Acclimation to Heat During Incubation. 2. Embryo Composition and Residual Egg Yolk Sac Fatty Acid Profiles in Chicks

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ABSTRACT The aim of the research was to evaluate embryo composition and changes in egg yolk fatty acid composition during embryonic development as a function of incubation temperature and age of breeders. Eggs obtained from a common breeder stock at 3 ages: 32 (younger), 42 (mid age), and 65 (older) wk were divided into 2 groups and placed into 2 incubators: the control and the second where eggs were heat-acclimated (HA) at 38.5°C for 6 h daily from d 10 to 18 of incubation. Body composition of embryos and chicks were measured on d 14, 18, and at hatch, respectively. Fatty acid profiles of yolk and residual egg yolk sac of chicks were analyzed before incubation and at hatch, respectively. Moisture content of embryos was highest on d 14 and then decreased regardless of parental age and incubation temperature. Moisture content of chicks at hatch from 42- and 65-wk parents were lower than those of chicks from 32-wk parents, whereas the trend in chick fat content was opposite. Incubation temperature had no effect on composition of chicks. Consistently lower *cis*-4,7,10,13,16,19-eicosapentaenoic (docosahexaenoic acid, DHA; 22:6n-3) and *cis*-11,14,17-eicosatrienoic (20:3n-3) fatty acids in the residual yolk sac of chicks than in egg yolks before incubation may have resulted from preferential uptake from the yolk. The DHA content in the residual yolk sac was considerably higher in chicks from older parents incubated at HA, whereas, in contrast, levels of 18:3n-3 were lower. Also, chicks from younger parents in the HA treatment had lower transported 18:3n-3 and higher levels of transported DHA. It may be concluded that this process observed during the high incubation temperature may be related to a protective strategy and thus contributes to postnatal heat adaptation.

Key words: parental age, heat acclimation, embryo composition, yolk sac composition, heat stress

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INTRODUCTION

Yolk, the main energy source for the developing embryo, supplies more than 90% of the total energy requirements of the embryo by oxidation of yolk lipids (Speake et al., 1998). Weight and percentage of dry matter accumulation of embryos are related to the fatty acid composition of the yolk (Peebles et al., 1999). Thus, yolk and its fatty acid content are essential for meeting the nutritional requirements of developing embryos. The $C_{20–22}$ polyunsaturated fatty acids (**PUFA**) of both n-3 and n-6 series, in particular docosahexaenoic acid (**DHA**, 22:6n-3) and arachidonic acid (20:4n-6), are important components of neural tissues with phospholipids having essential roles in embryonic development (Speake et al., 1998; Speake and Wood, 2005). Maldjian et al. (1996) studied the fatty acid composition of the total phospholipids of the chicken brain and concluded that chicken brain phospholipids contain approximate proportions of 8 and 17% of 20:4n-6 and 22:6n-3, respectively.

Age of hen (Nielsen, 1998; Latour et al., 2000) influences the fatty acid composition of eggs and lipid utilization by embryos. The rate of lipid mobilization from the yolk into the yolk sac membrane is lower in younger than older breeders (Noble and Connor, 1984; Noble et al., 1986; Tullett and Noble 1989; O'Sullivan et al., 1991), with the percentage of lipid and dry matter in embryos increasing with age of parents. Similarly, in duck embryos, Braun et al. (2001) reported that both the relative number and size of liver lipid droplets in embryos increased with age of breeder.

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Palmitoleic acid (16:1n-7) levels, relative to total fatty acids in yolks, were higher in unincubated eggs from 36 wk than 51- and 64-wk breeders (Latour et al., 1998). Also, relative stearic (18:0) and arachidonic (20:4n-6) acid levels were higher in eggs from breeders at 26 than 28 and 30 wk (Burnham et al., 2001), whereas $C_{20–22}$ series were higher in eggs from breeders at 21 than 57 wk (Nielsen, 1998). Variation in fatty acid composition of egg yolk occurs during the incubation; Latour et al. (1998) noted that incubation effects on yolk palmitoleic (16:1n-7), oleic (18:1n-9), arachidonic (20:4n-6), and to a lesser degree linoleic (18:2n-6) acids concentrations in eggs from younger than older parents.

