Captivity Diets Alter Egg Yolk Lipids of a Bird of Prey (the American Kestrel) and of a Galliforme (the Red-Legged Partridge)

Peter F. Surai^{1,*} Brian K. Speake¹ Gary R. Bortolotti² Juan J. Negro³

¹Avian Science Research Centre, Scottish Agricultural College, Auchincruive, Ayr KA6 5HW, United Kingdom; ²Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, Saskatchewan S7N 5E2, Canada; ³Consejo Superior de Investigaciones Cientificas, Estacion Biologica de Donana, 41013 Sevilla, Spain

Accepted 8/7/00

ABSTRACT

The salient feature of the fatty acid profile of kestrel eggs collected in the wild was the very high proportion of arachidonic acid (15.2% \pm 0.7% of fatty acid mass, n = 5) in the phospholipid fraction of the yolk. Kestrels in captivity fed on dayold chickens produced eggs that differed from those of the wild birds in a number of compositional features: the proportion of linoleic acid was increased in all the lipid fractions; the proportion of arachidonic acid was increased in volk phospholipid and cholesteryl ester; the proportion of α -linolenic acid was decreased in all lipid classes, and that of docosahexaenoic acid was decreased in phospholipid and cholesteryl ester. Partridge eggs from the wild contained linoleic acid as the main polyunsaturate of all the yolk lipid fractions. Captive partridges maintained on a formulated diet very rich in linoleic acid produced eggs with increased levels of linoleic, arachidonic, and n-6 docosapentaenoic acids in the phospholipid fraction; reduced proportions of α -linolenic acid were observed in all lipid classes, and the proportion of docosahexaenoic acid was markedly reduced in the phospholipid fraction. Thus, captive breeding of both the kestrel and the partridge increases the n-6/n-3 polyunsaturate ratio of the yolk lipids.

Introduction

The β -oxidation of fatty acids derived from yolk lipids provides the avian embryo with more than 90% of the energy required for development. The yolk lipids are also the source of phospholipid and cholesterol for the biogenesis of cell membranes. During development, the membrane phospholipids of the different tissues acquire distinct fatty acid compositions. For example, the phospholipids of the embryo's brain and retina become highly enriched with docosahexaenoic acid (22:6n-3), whereas those of the heart and liver display very high proportions of arachidonic acid (20:4n-6). These long-chain polyunsaturates are believed to play essential roles in the development and function of the tissues and can either be supplied preformed in the yolk or can be synthesized in the embryo from their yolk-derived C₁₈ precursors. Thus, linoleic (18:2n-6) and α -linolenic (18:3n-3) acids can be converted to 20:4n-6 and 22:6n-3, respectively, by elongation and desaturation reactions. It is, therefore, evident that the yolk lipids must contain polyunsaturates of both the n-6 and n-3 families in amounts and proportions appropriate for the demands of the embryonic tissues. The composition of yolk lipids and their utilization by the avian embryo have been described in several review articles (Noble and Cocchi 1990; Kuksis 1992; Speake et al. 1998a, 1998b; Speake and Thompson 1999).

The polyunsaturate profiles of the yolk vary greatly between avian species, largely due to differences in diet. Thus, the yolk of the granivorous chicken contains 18:2n-6 as its main polyunsaturate since this is the predominant fatty acid of the lipids stored in grains and seeds (Maldjian et al. 1996). By contrast, 18:3n-3 derived from chloroplast lipids is the characteristic polyunsaturate of the yolk lipids of herbivorous birds such as the goose (Speake et al. 1999b) and ostrich (Noble et al. 1996). Piscivores such as the emperor penguin produce eggs enriched with 22:6n-3 and eicosapentaenoic acid (20:5n-3) due to the preponderance of these fatty acids in fish lipids (Speake et al. 1999a). Superimposed onto these dietary effects are phylogenetic differences between avian species. For example, chickens, turkeys, ducks, and geese maintained on identical diets produce eggs with very different fatty acid profiles (Maldjian et al. 1996; Surai et al. 1999).

Several studies have demonstrated that certain avian species maintained in captivity produce eggs with markedly different fatty acid profiles than those of their counterparts in the wild; for instance, the yolk lipids of commercially raised ostriches (Noble et al. 1996), pheasants, and geese (Speake et al. 1999*b*)

^{*} Corresponding author; e-mail: p.surai@au.sac.ac.uk.

Physiological and Biochemical Zoology 74(2):153-160. 2001. © 2001 by The University of Chicago. All rights reserved. 1522-2152/2001/7402-0058\$03.00

are relatively deficient in 18:3n-3, while the eggs of ducks in captivity contain exceptionally low levels of 22:6n-3 (Maldjian et al. 1996). It appears that the natural diets selected by these birds in the wild provide greater amounts of n-3 polyunsaturates than are present in the feeds that are formulated for avian species in captivity. The suggestion that the low hatchability and neonatal viability characteristic of some species of captivebreeding birds may be due to inadequate amounts of n-3 fatty acids in the yolk (Noble et al. 1996) is feasible but has yet to be tested in controlled feeding trials.

