Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part B 147 (2007) 102–109
CBP
www.elsevier.com/locate/cbpb

Metabolic fates of yolk lipid and individual fatty acids during embryonic development of the coot and moorhen

Athanasios C. Pappas [a], Filiz Karadas [b], Nicholas A.R. Wood [c], Brian K. Speake [a,*]

[a] *Avian Science Research Centre, SAC, Auchincruive, Ayr KA6 5HW, UK*
[b] *Department of Animal Science, University of Yüzüncü Yil, 65080 Van, Turkey*
[c] *Waterfowl Consulting and Research, Devon EX18 7NB, UK*

Received 7 December 2006; received in revised form 20 December 2006; accepted 30 December 2006
Available online 12 January 2007

Abstract

There is currently little information regarding the metabolic fates of yolk lipid and individual fatty acids during embryonic development of free-living avian species. Here we report the pattern of lipid utilization during embryonic development of the coot (*Fulica atra*) and the moorhen (*Gallinula chloropus*), two related species producing precocial offspring from eggs with a distinctive fatty acid composition and with an incubation period similar to that of the chicken. By the time of hatching, the proportions of the initial yolk lipid that had been transferred to the embryo were 88.2% and 79.8% for the coot and moorhen respectively. During the whole incubation period, 42.9% and 40.0% of the initial yolk lipid of the coot and moorhen respectively were lost from the system due to oxidation for energy, equating to 47.8% and 50.0% respectively of the actual amount of lipid transferred over this time. Thus, the lipid received by the embryos of both species is partitioned almost equally between the alternative fates of energy metabolism and incorporation into tissue lipids. In the coot, this 50:50 split between oxidation and tissue formation was maintained during the hatching process. The proportions of arachidonic (20:4n-6) and docosahexaenoic (22:6n-3) in the yolk lipids of these species were 2.5-3.5 times higher than in eggs of domestic poultry. In contrast to the situation in the chicken, there was no preferential uptake of 22:6n-3 from the yolk during coot and moorhen development. The fatty acid compositions of the whole body lipids of the coot and moorhen hatchlings were almost identical to those of the initial yolks indicating that, unlike the chicken, these species display relatively little overall biomagnification of 20:4n-6 and 22:6n-6 during development. It is suggested that the yolk fatty acid profiles of the coot and moorhen are particularly well matched to the requirements of the embryo, reducing the need for selective uptake of 22:6n-3 and for the overall biomagnification of 22:6n-3 and 20:4n-6.

Keywords: Arachidonic acid; Coot; Egg; Docosahexaenoic acid; Fatty acids; Lipid; Moorhen; Yolk

1. Introduction

The use of yolk-derived fatty acids is a central feature of avian embryonic metabolism due, in part, to an almost total reliance on the β-oxidation pathway to provide the energy for development (Speake et al., 1998). Furthermore, embryonic tissue growth and differentiation are dependent on a continuous supply of fatty acids for the biosynthesis of cell membrane phospholipids. The phospholipid fatty acid profiles of the various tissues of the embryo are highly distinctive. For example, the phospholipids of the avian brain and retina acquire exceptionally high proportions of docosahexaenoic acid (22:6n-3) during the course of development (Anderson et al., 1989; Cherian and Sim, 1992; Ruiz-Gutierrez et al., 1996; Speake and Wood, 2005). Similarly, the long chain omega-6 polyunsaturate, arachidonic acid (20:4n-6), is a major component of the phospholipids of the developing heart, kidney, liver and brain (Speake et al., 1998; Decrock et al., 2002). Incorporation of yolk-derived fatty acids into adipocyte triacylglycerol to form embryonic fat stores represents an additional fate of yolk-derived fatty acids that is quantitatively important in some avian species (Farkas et al., 1996; Groscolas et al., 2003).

Fatty acids are present in yolk in the esterified form, mainly as components of triacylglycerol and phospholipid molecules

* Corresponding author. Tel.: +44 1292 443535; fax: +44 1292 525098.
E-mail address: Brian.Speake@googlemail.com (B.K. Speake).

1096-4959/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2006.12.009

(Speake et al., 1998). During development, these lipids are absorbed by the yolk sac membrane and delivered into the embryonic circulation. Following their uptake by the developing tissues, the yolk-derived fatty acids are variously utilized for energy, membrane biogenesis, and fat store formation (Speake et al., 1998).