Recent studies have focused on acclimation of poultry to extreme posthatch temperatures by exposing embryos to lower or higher incubation temperatures (e.g., Tzschentke and Basta, 2002; Yahav et al., 2004b; Yalçın et al., 2005). Feast et al. (1998) reported reduced yolk lipid uptake in embryos exposed to lower incubation temperatures. Although physiological responses of embryos to higher incubation temperatures (e.g., increased allantoic fluid temperature, lower plasma corticosterone level at internal pipping stage, decreased heat production at hatch, similar plasma triiodothyronine and corticosterone levels to control chicks at hatch) have been reported (Janke et al., 2002; Moraes et al., 2004; Yahav et al., 2004a), information is lacking on the effect of higher incubation temperature on embryo composition and residual fatty acid profiles of chicks. The present study aimed to evaluate embryo composition as well as to evaluate the changes in egg yolk fatty acid composition during embryonic development as a function of incubation temperature and age of breeders.

MATERIALS AND METHODS

Detailed procedures on eggs and hatching conditions used in this study were previously reported (Yalçın et al., 2008). Briefly, a total of 1,665 eggs were obtained from replicated flocks of Ross 308 breeders at 32 (younger), 42 (mid age), and 65 (older) wk of age. Those 6 flocks (2 flocks for each age) were from the same farm with similar husbandry and fed on the same nutrient density diet. All eggs were collected at the same day. Egg weights averaged 53.4, 61.4, and 64.6 g for 32-, 42-, and 65-wk parental flocks, respectively. Breeder diets were formulated to contain 2,750 kcal/kg of ME and 16% protein. The diet did not contain vegetable oil.

There were 276 eggs/breeder age per flock. Before incubation, 5 eggs from each parental age and flock were sampled for analysis of yolk fatty acid content.

Eggs were divided equally into 2 groups and placed into 2 incubators. One incubator was maintained at 37.8°C from d 1 to 18 of incubation (CONT). In the second incubator eggs were heat-acclimated (HA) at 38.5°C for 6 h daily from d 10 to 18 of incubation. From d 18 to hatch, the temperature was 37.5°C for both groups. Relative humidity was maintained at 65% in both incubators. There were 3 replicate trays in each subgroup (46 eggs/tray).

On d 14 and 18 of incubation, after 6 h of daily heat treatment, 5 eggs/parental age per flock per incubation temperature were randomly selected (30 eggs/incubation temperature). Eggs were opened, the embryos removed, and they were cleaned of yolk sac and membrane. They were then killed by cervical dislocation. The procedure was repeated on day of hatch with 5 randomly selected chicks/parental age per flock per incubation temperature subclass, after which chicks were dried in the incubators. Yolk sacs of chicks were removed for fatty acid determinations. Embryos, chicks, and yolk sacs were weighed and stored at –20°C before analyses.

Embryos and chicks were dried at 110°C for 24 h and their dry matter calculated as the differences between wet and dry weights divided by wet weight. Embryo N content was determined by the Kjeldahl method, and CP was calculated as N × 6.25. Embryo and chick lipid contents were determined by AOAC (1990). Protein and fat content were expressed as percentage of total dry matter.

Fatty Acid Analyses of Yolk Sac of Chicks

An aliquot of egg yolk was weighed, and lipid was extracted with chloroform:methanol (2:1, vol/vol; Folch et al., 1957) and total lipids methylated by sodium methoxide in ethanol. One hundred milligrams of lipid extracted from egg yolk was placed in a 15-mL screw-cap tube, 0.2 mL of NaOH-MeOH (0.5 *N*) was added, vortex-mixed, and heated at 100°C for 5 min. The tube was cooled in cold water, 0.5 mL of HCl-MeOH (4%) was added to the sample, vortex-mixed, and held at room temperature for 5 min. Approximately 5 mL of isooctane and 3 mL of distilled water were added to extract the fatty acid methyl esters (**FAME**). The mixture was shaken for 10 min, centrifuged at 4,000 × *g* for 5 min, and then the upper phase was collected, dried with sodium sulfate, and concentrated with nitrogen gas for gas chromatograph (**GC**) analysis.