There is little information currently available that describes the lipid composition of eggs of carnivorous birds. The American kestrel (*Falco sparverius*) is an appropriate species for such investigations because it is widely available both in the wild and in captivity (Bird 1980). The red-legged partridge (*Alectoris rufa*), by contrast, is largely vegetarian and is a member of the Galliforme order, which includes the domestic chicken. Captive breeding of the red-legged partridge is an emerging industry, particularly in Spain, but no information on the relationship between diet and egg composition is currently available. The aim of our study is, therefore, to document for the first time the natural fatty acid profile of the eggs of a carnivorous bird, the American kestrel, and to evaluate the consequences of the diets provided to kestrels and to red-legged partridges in captivity on egg lipid composition.

Material and Methods

Egg Collection

Eggs of wild kestrels were collected in 1999 from nest boxes provided for research purposes in the vicinity of Saskatoon, Saskatchewan, Canada. One egg per clutch was collected within 1–2 d after it was laid. Eggs of captive kestrels were collected in 1998 at the Avian Science and Conservation Centre of McGill University, Montreal, Quebec, Canada. These were collected within 1 d after they were laid, from a communal pen housing 13 females (i.e., the birds were not mated). The laying dates indicate that the five eggs collected were from five different females. The kestrels in captivity were fed exclusively on dayold chickens hatched from eggs laid by hens fed a standard corn–soybean meal diet.

Eggs of wild partridges originated from nests found on agricultural land in Andalucia (southern Spain) and were collected in May 1999 with permission of the environmental authorities. Eggs from captive partridges were obtained from a game farm in Andalucia in April and May 1999. The eggs from both the wild and captivity were collected 1–3 d after laying, thereby minimizing embryonic development. Immediately on collection, the whole eggs were frozen and stored at -2° C. The yolk was later removed intact from the egg, taking advantage of the fact that albumin thaws more quickly than yolk (Lipar et al. 1999).

Captive partridges were supplied with a commercial diet

(containing maize, wheat, barley, soybean meal, and sunflower seeds) formulated for this species. The fatty acid compositions of the formulated partridge feed and of the newly hatched chickens fed to the captive kestrels are shown in Table 1. Frozen yolk samples of both species were packed in dry ice and transported by air from Canada and Spain to the laboratory of P. F. Surai in the United Kingdom for analysis.

Lipid Analysis

Total lipid from egg yolk was extracted by homogenization in a suitable excess of chloroform/methanol (2:1, v/v). The mass of total lipid was determined gravimetrically on a portion of the extract after evaporation of the solvent. The major lipid classes in the extract (triacylglycerol, phospholipid, cholesteryl ester, free cholesterol, free fatty acid) were separated by thin-layer chromatography on silica gel G using a solvent system of hexane/diethyl ether/formic acid (80:20:1, v/v). Visualization of the bands and elution of the lipid classes from the silica were performed as described previously (Noble et al. 1996). The isolated phospholipid fraction was further subfractionated by thin-layer chromatography on silica gel D using a solvent system of chloroform/methanol/acetic acid/ water (25:15:4:2, v/v). The major phospholipid classes, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and sphingomyelin, were visualized and eluted from the silica (Noble et al. 1996).

The isolated acyl-containing lipid and phospholipid classes were transmethylated to form fatty acid methyl esters as described previously (Noble et al. 1996). Analysis of the fatty acid methyl esters was performed by gas–liquid chromatography using a capillary column (Carbowax, 30 m × 0.25 mm, film thickness 0.25 μ m; Alltech) in a CP9001 Instrument

Table 1: Fatty acid profiles of the diets provided to the captive kestrels and partridges

	Kestrels' Diet	Partridges' Diet		
Fatty acid:				
16:0	26.5 ± 1.8	$17.9 \pm .4$		
18:0	$10.5 \pm .6$	$4.7 \pm .2$		
18:1n-9	$35.9 \pm .4$	$26.4 \pm .5$		
18:1n-7	$2.0 \pm .1$	$1.6 \pm .1$		
18:2n-6	17.5 ± 1.2	$44.1 \pm .5$		
18:3n-3	$1.1 \pm .2$.3 ± .0		
20:4n-6	$3.6 \pm .4$	$.2 \pm .0$		
22:6n-3	$2.9 \pm .4$.0		
Ratio n-6/n-3	5.3	148.0		

Note. Values (% w/w of fatty acids) are means \pm SE of n = 4-d-old chickens (kestrels' diet) or n = 4 batches of partridge feed. Total lipid formed 3.6% \pm 0.4% and 4.3% \pm 0.2% of the wet mass of the kestrels' and partridges' diets, respectively.

(Chrompack) connected to an EZ Chrom Data System (Scientific Software). The column was maintained at 185°C for 2 min following injection of the sample, then increased at 5°C/min for 9 min, and maintained at 230°C for a further 24 min. The EZ Chrom Data System enabled the expression of the fatty acid compositions in terms of percentage (w/w) of fatty acids of the lipid class. Peaks were identified by comparison with the retention times of standard fatty acid methyl ester mixtures (Sigma, Poole) and were quantified by comparison with a 15:0 standard. The amounts of each lipid and phospholipid class present in the extracts were calculated from the amount and composition of the fatty acyl groups derived from each class together with the acyl group contribution to the molecular weights of these compounds. The amount of free cholesterol in the extracts was determined using an enzymatic-colorimetric kit system (Boehringer, Lewes).

