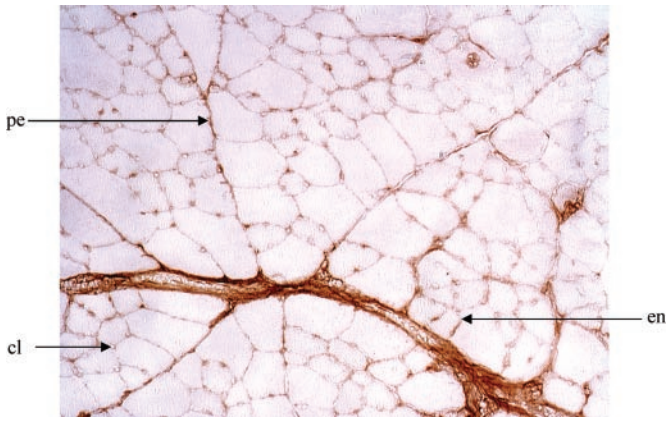

Figure 5. Lipoprotein lipase (LPL)-related immunoreactivity on cross sections of pectoralis major muscle of Muscovy duck ad libitum-fed at the level of perimysium (pe), endomysium (en), and capillary lumen (cl). Magnification × 10. Color version available in the online PDF.

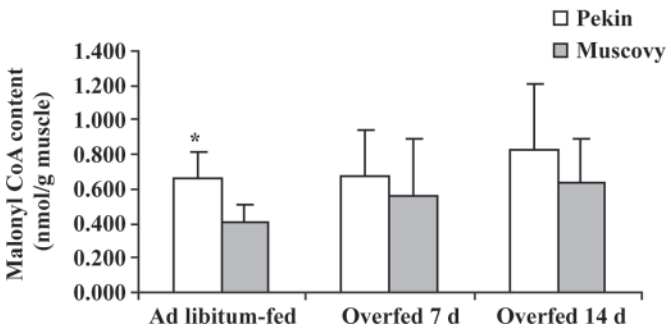

Figure 6. Malonyl coenzyme A (CoA) content in the pectoralis major muscle of Muscovy and Pekin ducks ad libitum-fed or overfed 7 or 14 d and killed 2 to 4 h after the last meal (means ± SE, n = 6). * $P \leq 0.05$: differences between species for each dietary level.

ments on lean and fat chickens in which no genotype effect was observed in the PM muscle (Skiba-Cassy et al., 2007). The oxidation of FA seemed to be higher in the liver of Pekin ducks also exhibiting a higher hepatic CPT1A gene expression than Muscovy ducks (Hérault et al., 2008). On the another hand, we showed that liver slices from ad libitum-fed Pekin ducks incubated with [1-^{14}C]-linoleic acid produced more ketone bodies than those from Muscovy ducks (Saez et al., 2009). Therefore, potentially more ketone bodies could be produced by liver and used as an energy source by PM muscle of Pekin duck and by consequence increase SCOT gene expression in muscle. These present results associated with FAS expression, which was higher in Pekin ducks, suggest that the i.m. fattening could be facilitated by a better muscular lipogenesis than in Muscovy ducks and by consequence more available fatty acid could stimulate the oxidation capacity of the muscle tissue. Our data were in agreement with those of Chartrin et al. (2006b) showing the highest lipogenic activity and the highest oxidative energy metabolism in PM muscle of Pekin ducks by comparison with Muscovy ducks. These data are also in agreement with the conclusions of Gondret et al. (2004) in rabbits, showing that muscle fat content variation resulted from a reciprocal balance between catabolic and anabolic fatty acid fluxes.

Both species and overfeeding produce the same effect: a 2-fold higher IMF content in Pekin ducks versus Muscovy ducks or in overfed ducks versus ad libitum-fed ducks. However, it seems that during overfeeding, duck muscles store lipids neosynthesized by liver, but the muscle lipid metabolism is less affected. On the reverse, the difference observed between Pekin and Muscovy ducks for the IMF content could partly be induced by difference in muscle lipid metabolism and particularly the number of i.m. adipocytes (indicated by A-FABP expression; Jurie et al., 2007) and balance between fatty acid fluxes (uptake from plasma, synthesis, and oxidation).

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