A gas chromatograph (Agilent 6890N Series) equipped with an autosampler (Model 7683B), GC ChemStation, and flame ionization detector (Agilent Technologies, Inc., Wilmington, DE) was used to analyze the FAME. The GC was operated at a temperature of 150°C for 2 min, followed by heating at 3°C/min to 210°C and holding for 20 min. A DB-23 column was used for the analysis (60 m, 0.25-mm i.d., 0.25-μm film thickness; Agilent Technologies Inc.). The injector and flame ionization detector were maintained at 220 and 250°C, respectively. Identification of sample FAME was achieved by comparing the retention times to FAME standards (Mixture ME-100; Greyhound Chromatography and Allied Chemicals, Birkenhead, Merseyside, UK).

Statistical Analyses

Data from the 2 flocks/breeder age were pooled, because differences between flocks/breeder age were not significant. One-way ANOVA was performed to analyze

Table 1. Mean percentage of moisture, protein, and fat content of embryos by age, incubation temperature,[1] and parental age

Embryonic age, d	Treatments		Embryo		
			Moisture	Protein	Fat
			%		
14	Incubation temperature		<0.001	NS	NS
		Control	87.2[b]	73.8	20.7
		High	88.3[a]	74.6	20.9
		SEM	0.2	3.1	0.8
	Parental age (wk)		<0.001	NS	NS
		32	88.4[a]	74.9	20.9
		42	87.4[b]	73.4	21.1
		65	87.4[b]	75.9	20.5
		SEM	0.2	3.9	1.0
18	Incubation temperature		NS	NS	NS
		Control	83.0	69.0	24.0[b]
		High	82.8	68.0	26.8[a]
		SEM	0.1	1.7	0.8
	Parental age (wk)		NS	NS	NS
		32	83.3	68.3	24.3[b]
		42	82.8	67.7	27.3[a]
		65	82.8	69.4	25.1[ab]
		SEM	0.17	2.1	0.9
Hatch	Incubation temperature		NS	NS	NS
		Control	80.5	71.8	21.3
		High	81.4	69.2	22.8
		SEM	0.2	1.1	0.8
	Parental age (wk)		0.002	NS	0.001
		32	81.6[a]	71.5	18.5[b]
		42	80.7[b]	69.2	23.9[a]
		65	80.4[b]	69.7	23.9[a]
		SEM	0.2	1.0	0.9

[a,b]Means in the same column within a measurement with no common superscript differ significantly ($P < 0.05$).

[1]Control = incubated at 37.8°C throughout; high = exposed to heating at 38.5°C 6 h/d from 10 to 18 d of incubation.

the effects of breeder age on fatty acid composition before incubation (SAS, 1999). Comparisons of effects of parental age and incubation temperature on fatty acid composition were made by 2-way ANOVA. Multiple mean comparisons were made by Tukey's studentized range test. Statements of statistical significance were based on $P < 0.05$ unless otherwise stated.

RESULTS

Embryo Composition

Mean moisture content was 87.7, 82.9, and 81.0% on d 14, 18, and at hatch, respectively (Table 1). Incubation temperature and parental age significantly affected embryo moisture content on d 14. A significant incubation temperature × parental age interaction showed that although HA resulted in an increase in moisture content, it was the case only for embryos from younger parents with values for 42- and 65-wk parents similar for HA and CONT embryos (Table 2). On d 18, there was no parental age effect on embryo moisture for CONT. At HA, the highest moisture content was obtained for embryos from 32-wk parents. At hatch, incubation temperature had no effect on moisture content; however, moisture was higher for embryos from 32-wk than 42- and 65-wk parents.

Mean protein content of embryos was 74.2% on d 14. It decreased to 68.5% on d 18 and was 70.5% at hatch. Neither incubation temperature nor parental age had an effect on protein content of embryos from 14 d of incubation to hatch (Table 1).