The quantitative partitioning of yolk fatty acids between energy generation and incorporation into tissue structures has been studied in considerable detail during the embryonic development of the domestic chicken (Noble and Cocchi, 1990; Lin et al., 1991; Cherian and Sim, 1993). A series of key findings has emerged from these studies. Firstly, only 75–80% of the original yolk lipid is transferred to the chicken embryo prior to hatching: the remainder forms the residual yolk that is retracted into the body of the chick, providing a source of nutrients for several days post hatch (Noble and Cocchi, 1990). Secondly, approximately 50% of the initial yolk lipid is oxidized for energy during embryonic development, with the remaining 50% being recovered in the body tissues and residual yolk of the newly hatched chicken (Lin et al., 1991). Thirdly, the functional importance of 22:6n-3 and 20:4n-6 in cell membrane phospholipids is reflected in the preferential incorporation of these long chain polyunsaturates into the tissue lipids, with lower proportions of these two fatty acids being used for energy. Thus, 70–80% of the 22:6n-3 and 20:4n-6 initially present in the yolk are recovered in the tissues of the newly hatched chicken, compared with only about 50% for all the other major fatty acids (Lin et al., 1991). A fourth finding is that 22:6n-3 is preferentially transferred from the yolk to the embryo (Maldjian et al., 1995). Thus, as a result of its selective transfer during the embryonic period, the proportion of 22:6n-3 (as % w/w of total fatty acids) in the residual yolk of the newly hatched chicken is far less than that in the initial yolk whereas the proportions of all the other fatty acids show little or no change (Noble and Cocchi, 1990; Lin et al., 1991; Cherian and Sim, 1992). It is suggested that the selective uptake of 22:6n-3 is necessary to ensure an adequate supply of this fatty acid to the developing nervous system of the chicken (Speake et al., 1998).

Such in depth information is, however, lacking for most other avian species, particularly for birds breeding in the wild. This limitation precludes any comparative evaluation of the effects of interspecies differences in developmental strategies and yolk fatty acid compositions on the pattern of lipid utilization by the embryo. An exception is the embryo of the king penguin (*Aptenodytes patagonicus*), where the metabolic fate of yolk fatty acids has been investigated in detail (Decrock et al., 2001; Groscolas et al., 2003). The distribution of yolk lipid among the various developmental fates in the king penguin embryo was found to differ markedly from the situation described for the chicken. For example, 83% of the transferred fatty acid was oxidized for energy by the penguin embryo with only 17% incorporated into the tissue lipids. Moreover, 20:4n-6 was selectively incorporated into tissue lipids of the penguin embryo so that less 20:4n-6 was used for energy in comparison with the other fatty acids but, unlike the situation in the chicken, no such preference was observed for 22:6n-3. Most notably, 22:6n-3 was not preferentially taken up from the yolk by the penguin embryo, since the proportion (% w/w of total fatty acids) of 22:6n-3 in the lipid of the residual yolk was identical to that in the initial yolk (Speake et al., 2003a).

These findings suggest that interspecies differences in embryonic physiology, and in the natural proportions of 22:6n-3 and 20:4n-6 in the yolk, can profoundly affect the patterns of embryonic fatty acid utilization. To further investigate these possibilities, we have quantified the various fates of yolk fatty acid during development of the coot and moorhen. These species were chosen because the proportions of 22:6n-3 in their egg lipids are intermediate between those in the chicken and penguin. Also, eggs of both coot and moorhen contain higher proportions of 20:4n-6 compared with those of both chicken and penguin (Speake et al., 2003a and unpublished data).

2. Materials and methods

Freshly laid eggs of the coot (*Fulica atra*) and the moorhen (*Gallinula chloropus*) were collected from nests in a natural wetland habitat near Sherborne, Dorset, UK (51°0′N, 2°30′W). Egg collection was approved by the Licensing Section, English Nature, Peterborough, UK, under license numbers 20021118, 20040996 and 20041576 (to N.A.R Wood). Coot eggs (3 per clutch) were collected from 8 clutches. Moorhen eggs (2 per clutch) were collected from 8 clutches. In the case of the coot samples, one egg from each clutch was taken for analysis of fresh egg contents and the other 2 eggs from that clutch were incubated in a bench-top incubator at 37.5 °C with automatic egg turning. One of these eggs was removed for sampling after 19 days of incubation, just prior to the start of the hatching process. The remaining egg was incubated for 22 days when the chick hatched. The newly hatched chick was sampled within 8 h of emerging from the shell. The weights of the eggs selected for analysis of fresh egg contents did not differ significantly from the initial weights of the eggs that were incubated. The moorhen eggs were treated in the same way, except only the fresh egg and the newly hatched chick from each clutch were available (due to the limited number of eggs permitted under the collection

Table 1
Weights of yolk and embryo/chick during development of the coot and moorhen

	Coot	Moorhen
Mass (g)		
Whole egg	33.29±1.11	23.72±0.81
Yolk (initial)	11.81±0.44[a]	8.19±0.40[a]
Yolk (residual–late embryo)	5.05±0.31[b]	N/A
Yolk (residual–newly hatched chick)	2.24±0.31[c]	1.95±0.20[b]
Late embryo body (minus yolk sac)	17.84±0.60[d]	N/A
Hatched chick body (minus yolk sac)	20.38±0.75[e]	13.90±0.57[c]
% of yolk transferred		
Late embryo	57.3±2.0[x]	N/A
Hatched chick	80.8±2.2[y]	76.2±2.0

Values are means±S.E.M. ($n=7$ for coot and 8 for moorhen samples).
[a–e]Within a column, values for yolk and body weights with different superscripts are significantly different: $P<0.05$.
[x,y]Within a column, values for percentage yolk transfer (coot only) are significantly different: $P<0.01$.
Abbreviation: N/A indicates data not available.