Statistical comparison between respective wild and captive samples was performed by two-tailed unpaired Student's *t*-test following arcsine transformation of data expressed as percentages in order to make the variance independent of the mean (Snedecor and Cochrane 1967).

Results

Fatty Acid Profiles of the Lipid Classes in Yolks of Wild and Captive Kestrels

The major polyunsaturates of the phospholipid fraction of the eggs from wild kestrels were 20:4n-6 followed by 18:2n-6 (Table 2). In the triacylglycerol fraction, 18:2n-6 was the major polyunsaturate, although a substantial proportion of 18:3n-3 was

also observed. Cholesteryl ester was characterized by a high content of 18:2n-6 with lower proportions of 18:3n-3 and 20: 4n-6. The fatty acid profiles of yolk lipids from birds in captivity differed from those of their wild counterparts in many respects. In all three lipid classes, the eggs laid in captivity contained higher proportions of 18:2n-6 and lower levels of palmitoleic acid (16:1n-7) and 18:3n-3; the phospholipid and cholesteryl ester also displayed increased proportions of 20:4n-6 and less 20:5n-3 and 22:6n-3 compared with eggs from the wild. The proportion of oleic acid (18:1n-9) was also augmented in the cholesteryl ester of the eggs from the captive birds. As a result of these differences, the n-6/n-3 ratio was far higher in the yolk lipids of the captive kestrels.

While all the phospholipid classes were rich in 20:4n-6, the highest proportions of 22:6n-3 were found in PE and PS (Table 3). The total phospholipid of the eggs of captive kestrels contained less PC and more PS compared with eggs from the wild birds. For PC and PE, the effects of captive breeding were to increase the proportions of 18:2n-6 and 20:4n-6 and to decrease those of 16:1n-7, 18:3n-3, and 20:5n-3; PC contained less of 22:6n-3, and in PS the proportion of 20:4n-6 was increased and those of 18:3n-3, 20:5n-3, and docosapentaenoic acid (22: 5n-3) were reduced.

Fatty Acid Profiles of the Lipid Classes in Yolks of Wild and Captive Partridges

The phospholipid of eggs of the wild partridge contained 18: 2n-6 as the main polyunsaturate, with lower levels of 20:4n-6 and 22:6n-3 (Table 4). In the triacylglycerol and cholesteryl

Table 2: Fatty acid profiles of the yolk lipids of wild and captive kestrels

	Triacylglycerol		Phospholipic	1	Cholesteryl Ester	
	Wild	Captive	Wild	Captive	Wild	Captive
% w/w of total lipid	58.6 ± .8	$60.4 \pm .8$	31.8 ± 1.2	28.2 ± 1.2	$4.0 \pm .4$	5.7 ± .6
Fatty acid:						
16:0	$27.4 \pm .4$	$28.3 \pm .1$	$23.6 \pm .2$	$22.5 \pm .5$	$11.4 \pm .3$	$10.2 \pm .5$
16:1n-7	$4.0 \pm .1$	$1.6~\pm~.1^{*}$	$1.1 \pm .1$	$.4 \pm .1^{**}$	$2.7 \pm .1$	$1.1 \pm .1^{*}$
18:0	$3.0 \pm .1$	$3.4 \pm .1$	$19.0 \pm .2$	$20.6 \pm .7$	$7.8 \pm .9$	5.6 ± .8
18:1n-9	$39.8 \pm .5$	$40.6 \pm .9$	$15.8 \pm .2$	$14.4 \pm .6$	$29.4 \pm .9$	$36.7 \pm 1.8^{**}$
18:1n-7	$2.6 \pm .1$	$2.4 \pm .1$	$2.0 \pm .1$	$2.1 \pm .1$	$2.3 \pm .1$	$2.4 \pm .1$
18:2n-6	$12.7 \pm .4$	$19.4 \pm .8^{*}$	$12.1 \pm .1$	$14.7 \pm .4^{*}$	24.6 ± 1.4	$30.8 \pm .9^{**}$
18:3n-3	$5.1 \pm .4$	$.4 \pm .0^{*}$.6 ± .1	$.1 \pm .0^{**}$	$4.8 \pm .3$	$.7 \pm .0^{*}$
20:4n-6	$1.0 \pm .1$	$1.2 \pm .1$	15.2 ± .7	$18.6 \pm .4^{**}$	$4.2 \pm .4$	$5.8 \pm .3^{**}$
20:5n-3	$.4 \pm .0$	$.0^{*}$	$1.6 \pm .1$	$.2 \pm .0^{*}$	$1.6 \pm .1$	$.4 \pm .0^{*}$
22:6n-3	.9 ± .1	.7 ± .1	$5.5 \pm .1$	$3.7 \pm .2^{*}$	$1.8 \pm .1$	$1.2 \pm .1^{**}$
Ratio n-6/n-3	2.1	17.2	3.6	8.3	3.5	15.9

Note. Values (% w/w of fatty acids in lipid class) are means \pm SE of n = 5 eggs. Total lipid formed 29.1% \pm 0.5% and 30.1% \pm 1.2% of the wet mass of the wild and captive birds' yolks, respectively. Free cholesterol formed 3.5% \pm 0.2% and 3.3% \pm 0.3% of the total lipid mass of the wild and captive birds' yolks, respectively.