Fat content of embryos changed with age of embryo, increasing from 20.8 to 25.4% between d 14 and 18 and then decreasing to 22.1% from d 18 to hatch. There were no effects of incubation temperature and parental age on fat content of embryos on d 14. On d 18, fat content of HA embryos increased. A significant interaction between incubation temperature and parental age showed that there were no differences for fat content of embryos for CONT eggs; however, fat content of embryos from 42-wk parents increased at HA temperatures (Table 2). On day of hatch, fat content was higher for embryos from 42- and 65-wk than 32-wk parents (Table 1).

Egg Yolk Fatty Acid Composition Before Incubation

Mean fatty acid compositions of egg yolk before incubation by parental age are presented in Table 3. Palmitic (16:0), oleic (18:1n-9), and linolenic (18:2n-6) acids were the major fatty acids, ranging from 17.94 to 36.88%. This was followed by stearic (18:0), palmitoleic (16:1n7), and *cis*-11,14,17-eicosatrienoic (20:3n-3) acids ranging from 2.21 to 9.53%, with the remaining fatty acids <1.0%.

Total saturated fatty acids were lower in eggs from 65-wk parents than in eggs from 32- and 42-wk parents.

Table 2. Means for the interaction between incubation temperature[1] and parental age on % moisture of embryos at 14 and 18 d of incubation and fat at 18 d of incubation

Embryonic age, d	Embryo composition, %	Incubation temperature	Parental age, wk 32	42	65	SEM
14	Moisture	Control	87.4[a]	87.2[a]	86.9[a]	0.2
			*	NS	NS	
		High	89.3[a]	87.6[b]	87.9[b]	0.2
18	Moisture	Control	82.9[a]	83.4[a]	83.0[a]	0.2
			NS	*	NS	
		High	83.6[a]	82.4[b]	82.6[ab]	0.2
	Fat	Control	23.6[a]	24.2[a]	25.3[a]	1.2
			NS	*	NS	
		High	25.0[b]	30.5[a]	24.8[b]	1.3

[a,b]Means in the same row within an incubation temperature with no common superscript differ significantly ($P < 0.05$).

[1]Control = incubated at 37.8°C throughout; high = exposed to heating at 38.5°C 6 h/d from 10 to 18 d of incubation.

*Means in the same column within a parental age differ significantly ($P < 0.05$).

Within saturated fatty acids, palmitic acid (16:0) content of egg followed the same pattern as total saturated fatty acids. Conversely, heptadecanoic acid (17:0) was lower in eggs from 32- than 65-wk parents. Parental age had no effect on myristic (14:0), pentadecanoic (15:0), stearic (18.0), and arachidic (20:0) acid content.

Although egg total monounsaturated fatty acid composition was similar across parental ages, palmitoleic (16:1n-7) acid was higher in eggs from the younger parents, and conversely, *cis*-11-eicosenoic acid was higher in eggs from the older parents. Total PUFA was lower in eggs from 32-wk than 42- and 65-wk parents. Linoleic acid (18:2n-6), as a major PUFA in egg, changed with parental age similar to total PUFA. *Cis*-11,14-eicosadienoic acid (20:2n-6) and *cis*-11,14,17-eicosatrienoic (20:3n-3) contents of yolk were higher for eggs from 65-wk than 32- and 42-wk parents. Parental age had no effect on DHA (22:6n-3) and eicosapentaenoic acid (20:5n-3) contents of egg. The ratio between saturated and unsaturated fatty acids was higher in eggs from younger parents than in eggs from either 42- and 65-wk parents.

Residual Yolk Sac Fatty Acid Composition of Chicks at Hatch

When yolk fatty acid profiles were compared before and after incubation, there was no change in the total saturated fatty acids (35.21 vs 35.23%; data not shown in tables). However, total monounsaturated fatty acid content of yolk was 34.9% before incubation and increased to 36.5% after incubation in the yolk sac of embryos. This increase resulted mainly from an increase in the oleic acid (18:1n-9) content in the yolk sac of chicks (34.7 vs 36.3%). Compared with before incubation, DHA (22:6n-3) and

Table 3. Mean fatty acid profiles (wt %) of egg yolk before incubation by parental age

