Effects of age on lipid deposition in breast muscle of mule ducks

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Introduction

The effects of age on lipid levels in poultry muscle depend on the species. In chickens, lipid levels in breast and thigh muscles remain stable or decrease with age, at least until 100 days (Rabot, 1998) and then, according to the few reports available for older chickens, lipid levels possibly increase (Marion and Miller, 1968; Grey *et al.*, 1983). Wangen *et al.* (1972) reported that lipid levels in breast muscle remained unchanged in turkeys killed at intervals of 4 weeks from 4 to 28 weeks, whereas they increased in thigh muscle after 16 weeks of age. Komprda *et al.* (2002) found significant increases in lipid levels in breast and thigh muscles of turkeys slaughtered at one-week intervals between 10 and 25 weeks of age. In ducks, only Setiawan *et al.* (1994) and Baéza *et al.* (2000; 2002) showed an increase in lipid content of breast muscle of mule and Muscovy ducks between 6-8 and 12-13 weeks of age. The aim of this study was therefore to determine the effects of age (from 1 day post-hatching to 98 days of age) on lipid deposition in breast muscle (quantity and quality, localisation) of mule ducks compared to muscle energy metabolism (glycolytic and oxidative), plasma levels of lipids, and muscle capacity for lipid uptake (characterized by lipoprotein lipase activity).

Material and methods

We used male mule ducks provided by Grimaud (Roussay, France). The birds were reared and ad libitum fed at the Experimental Station for Waterfowl Breeding (INRA Artiguères, France). Feed consumption was recorded for each period. At 1, 14, 42, 75, 84, 91 and 98 days of age, 10 ducks per group, deprived of feed overnight, were randomly selected and weighed. Blood samples were collected on EDTA and plasma samples were stored at -20°C. Ducks were then sacrificed by cervical dislocation. Immediately after bleeding, a breast muscle (*Pectoralis major*) was excised and weighed. One sample of *P. major* was quickly frozen in isopentane, cooled with liquid nitrogen (Chartrin *et al.*, 2005) and stored at – 80°C prior to determination of enzymatic activity and histochemical analysis. A sample of *P. major* muscle was also frozen and stored at -20°C for chemical analysis.

Plasma triglyceride (TG), total cholesterol (CT) and phospholipid (PL) levels were quantified by colorimetric enzymatic methods using kits provided by Bio-Mérieux and Bio-direct. The levels of triglycerides (stained with red oil = RO), and total lipid (stained with Sudan black = SB), were determined in muscle fibres according to type as previously described by Chartrin *et al.* (2005): fast-twitch fibres with glycolytic energy metabolism (αW) or oxydo-glycolytic energy metabolism (αR). Staining intensities were expressed as luminance (Lc RO and Lc SB), luminance being negatively correlated with lipid and triglyceride contents. We also evaluated the relative surface occupied by adipocytes on cross-sectional areas of each muscle sample as described by Chartrin *et al.* (2005) and the results were expressed as relative surface occupied by adipocyte cluster per observed field (%).

Total lipids were determined according to Folch *et al.* (1957). Lipid classes were determined by thin-layer chromatography according to Mares *et al.* (1983). Fatty acid composition was determined after transmethylation of lipids (Morrisson and Smith, 1964) by gas chromatography as previously described by Chartrin et al. (2006). LPL activity was determined in the *P. major* muscle according to Hocquette *et al.* (1998).

The activity of the enzymes for glycolytic (lactate dehydrogenase, LDH) and oxidative (β-hydroxyacyl CoA dehydrogenase, HAD and citrate synthase, CS) muscle energy metabolism was assayed in *P. major* muscle (Bass *et al.*, 1969).

Data were analysed by analysis of variance using the General Linear Model procedure of SAS (1989).

Results and discussion

The kinetics of fat deposition in breast muscle might be divided into two periods, i.e. before and after 42 days of age, the greatest effects of age occurring between 14 and 42 days when growth of breast muscle was maximal (weight increased 27-fold, CSA increased 15 and 18-fold in αR and αW fibre types, respectively). The energy metabolism of birds is based on the use of lipids stored in egg yolk until hatching (Klasing, 1998). Total lipid content in the breast muscle of mule ducks was therefore high at 1 day of age (2.9 g/100 g muscle) and mainly composed of phospholipids (50% of total lipids) and cholesterol (29%), reflecting the high phospholipid and cholesterol plasma levels. Egg yolk is rich in oleic acid (C18:1), explaining the high oleic acid levels measured in the breast muscle of mule ducks at 1 day of age (41% of total fatty acids). LPL activity was high at 1 and 14 days of age, allowing breast muscle to import blood lipids. Lipids were mainly stored in adipocytes in the breast muscle of 1-day-old ducklings which could be a specificity of birds as in most mammals, triglycerides are mainly stored in the fibre cytoplasm at birth and adipocytes develop later (Gondret *et al.*, 1998). By contrast, total lipid and triglyceride levels in muscle fibres were lower at hatching than at older ages. Breast muscle development occurred mainly after 14 days of age. Levels of enzyme activity reflecting energy metabolism (CS, HAD, LDH) were therefore low at hatching as previously reported in mammals.