* P < 0.001; comparison with wild birds' eggs.

** P < 0.01; comparison with wild birds' eggs.

	PC		PE		PS	
	Wild	Captive	Wild	Captive	Wild	Captive
% w/w of phospholipid	76.0 ± 1.4	$69.7 \pm 1.8^{***}$	13.7 ± .8	13.4 ± .8	6.5 ± 1.4	$12.0 \pm 1.6^{***}$
Fatty acid:						
16:0	$25.9 \pm .2$	$24.6 \pm .9$	$10.7 \pm .3$	$9.0 \pm .7$	$6.0 \pm .9$	7.1 ± 1.8
16:1n-7	$1.3 \pm .0$	$.4$ \pm $.0^{*}$	$.7 \pm .0$	$.4~\pm~.0^{*}$	$.3 \pm .0$	$.2 \pm .0$
18:0	$18.5 \pm .2$	19.9 ± .8	$24.5 \pm .5$	$26.0 \pm .5$	$23.1 \pm .4$	$23.9 \pm .9$
18:1n-9	$16.5 \pm .2$	$15.4 \pm .7$	9.3 ± .3	$9.2 \pm .2$	$7.6 \pm .6$	$7.4 \pm .4$
18:1n-7	$2.1 \pm .2$	$2.3 \pm .1$	$1.3 \pm .1$	$1.3 \pm .1$	$1.0 \pm .1$	$1.2 \pm .2$
18:2n-6	$12.5 \pm .1$	$15.6 \pm .5^{*}$	$7.5 \pm .5$	$8.9 \pm .3^{***}$	$6.6 \pm .4$	$7.7 \pm .5$
18:3n-3	$.5 \pm .0$	$.0^{*}$	$.8 \pm .0$	$.2 \pm .0^{*}$	$.9 \pm .0$	$.2 \pm .0^{*}$
20:4n-6	$14.6 \pm .7$	$17.7 \pm .8^{***}$	$17.4 \pm .4$	$22.1 \pm .5^{*}$	$20.1 \pm .8$	$28.9 \pm 2.7^{***}$
20:5n-3	$1.5 \pm .1$	$.2 \pm .0^*$	$2.3 \pm .1$	$.3 \pm .0^*$	$1.4 \pm .1$	$.4 \pm .1^{*}$
22:5n-3	.0	.0	.0	.0	$4.1 \pm .2$	$1.4 \pm .3^{*}$
22:6n-3	$3.6 \pm .2$	$1.9 \pm .2^{*}$	$18.2 \pm .5$	$16.4 \pm .9$	$23.2~\pm~1.8$	16.9 ± 2.8
Ratio n-6/n-3	4.8	15.8	1.2	1.8	.9	1.9

Table 3: Fatty acid profiles of yolk phospholipid classes in eggs of wild and captive kestrels

Note. Values (% w/w of fatty acids in phospholipid class) are means \pm SE of n = 5 eggs. Sphingomyelin formed 3.9% \pm 0.5% and 5.1% \pm 0.7% of the total phospholipid mass of the wild and captive birds' eggs, respectively. Abbreviations: PC, phosphatidylcholine; PE, phosphatidyle-thanolamine; PS, phosphatidylserine.

* P < 0.001; comparison with wild birds' eggs.

*** P < 0.05; comparison with wild birds' eggs.

ester of these eggs, 18:2n-6 was again the predominant polyunsaturate, and substantial proportions of 18:3n-3 were also observed. The cholesteryl ester also contained some 20:4n-6 and 22:6n-3. The eggs of the captive partridges contained a slightly higher proportion of triacylglycerol and a lower level of phospholipid compared with the eggs from the wild birds. The eggs of the captive birds were characterized by increased proportions of 18:1n-9, 18:2n-6, 20:4n-6, and 22:5n-6 and reduced proportions of 18:3n-3 and 22:6n-3 in the phospholipid fraction. The proportions of 18:3n-3 were reduced in the triacylglycerol and cholesteryl ester fractions of the eggs laid in captivity, and the level of 22:6n-3 in cholesteryl ester was also reduced. The overall consequence of captive breeding was to produce major increases in the n-6/n-3 ratio in the lipid classes of the yolk.

PC contained relatively low proportions of the C_{20-22} n-6 and n-3 polyunsaturates, while PE and PS displayed much greater proportions of these fatty acids (Table 5). The eggs of the captive birds contained a greater proportion of PC at the expense of PS. In all three phospholipid classes, the eggs from the captive partridges displayed lower proportions of 18:3n-3 and 22:6n-3 and higher proportions of 22:5n-6 compared with the eggs of the wild birds; also, in the captive samples, 18:2n-6 was enhanced in PE and PS, 20:4n-6 was elevated in PE and PC, and 18:1n-9 was increased in the PE fraction. As a consequence of these differences, the n-6/n-3 ratio in all three phospholipid fractions was higher in the captive state.