Fatty acids		Parent age, wk 32	42	65	SEM	Significance (*P*-values)
Myristic acid	14:0	0.37	0.37	0.35	0.02	NS
Pentadecanoic	15:0	0.07	0.08	0.08	0.01	NS
Palmitic	16:0	25.53[a]	25.48[a]	24.49[b]	0.23	0.006
Palmitoleic	16:1n-7	3.58[a]	2.86[b]	2.50[b]	0.15	<0.001
Heptadecanoic	17:0	0.21[b]	0.25[ab]	0.28[a]	0.01	0.002
Stearic	18:0	9.02	9.53	9.39	0.21	NS
Oleic (*cis*-9)	18:1n-9	35.00	33.84	36.88	0.85	0.048
Linoleic (*cis*-9,12)	18:2n-6	17.95[b]	21.57[a]	20.44[a]	0.57	<0.001
Linolenic	18:3n-3	0.73	0.80	0.79	0.05	NS
Arachidic	20:0	0.04	0.04	0.05	0.01	NS
Cis-11-eicosenoic	20:1n-9	0.21[b]	0.20[b]	0.25[a]	0.01	0.048
Cis-11,14-eicosadienoic	20:2n-6	0.27[b]	0.28[b]	0.39[a]	0.02	0.049
Cis-8,11,14-eicosatrienoic	20:3n-6	0.08	0.06	0.07	0.01	NS
Cis-11,14,17-eicosatrienoic	20:3n-3	2.21[b]	2.26[b]	2.68[a]	0.16	0.001
Arachidonic	20:4n-6	0.03	0.03	0.05	0.01	NS
Cis-5,8,11,14,17-eicosapentaenoic	20:5n-3	0.02	0.02	0.02	0.01	NS
Cis-4,7,10,13,16,19-eicosapentaenoic	22:6n-3	0.91	0.94	0.93	0.04	NS
Saturated		35.25[a]	35.76[a]	34.64[b]	0.28	0.032
Monounsaturated		35.24	34.04	36.57	0.85	NS
Polyunsaturated (PUFA)		22.19[b]	25.96[a]	25.32[a]	0.61	<0.001
Saturated:PUFA		1.57[a]	1.37[b]	1.36[b]	0.07	0.022

[a,b]Means in the same row with no common superscript differ significantly ($P < 0.05$).

Table 4. Mean fatty acid profiles (wt %) of residue egg yolk of chicks at hatch by parental age and incubation temperature[1]

		Parental age (PA), wk				Incubation temperature (IT)			*P*-values		
Fatty acids		32	42	65	SEM	Control	High	SEM	PA	IT	PA × IT
Myristic acid	14:0	0.37	0.38	0.37	0.01	0.36	0.38	0.01	NS	NS	NS
Pentadecanoic	15:0	0.07[b]	0.09[a]	0.08[ab]	0.01	0.08	0.09	0.01	0.041	0.016	NS
Palmitic	16:0	24.95	25.46	24.89	0.23	24.88	25.43	0.19	NS	NS	0.009
Palmitoleic	16:1n-7	3.08[a]	2.81[ab]	2.54[b]	0.12	2.61	3.03	0.09	0.012	0.004	NS
Heptadecanoic	17:0	0.21[b]	0.26[a]	0.26[a]	0.01	0.24	0.24	0.01	<0.001	NS	NS
Stearic	18:0	9.42	9.51	9.41	0.20	9.68	9.22	0.16	NS	NS	NS
Oleic (*cis*-9)	18:1n-9	35.82[b]	35.87[b]	36.99[a]	0.32	36.68	35.78	0.26	0.016	0.016	0.017
Linoleic (*cis*-9,12)	18:2n-6	21.66	21.00	20.85	0.44	21.71	20.64	0.36	NS	0.044	NS
Linolenic	18:3n-3	0.71[b]	0.81[a]	0.71[b]	0.02	0.75	0.74	0.02	0.004	NS	0.031
Arachidic	20:0	0.06	0.07	0.06	0.01	0.07	0.07	0.01	NS	NS	NS
Cis-11-eicosenoic	20:1n-9	0.25	0.24	0.23	0.09	0.24	0.23	0.01	NS	NS	0.033
Cis-11,14-eicosadienoic	20:2n-6	0.33	0.32	0.29	0.01	0.32	0.31	0.01	NS	NS	0.045
Cis-8,11,14-eicosatrienoic	20:3n-6	0.06	0.05	0.06	0.01	0.05	0.06	0.01	NS	NS	0.002
Cis-11,14,17-eicosatrienoic	20:3n-3	1.70	1.58	1.72	0.06	1.64	1.69	0.05	NS	NS	NS
Arachidonic	20:4n-6	0.03	0.03	0.03	0.01	0.03	0.03	0.01	NS	NS	NS
Cis-5,8,11,14,17-eicosapentaenoic	20:5n-3	0.02	0.02	0.02	0.01	0.02	0.02	0.01	NS	NS	NS
Cis-4,7,10,13,16,19-eicosapentaenoic	22:6n-3	0.29[ab]	0.27[b]	0.35[a]	0.02	0.29	0.31	0.02	0.040	NS	<0.001
Saturated		35.00	35.77	35.06	0.22	35.30	35.21	0.19	NS	NS	<0.001
Monounsaturated		39.13	38.94	39.77	0.34	39.53	39.03	0.28	NS	NS	NS
Polyunsaturated (PUFA)		27.21	24.01	24.18	0.60	26.03	24.26	0.7	NS	NS	NS
Saturated:PUFA		1.30	1.47	1.46	0.06	1.35	1.47	0.04	NS	NS	NS