We observed a decrease in total lipid content in breast muscle of mule ducks induced mainly by a decrease in phospholipids and cholesterol between 1 and 42 days of age. In terms of fatty acid composition, the greatest decrease was in oleic acid. Triglyceride content in fibres increased in breast muscle of mule ducks at day 14 whereas the surface occupied by adipocytes decreased at day 42. As CS and HAD activity increased it can be hypothesised that muscle fibres use the lipids stored in adipocytes to supply their energy requirements for growth. The LPL activity of breast muscle decreased, in agreement with the decrease in LPL activity in adipose tissues of Aylesbury ducks (*Anas platyrhynchos*) reported by Evans (1972). It seems that LPL activity in breast muscle depends on plasma levels of lipids.

We observed an increase in total lipid content in breast muscle of mule ducks, induced by an increase mainly in triglycerides, but also in phospholipids, between 42 and 98 days of age. At 98 days of age, triglycerides represented 58% of total lipids. Setiawan *et al.* (1994) and Baéza *et al.* (2000; 2002) also reported an increase in total lipid content in the breast muscle of mule ducks between 42 or 56 and 91 days of age and in the breast muscle of Muscovy ducks between 56 and 84 days of age, respectively. Analysis of fatty acid composition showed that the main increase involved MUFA (particularly oleic acid) and SFA (particularly palmitic acid) provided by lipogenesis in the liver (Klasing, 1998) or feed. During this second period, lipids were first stored in muscle fibres: the triglyceride content increased until 75 and 84 days of age in αR and αW fibre types, respectively. Later, lipids were stored in adipocytes which occupied an increasing surface from 75 days of age. LPL activity increased in the breast muscle of mule ducks between 42 and 75 days of age allowing an increased uptake of circulating lipids, then remained stable. HAD, LDH and CS activity increased until 75, 91 and 98 days of age, respectively. LDH activity was much higher than CS and HAD activity. These findings are in agreement with previous reports in Muscovy ducks by Baéza *et al.* (1998) and confirm that oxidative metabolism is prevalent in breast muscle at the beginning of postnatal development whereas glycolytic metabolism takes over in later development.

Conclusion

The original contribution of this study was to describe the kinetics of lipid deposition in relation to its localisation (adipocytes and muscle fibres) in the breast muscle of ducks during growth post-hatch. This has not been reported for any domesticated bird to date.

Two periods can be defined for age effects on intramuscular lipids in breast muscle:

- 1 to 42 days of age when lipids stored in the adipocytes during embryonic life are used by the muscle fibres for growth and energy requirements,
- 42 to 98 days of age when the muscle again stores lipids, first in fibres then in adipocytes.

Table: Age effect on the breast muscle weight from mule ducks (g), the breast muscle level in total lipids, triglycerides, phospholipids, cholesterol ester, free cholestérol (mg/100 g muscle), saturated, mono- and poly-unsaturated fatty acids (SFA, MUFAI, PUFA, g/100 g muscle), the enzyme activity of lactate dehydrogenase (LDH), β-hydroxyacyl Co A dehydrogenase (HAD), citrate synthase (CS, UI/g muscle) and lipoproteine lipase (LPL en nmol/min/g muscle), the relative surface (%) occupied by adipocytes on a cross-sectional area of muscle, the cross-sectional area (CSA, μm^2) of muscle fibres and their luminance induced by triglyceride (Lc RH) or total lipid (Lc NS) staining (mean, n = 10)

Age (days)	1	14	42	75	84	91	98
Breast muscle weight	0.31 d	2.47 d	67.35 c	252.54 b	308.96 a	310.65 a	313.65 a
Total lipids	2930 b	2300 d	1270 e	2740 c	2830 bc	3110 b	3890 a
Triglycerides	619 d	1140 c	170 e	1146 c	1347 bc	1569 b	2263 a
Phospholipids	1468 ab	898 d	929 d	1225c	1362 b	1401 ab	1520 a
Cholesterol ester	385 a	11 b	3 c	1 c	nd	nd	nd
Free cholesterol	458 a	251 c	166 d	368 b	121 d	140 d	107 d
SFA	0.98 b	0.78 bc	0.49 c	0.98 b	1.01 b	1.37 a	1.40 a
MUFA	1.37 ab	1.12 bc	0.45 d	1.03 c	1.10 bc	1.25 b	1.63 a
PUFA	0.57 bc	0.40 c	0.33 c	0.73 b	0.72 b	0.76 b	0.86 a
LDH	87 f	135 e	460 d	588 b	569 b	696 a	535 c
HAD	2.49 c	3.20 c	2.21 c	14.78 a	11.75 ab	12.22 ab	9.85 b
CS	6.63 d	5.72 d	9.45 c	10.75 bc	11.68 ab	12.48 ab	12.75 a
LPL	565 a	573 a	93 c	236 b	190 bc	201 bc	160 bc
Adipocyte surface	2.71 a	2.11 a	0.54 c	0.22 c	0.39 c	1.02 bc	1.33 b
CSA of αR fibres	8 c	14 c	212 b	625 ab	659 ab	682 a	767 a
Lc NS	123 a	114 a	110 a	79 b	75 b	75 b	90 b
Lc RH	137 a	96 b	87 b	67 c	62 c	61 c	83 b
CSA of αW fibres	12 d	30 d	550 c	1561 b	1525 b	1678 b	2143 a
Lc NS	124 a	120 a	128 a	120 a	78 b	107 a	101 ab
Lc RH	143 a	99 b	93 b	77 bc	62 c	71 bc	88 bc

αR and αW fibres: fast-twitch fibres with oxydo-glycolytic or glycolytic energy metabolism, respectively.
The luminance is negatively correlated with lipid and triglyceride levels in fibres.
a-e: significant age effect, $P < 0.05$; nd = not detected

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