Discussion

There are approximately 9,000 extant species of birds adapted to a wide range of habitats and exhibiting great diversity in their dietary preferences (Klasing 1998; Speake and Thompson 1999). Depending on the species, birds in the wild may be predominantly granivorous, herbivorous, frugivorous, insectivorous, carnivorous, or piscivorous or may exploit several of these trophic modes in combination (Klasing 1998). Each of these dietary patterns provides a distinctive profile of polyunsaturated fatty acids; thus, 18:2n-6, 18:3n-3, and 22:6n-3 are, respectively, typical of granivory, herbivory, and piscivory (Speake and Thompson 1999). Feeding trials performed on domestic poultry have demonstrated that the polyunsaturate profile of the yolk is largely a reflection of the fatty acid composition of the diet provided to the laying hen, although genetic differences between avian species also play a determining role (Speake et al. 1998a; Surai et al. 1999). Thus, it may be expected that the yolk fatty acid profiles of different avian species may display a high degree of variation in accordance with their natural dietary preferences. The distinctive polyunsaturate signatures reported for the eggs of granivorous (Maldjian et al. 1996), herbivorous (Noble et al. 1996; Speake et al. 1999b), and piscivorous (Speake et al. 1999a) birds are consistent with this prediction.

The most striking aspect of the polyunsaturate profile of the yolk lipids of wild and captive kestrels was the very high proportion of 20:4n-6 in the phospholipid fraction. In eggs of the

	Triacylglycerol		Phospholipid		Cholesteryl Ester	
	Wild	Captive	Wild	Captive	Wild	Captive
% w/w of total lipid Fatty acid:	61.5 ± .2	$63.0 \pm .5^{***}$	31.6 ± .3	$29.4 \pm .2^{***}$	2.6 ± .4	3.1 ± .3
16:0	$28.4 \pm .7$	$28.4 \pm .9$	$21.4 \pm .2$	$21.1 \pm .7$	11.0 ± .6	12.9 ± .7
16:1n-7	$4.6 \pm .6$	6.9 ± 1.0	$1.3 \pm .2$	$1.9 \pm .3$	$2.6 \pm .3$	$3.4 \pm .5$
18:0	$5.4 \pm .3$	$5.4 \pm .6$	$22.0 \pm .3$	$21.6 \pm .6$	$7.6 \pm .8$	$8.8 \pm .6$
18:1n-9	$43.0 \pm .7$	$44.0~\pm~1.1$	$23.8 \pm .4$	$25.6 \pm .4^{***}$	$37.3 \pm .6$	37.4 ± 1.2
18:1n-7	$2.4 \pm .4$	$3.1 \pm .3$	$1.5 \pm .1$	$1.9 \pm .3$	$1.9 \pm .1$	$1.9 \pm .2$
18:2n-6	$11.1 \pm .4$	$9.4 \pm .9$	$12.2 \pm .2$	$15.6 \pm .6^{*}$	$25.3~\pm~1.6$	26.7 ± 1.2
18:3n-3	$2.3 \pm .2$	$.3 \pm .0^{*}$	$.6 \pm .0$	$.1~\pm~.0^{*}$	$4.2 \pm .4$	$1.1 \pm .3^{*}$
20:4n-6	$.2 \pm .0$	$.2 \pm .0$	$5.6 \pm .2$	$7.3 \pm .4^{**}$	$1.4 \pm .1$	$1.7 \pm .2$
22:5n-6	.0	.0	$.3 \pm .0$	$1.3 \pm .3^{**}$.0	.0
22:6n-3	$.2 \pm .1$	$.1 \pm .0$	$5.9 \pm .2$	$1.6 \pm .1^{*}$	$2.9 \pm .2$	$.8 \pm .1^{*}$
Ratio n-6/n-3	4.9	32.0	2.8	14.2	3.8	15.0

Table 4: Fatty acid profiles of the yolk lipids of wild and captive partridges

Note. Values (% w/w of fatty acids in lipid class) are means \pm SE of n = 5 eggs. Total lipid formed $32.4\% \pm 1.0\%$ and $33.5\% \pm 0.4\%$ of the wet mass of the wild and captive birds' yolks, respectively. Free cholesterol formed $2.9\% \pm 0.1\%$ and $3.2\% \pm 0.1\%$ of the total lipid mass of the wild and captive birds' yolks, respectively.

* P < 0.001; comparison with wild birds' eggs.

** P < 0.01; comparison with wild birds' eggs.

*** P < 0.05; comparison with wild birds' eggs.

domestic chicken (Maldjian et al. 1996) and of many wild birds that have been studied, including the ostrich (Noble et al. 1996), pheasant (Speake et al. 1999b), and emperor penguin (Speake et al. 1999a), 20:4n-6 forms about 5% of the fatty acid mass of the yolk phospholipid compared with 15%-18% in the kestrel eggs. However, the gull, Larus fuscus, consuming a varied diet in which earthworms predominate, produces eggs with a similar level of 20:4n-6 in phospholipid (Speake et al. 1996b) to that reported here for the kestrel. In the wild, kestrels consume a varied diet of small mammals, birds, amphibians, and arthropods, although the diet consists almost entirely of small mammals during the period of egg formation (Bird 1988; Bortolotti et al. 2000). The edible parts of many animals are a relatively rich source of 20:4n-6 (Li et al. 1998; Taber et al. 1998). For example, 20:4n-6 forms approximately 13%, 10%, 11%, and 5%, respectively, of the total fatty acyl mass of the liver, heart, brain, and skeletal muscle of the adult chicken (Phetteplace and Watkins 1989). It may therefore be proposed that the high proportion of 20:4n-6 in the egg phospholipid of the kestrel is a consequence of carnivory, although certain invertebrates may also contribute to the intake of this polyunsaturate.