[a,b]Means in the same row within parental age with no common superscript differ significantly ($P < 0.05$).

[1]Control = incubated at 37.8°C throughout; high: exposed to heating at 38.5°C 6 h/d from 10 to 18 d of incubation.

cis-11,14,17-eicosatrienoic (20:3n-3) contents reduced significantly from 0.9 to 0.3% and from 2.4 to 1.6%, respectively, whereas linoleic acid (18:2n-6) increased from 19.9 to 21.0% after incubation. However, total PUFA content was similar before (24.49%) and after incubation (24.18%).

The fatty acid composition of the residual yolk sac (Table 4) generally resembled that of yolk before incubation. Similar to yolk before incubation, palmitic (16:0), palmitoleic (16:1n-7), stearic (18:0), oleic (18:1 n-9), and linoleic (18:3 n-3) acids were the major components in the residual yolk, and the main $C_{20–22}$ polyunsaturates were *cis*-11,14,17-eicosatrienoic (20:3n-3) and DHA (22:6n-3).

Neither parental age nor incubation temperature influenced total saturated fatty acid compositions in residual yolk sac of chicks. A significant interaction between parental age and incubation temperature, however, resulted from reduced saturated fatty acid content of residual yolk sac of HA than CONT chicks from older parents (Table 5). Parental age significantly affected pentadecanoic (15:0) and heptadecanoic (17:0) acids in the residual yolk sac, being lower in chicks from 32-wk than 42-wk parents. The HA slowly increased pentadecanoic acid (15:0) content of residual egg yolk sac. Treatments had no effect on arachidic acid content (Table 4). A significant interaction for palmitic acid content of residual yolk sac resulted from chicks hatched from 32-wk parents having higher palmitic acid content in the yolk sac when incubated at HA (Table 5).

There was no effect of parental age and incubation temperature on total monounsaturated fatty acid content of residual yolk sac. Interaction effects were significant for oleic (18.1n-9) and *cis*-11-eicosenoic (20:1n-9) acid content. Parental age did not influence oleic acid (18.1n-9) content of yolk sac for eggs incubated at CONT, but HA increased oleic acid (18:1n-9) content in yolk residue of chicks from 65-wk parents. It was found that HA decreased 20:1n-9 content of residual yolk of chicks from younger parents.