The breeding of kestrels in captivity resulted in a considerable displacement of the yolk fatty acid profile away from that observed in the eggs of the wild birds. The main effects were to increase the proportions of n-6 polyunsaturates (18:2n-6 and 20:4n-6) at the expense of the n-3 fatty acids (18:3n-3, 20: 5n-3, and 22:6n-3), thereby elevating the n-6/n-3 ratio. The main polyunsaturate of the day-old chickens that were consumed by the kestrels in captivity was 18:2n-6, and it is feasible that the n-6/n-3 ratio of this diet exceeds that of the prey items selected in the wild. There is evidence that the n-6/n-3 ratio of meat and particularly of adipose tissue from animals in the wild is lower than that from domesticated varieties (Simopoulos 1999).

The proportions of 20:4n-6 and 22:6n-3 in the phospholipid fraction of eggs of the wild partridge were similar to values reported for the domestic chicken (Maldjian et al. 1996) and for the wild ostrich (Noble et al. 1996), pheasant, and Canada goose (Speake et al. 1999b). The major polyunsaturate in all the lipid fractions of the partridge eggs was 18:2n-6, with lower levels of 18:3n-3 also present. The diet of the wild partridge in Spain consists of wild and cultivated grasses (Graminae) and forage legumes (especially vetches or tares), supplemented at times by other seeds and fruits, and in late spring insects are eaten, especially ants and grasshoppers (Cramp and Simmons 1980). Thus, the provision of 18:2n-6 from the seeds and legumes plus some 18:3n-3 from grasses would appear to be consistent with the polyunsaturate profile of the yolk, with 20:4n-6 and 22:6n-3 being synthesized maternally from their respective C₁₈ precursors.

The partridges in captivity consumed a diet formulated from cereal grains (maize, wheat, barley) and seeds (sunflower); this diet was exceptionally rich in 18:2n-6 but contained only minor amounts of n-3 fatty acids. The effect of this diet on the egg lipids was to increase the proportions of n-6 fatty acids (18: 2n-6 and 20:4n-6) in phospholipid while reducing the proportions of n-3 polyunsaturates (18:3n-3 and 22:6n-3) in all

Table 5: Fatty acid profiles of york phospholipid classes in eggs of wild and captive partridges							
	PC		PE		PS		
	Wild	Captive	Wild	Captive	Wild	Captive	
% w/w of total phospholipid	67.2 ± 1.6	$74.4 \pm .9^{**}$	20.8 ± 1.8	16.6 ± .8	8.8 ± .6	$5.6 \pm .8^{***}$	
Fatty acid:							
16:0	$23.4 \pm .4$	$22.6 \pm .6$	13.2 ± .5	$12.5 \pm .5$	$9.9~\pm~1.4$	$11.1 \pm .7$	
16:1n-7	$1.5 \pm .1$	$2.2 \pm .2$.6 ± .1	$1.2 \pm .3$.6 ± .1	$1.0 \pm .2$	
18:0	$20.9 \pm .3$	$20.3 \pm .5$	$24.6 \pm .6$	$22.2 \pm .6$	32.2 ± 2.2	$29.9 \pm .8$	
18:1n-9	$26.6 \pm .4$	$27.2 \pm .3$	$15.4 \pm .4$	$19.5 \pm .4^{*}$	9.1 ± .3	$14.4 \pm .9$	
18:1n-7	$1.5 \pm .1$	$1.9 \pm .2$	$1.1 \pm .1$	$1.7 \pm .2$	$1.1 \pm .1$	$1.5 \pm .2$	
18:2n-6	16.3 ± .3	$15.9 \pm .4$	$11.2 \pm .2$	$13.2 \pm .6^{***}$	9.6 ± .3	$13.4 \pm .5^*$	
18:3n-3	$.7 \pm .0$	$.1~\pm~.0^{*}$	$1.6 \pm .1$	$.3 \pm .0^{*}$	$.8 \pm .0$	$.1~\pm~.0^{*}$	
20:4n-6	$3.7 \pm .4$	$5.8 \pm .3^{**}$	$10.0 \pm .8$	$15.0 \pm .4^{*}$	$15.7 \pm .4$	$16.5 \pm .9$	
22:5n-6	$.3 \pm .0$	$1.1 \pm .1^{*}$.6 ± .1	$4.5 \pm .3^{*}$	$.8 \pm .0$	$3.2 \pm .4^{*}$	
22:6n-3	$3.1 \pm .3$	$1.0~\pm~.1^{*}$	16.4 ± .5	$5.4 \pm .6^{*}$	13.4 ± 1.4	$3.4 \pm .3^{*}$	
Ratio n-6/n-3	5.3	22.8	1.2	5.7	1.8	9.5	

Table 5: Fatty acid profiles of yolk phospholipid classes in eggs of wild and captive partridges

Note. Values (% w/w of fatty acids in phospholipid class) are means \pm SE of n = 5 eggs. Sphingomyelin formed 3.2% \pm 0.7% and 3.5% \pm 0.5% of the total phospholipid mass of the wild and captive birds' eggs, respectively. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.

* P < 0.001; comparison with wild birds' eggs.

** P < 0.01; comparison with wild birds' eggs.

*** P < 0.05; comparison with wild birds' eggs.

the lipid classes. Again, this resulted in major increases in the n-6/n-3 ratio. Most notably, the proportion of 22:6n-3 in yolk phospholipid was only 1.6% in eggs from the captive birds compared with 5.9% in eggs of the wild partridges. A similar low level of 22:6n-3 in the yolk of commercially reared ducks resulted in a reduced proportion of this fatty acid in the brain of the embryo when compared with embryos of wild ducks (Maldjian et al. 1996; Speake et al. 1996*a*). This may imply that the yolk of the captive partridges may provide insufficient 22: 6n-3 to support optimal brain function in the hatchling. Another noteworthy feature was the presence of the n-6 docosapentaenoic acid (22:5n-6) in the yolk phospholipid of the captive partridges since this fatty acid is regarded as an indicator of n-3 deficiency (Neuringer et al. 1988; Anderson et al. 1989).

A problem inherent in ascribing a characteristic yolk fatty acid profile to a species in the wild is the variation that may result from the diversity of dietary items available to different females during vitellogenesis. Ideally, it would have been useful to obtain eggs from a large number of females to assess the extent of such variation. It is, however, essential to follow current ethical guidelines designed to minimize the number of eggs collected in the wild and to prevent any reduction in population size. In the case of the wild kestrels, each of the five eggs was from a different clutch, yet the variation in fatty acid composition was small, suggesting that the different females in this location were obtaining similar diets. Although there is considerable information available describing the food items eaten by the American kestrel and the red-legged partridge in the wild (Cramp and Simmons 1980; Bird 1988; Bortolotti et al. 2000), it would have been useful to obtain samples of these items for analysis to provide a direct comparison between the fatty acid profiles of the diets and eggs.

In conclusion, this study provides the first description of the effects of carnivory on yolk fatty acid composition, supplies additional support for the concept that the characteristic dietary strategies of different avian species have produced a great diversity of yolk polyunsaturate profiles, and presents further evidence that diets currently formulated for birds in captivity may displace the egg's n-6/n-3 ratio away from that which is normally achieved in the wild and which in extreme cases could possibly have deleterious consequences for the development of the embryo and neonate. The deficiencies that we report here may be particularly relevant for improving the nutritional status of the red-legged partridge raised in captivity. This species is the main small-game species in Spain, and more than 2 million captive-bred individuals are released annually to reinforce local populations (Delibes 1992). Red-legged partridges are, however, difficult to breed in captivity, partly due to a high mortality of newly hatched birds in the aviaries (Nadal 1992). The reasons for this mortality are not fully understood, but composition of the feed provided to the breeders could be a factor. Although the rearing of red-legged partridges is becoming an important industry in Spain, little research effort has been devoted to improving the standards of captive rearing. This article is the first example in which egg characteristics are related to the composition of the feed provided to the laying hen of this species.

Previous work has shown that eggs of captive alligators (No-

ble et al. 1993) and ostriches (Noble et al. 1996) contain reduced proportions of n-3 fatty acids and also display a greater incidence of embryonic mortality when compared with eggs laid by their wild counterparts, although a direct causal relationship between yolk fatty acids and hatching success has yet to be established. With regard to the kestrel and partridge, it would be of interest to compare the hatchabilities of eggs produced in the wild versus captivity and to evaluate the effects of supplementing the diets of the captive females with n-3 fatty acids.

Acknowledgments

We are grateful to the Scottish Executive Rural Affairs Department (to P.F.S. and B.K.S.) and the Natural Sciences and Engineering Council of Canada (to G.R.B.) for financial support. Russ Dawson, Kim Fernie, Ian Ritchie, and David Bird were instrumental in providing kestrel eggs. We thank Saskatchewan Environment and Resource Management and the Canadian Wildlife Service for permits for egg collection. We also thank Paloma Prieta and the staff of the partridge farm at Lugar Nuevo (Consejeria de Medio Ambiente, Junta de Andalucia) for providing the eggs of captive partridges and Rafael Villafuerte, director of Instituto de Investigación en Recursos Cinegéticos, for collecting the eggs of wild partridges.