Total PUFA contents of yolk sac of chicks were similar among parental ages and between incubation temperatures. Interactions between these 2 main effects were significant for linolenic acid (18:3n-3); *cis*-11,14-eicosadienoic (20:2n-6); *cis*-8,11,14-eicosatrienoic acids (20:3n-6); and DHA (22:6n-3) contents. Linoleic acid (18:3n-3) was higher in the residual yolk sac of chicks from 32-wk parents incubated at HA than the CONT, whereas the pattern was reversed for chicks from 65-wk parents. Conversely, HA decreased 20:3n-6 and 22:6n-3 contents of residual yolk of chicks from younger parents but increased those fatty acid contents in yolk sac of chicks from older parents. *Cis*-11,14-eicosadienoic acid (20:2n-6) content increased in eggs from 42-wk parents when eggs were incubated at HA.

DISCUSSION

Our results that moisture content of embryos was highest on d 14 and then decreased regardless of parental age and incubation temperature were consistent with those of Peebles et al. (1999). They reported that after d 14 the relative contribution of dry matter to embryo mass increased considerably. As moisture decreased, fat content increased, which also supported their findings. That higher moisture content of acclimated embryos on d 14 was associated with lower embryo weights is consistent with the suggestion that embryo growth was depressed during the first 4 d of acclimation (Yalçın et al., 2008) and that growth is directly related to accumulation of dry

Table 5. Means for the interaction between incubation temperature[1] and parental age of fatty acid profiles (wt %) in residual yolk sac of chicks at hatch

Fatty acids		Incubation temperature	Parental age, wk 32	42	65	SEM
Palmitic	16:0	Control	24.15[b]	25.35[a]	25.13[a]	0.33
			*	NS	NS	
		High	25.76[a]	25.56[a]	24.64[b]	0.33
Oleic (*cis*-9)	18:1n-9	Control	36.59[a]	36.77[a]	36.69[a]	0.43
			NS	NS	NS	
		High	35.05[b]	34.97[b]	37.31[a]	0.43
Linolenic	18:3n-3	Control	0.65[b]	0.81[a]	0.77[ab]	0.03
			*	NS	*	
		High	0.76[a]	0.80[a]	0.65[b]	0.03
Cis-11-eicosenoic	20:1n-9	Control	0.27[a]	0.23[b]	0.23[b]	0.01
			*	NS	NS	
		High	0.21[b]	0.25[a]	0.23[ab]	0.01
Cis-11,14-eicosadienoic	20:2n-6	Control	0.36[a]	0.29[b]	0.31[b]	0.02
			NS	*	NS	
		High	0.31[a]	0.34[a]	0.28[b]	0.02
Cis-8,11,14-eicosatrienoic	20:3n-6	Control	0.07[a]	0.05[b]	0.04[b]	0.00
			*	NS	*	
		High	0.05[a]	0.05[a]	0.06[a]	0.00
Cis-4,7,10,13,16,19-eicosapentaenoic	22:6n-3	Control	0.34[a]	0.28[a]	0.26[a]	0.03
			*	NS	*	
		High	0.25[b]	0.25[b]	0.45[a]	0.03
Saturated		Control	34.68[a]	35.48[a]	35.75[a]	0.33
			NS	NS	NS	
		High	35.51[ab]	36.06[a]	34.38[b]	0.33

[a,b]Means in the same row within an incubation temperature with no common superscript differ significantly ($P < 0.05$).

[1]Control = incubated at 37.8°C throughout; high = exposed to heating at 38.5°C 6 h/d from 10 to 18 d of incubation.

*Means in the same column within a parental age differ significantly ($P < 0.05$).

matter (Ricklefs and Starck, 1998). The results also suggested that fat accumulation at 18 d and at hatch in embryos and chicks, respectively, from 32-wk parents was less than those from 42- and 65-wk parents, which may be related to the smaller yolks in eggs from the 32-wk parents (Yalçın et al., 2008).

Incubation temperature had no effect on protein content of embryos, suggesting that the higher incubation temperature did not alter protein accumulation by the embryo. Moreover, protein-to-water ratios were similar among and between treatments during incubation. The protein in the dried samples was higher on d 14 of incubation and decreased until d 18, which occurred with an increase in fat content. The protein-to-fat ratio was lower on d 18 (2.65) than d 14 (3.56) of incubation with a small and nonsignificant change in protein from d 18 to hatch. The decrease in the fat content led to lower organic material and greater inorganic material at hatch. The decrease in relative fat content at hatch compared with d 18 may be related to the increased heat production in the embryos, linked to the hatching process.

Higher fat content in chicks from 42- and 65-wk parents would be expected due to an association of parental age with egg yolk fat. McNaughton et al. (1978), however, noted that when egg weights were the same there were no differences in yolk composition between 29- and 50-wk parents. When we analyzed for fat content excluding chick weight (using chick weight as a covariate) there was no change in the results observed for fat content. Thus, parental age influenced fat mobilization/utilization independent of chick weight, whereas incubation temperature had no apparent effect on this process. Similarly, Yafei and Noble (1990) reported a reduced yolk lipid absorption in embryos from the 23-wk parents. Yalçın et al. (2008) found heavier livers in embryos and chicks from older parents regardless of incubation temperature. Thus, the higher fat content of the embryos and chicks from older parents was associated with heavier liver weights of those embryos and chicks.

The pattern of change in the concentration of DHA (22:6n-3) and *cis*-11,14,17-eicosatrienoic (20:3n-3) in the residual yolk sac of chicks compared with egg yolks before incubation was markedly different from that of the other fatty acids. A consistent decrease observed in DHA and *cis*-11,14,17-eicosatrienoic fatty acids across the breeder ages (i.e., 60 and 30% less for DHA and *cis*-11,14,17-eicosatrienoic, respectively) may have been a result of prefential uptake from the yolk. Residual yolk sac 14.0, 20:0, 20:3n-3, 20:4n-6, and 20:5n-3 fatty acid contents were independent of parental age and incubation temperature. Pentadecanoic (15:0) and heptadeconoic (17:0) acids were more efficiently transported to the embryo from the yolk sac from breeders at 32 wk than those from 42 and 65 wk. The HA treatment decreased transported levels of pentadeconoic acid (15:0) and palmitoleic (16:1n-7) acids regardless of parental age. These changes in yolk fatty acid transportation may be due to activities of enzymes and lipoprotein transport (Speake et al., 1998).

Important observations in our experiment were changes in linolenic (18:3n-3) acid and DHA (22:6n-3) in yolk sacs of chicks from younger and older parents when eggs were incubated at higher temperatures. The DHA content in residual yolk sac was considerably higher in chicks from older parents incubated at HA, whereas, in contrast, levels of 18:3n-3 were lower. Also, chicks from younger parents in the HA treatment had lower transported 18:3n-3 and higher levels of transported DHA. It may be concluded that, although higher levels of 18:3n-3 were transported to the chick, they did not trigger its proper conversion to DHA in yolk sac of chicks from older parents when eggs were incubated at high temperatures. These results support previous conclusions (Maldjian et al., 1995; Speake et al., 1998; Speake and Wood, 2005) that DHA is preferentially taken up from the yolk, which is related to the specific requirements of tissue. Moreover, previous results indicated that heat-acclimated embryos and chicks, at the internal pipping stage and at hatch, exhibited adaptive physiological responses (Yalçın et al., 2008). Furthermore, actual environment during incubation influences the development of respective physiological control systems via changes in neuroorganization (Tzschentke and Plagemann, 2006). Tzschentke and Basta (2002) showed that exposure to higher incubation temperatures changed postnatal thermosensitivity of the preoptical area of anterior hypothalamus neurons in ducks. Because of the important role of DHA in the functional development of neural tissues, that this process occurred during the higher incubation temperature may be related to a protective strategy and thus contributes to heat adaptation by the chick. Shmeeda et al. (2002) also suggested that if DHA influenced membrane permeability properties, it would directly affect stress endurance during heat challenge and prevent dehydration of the cells. Because there were no flock effects within breeder age, the results may be attributed to the higher incubation temperature effects on yolk fatty acid uptake. The question is raised if the higher incubation temperature reduced the need of DHA of embryos from older parents. Our results also show that although HA had no effect of chick composition at hatch, residual yolk composition may be changed by heat acclimation of embryos during incubation and that breeder age may have additional effects.

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