Literature Cited

- Anderson G.J., W.E. Connor, J.D. Corliss, and D.S. Lin. 1989. Rapid modulation of the n-3 docosahexaenoic acid levels in the brain and retina of the newly hatched chick. J Lipid Res 30:433–441.
- Bird D.M. 1980. The kestrel as a laboratory research animal. Nature 229:300–301.
- ——. 1988. American kestrel. Pp. 253–290 in R.S. Palmer, ed. Handbook of North American Birds. Vol. 5. Yale University Press, New Haven, Conn.
- Bortolotti G.R., J.L. Tella, M.G. Forero, R.D. Dawson, and J.J. Negro. 2000. Genetics, local environment and health as factors influencing plasma carotenoids in wild American kestrels (*Falco sparverius*). Proc R Soc Lond B Biol Sci 267: 1433–1438.
- Cramp S. and K.E.L. Simmons. 1980. Handbook of the Birds of Europe, the Middle East and North Africa. Vol. 2. Hawks to Bustards. Oxford University Press, Oxford.
- Delibes J. 1992. Gestión de los cotos perdiz roja. Pp. 141–146 in F. la Caixa, ed. La Perdiz Roja: Gestión del Hábitat. Editorial Aedos, Barcelona.
- Klasing K.C. 1998. Comparative Avian Nutrition. CAB International, Wallingford.

- Kuksis A. 1992. Yolk lipids. Biochim Biophys Acta 1124: 205–222.
- Li D., A. Ng, N.J. Mann, and A.J. Sinclair. 1998. Contribution of meat fat to dietary arachidonic acid. Lipids 33:437–440.
- Lipar J.L., E.D. Ketterson, and V. Nolan. 1999. Intraclutch variation in testosterone content of red-winged blackbird eggs. Auk 16:231–235.
- Maldjian A., C. Cristofori, R.C. Noble, and B.K. Speake. 1996. The fatty acid composition of brain phospholipids from chicken and duck embryos. Comp Biochem Physiol 115B: 153–158.
- Nadal J. 1992. Problemática de las poblaciones de perdiz roja, bases ecoetológicas para tener éxito con las repoblaciones.
 Pp. 87–100 in F. la Caixa, ed. La Perdiz Roja: Gestión del Hábitat. Editorial Aedos, Barcelona.
- Neuringer M., G.J. Anderson, and W.E. Connor. 1988. The essentiality of n-3 fatty acids for the development and function of the retina and brain. Annu Rev Nutr 8:517–541.
- Noble R.C. and M. Cocchi. 1990. Lipid metabolism and the neonatal chicken. Prog Lipid Res 29:107–140.
- Noble R.C., R. McCartney, and M.W.J. Ferguson. 1993. Lipid and fatty acid compositional differences between eggs of wild and captive-breeding alligators (*Alligator mississippiensis*): an association with reduced hatchability? J Zool (Lond) 230: 639–649.
- Noble R.C., B.K. Speake, R. McCartney, C.M. Foggin, and D.C. Deeming. 1996. Yolk lipids and their fatty acids in the wild and captive ostrich (*Struthio camelus*). Comp Biochem Physiol 113B:753–756.
- Phetteplace H.W. and B.A Watkins. 1989. Effects of various n-3 lipid sources on fatty acid compositions in chicken tissues. J Food Comp Anal 2:104–117.
- Simopoulos A. 1999. New products from the agri-food industry: the return of n-3 fatty acids into the food supply. Lipids 34:S297–S301.
- Snedecor G.W. and W.G. Cochrane. 1967. Statistical Methods. Iowa State University Press, Ames.
- Speake B.K., C. Cristofori, R.J. McCartney, and R.C. Noble. 1996*a*. The relationship between the fatty acid composition of the lipids of the yolk and the brain of the duck embryo. Biochem Soc Trans 24:181S.
- Speake B.K., F. Decrock, P.F. Surai, and R. Groscolas. 1999*a*. Fatty acid composition of the adipose tissue and yolk lipids of a bird with a marine-based diet, the emperor penguin (*Aptenodytes forsteri*). Lipids 34:283–290.
- Speake B.K., R.J. McCartney, M. Feast, A. Maldjian, and R.C. Noble. 1996b. The relationship between the fatty acid profiles of the yolk and the embryonic tissue lipids: a comparison between the lesser black backed gull (*Larus fuscus*) and the pheasant (*Phasianus colchicus*). Comp Biochem Physiol 115B: 493–499.
- Speake B.K., A.M.B. Murray, and R.C. Noble. 1998a. Transport

and transformations of yolk lipids during development of the avian embryo. Prog Lipid Res 37:1–32.

- Speake B.K., R.C. Noble, and A.M.B. Murray. 1998*b*. The utilization of yolk lipids by the chick embryo. World's Poult Sci J 54:319–334.
- Speake B.K., P.F. Surai, R.C. Noble, J.V. Beer, and N.A.R. Wood. 1999*b*. Differences in egg lipid and antioxidant composition between wild and captive pheasants and geese. Comp Biochem Physiol B Comp Biochem 124: 101–107.
- Speake B.K. and M.B. Thompson. 1999. Comparative aspects of yolk lipid utilization in birds and reptiles. Poult Avian Biol Rev 10:181–211.
- Surai P.F., B.K. Speake, R.C. Noble, and M. Mezes. 1999. Species-specific differences in the fatty acid profiles of lipids of the yolk and of the liver of the chick. J Sci Food Agric 79:733–736.
- Taber L., C.H. Chiu, and J. Whelan. 1998. Assessment of the arachidonic acid content in foods commonly consumed in the American diet. Lipids 33:1151–1157.