Effect of Supplementing the Diet of Male Chickens With Oils Rich in n-6 Polyunsaturated Fatty Acids on the Fatty Acid Profiles of the Testis and Liver

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ABSTRACT : Since the n-6 polyunsaturated fatty acid, docosatetraenoic acid (22:4n-6), is a major functional constituent of avian spermatozoa, the effects of two dietary oils rich in fatty acids which are metabolic precursors of 22:4n-6 on the fatty acid profiles of testicular lipids were investigated during a 39 week period of supplementation from 21 to 60 weeks of age. The effects on liver lipids were determined for comparison. Dietary supplementation of male chickens with Arasco Oil, which provides a large amount of arachidonic acid (20:4n-6), increased the proportion of 20:4n-6 in liver phospholipid by almost 2.5-fold. Although liver phospholipid normally contains very little 22:4n-6, this proportion was significantly increased as a result of Arasco feeding, indicating that the conversion of 20:4n-6 to 22:4n-6 was occurring. The phospholipid of the testis contains much higher proportions of 20:4n-6 and particularly of 22:4n-6 than the liver; supplementation with Arasco Oil significantly increased the proportions of both these polyunsaturates in testis phospholipid but the magnitude of this effect was much lower than that which occurred in the liver. Dietary supplementation with Evening Primrose Oil which contains γ-linolenic acid (18:3n-6) resulted in significant increases in the proportions of 20:4n-6 and 22:4n-6 in liver phospholipid, although the extent of this increase was less than that produced by the Arasco Oil. By contrast, the feeding of Evening Primrose Oil did not alter the fatty acid composition of phospholipid in the testis. The findings raise the possibility that dietary supplementation with Arasco Oil may modulate the fatty acid profile of avian spermatozoa in a way which could potentially be beneficial for fertility. Moreover, the weights of the testes were almost doubled as a result of supplementation with Arasco Oil or Evening Primrose Oil. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 11 : 1518-1522*)

Key Words : Testis, Fertility, Polyunsaturated Fatty Acids, Docosatetraenoic Acid, Arachidonic Acid, Evening Primrose Oil

INTRODUCTION

Avian spermatozoa have a distinctive fatty acid composition, characterized by very high proportions of the long-chain highly polyunsaturated fatty acids of the n-6 family, arachidonic (20:4n-6) and docosatetraenoic (22:4n-6) acids (Darin-Bennett et al., 1974; Ravie and Lake, 1985; Surai et al., 1998). There is evidence that these polyunsaturates are essential for the formation and function of spermatozoa since their proportions in sperm phospholipid decrease with aging in the chicken, in parallel with declining spermatogenesis and reductions in the quality of the semen (Kelso et al., 1996, 1997; Cerolini et al., 1997). The proportion of 22:4n-6 in sperm phospholipid was significantly correlated with the fertilising ability of the semen throughout the reproductive lifetime of the male bird (Cerolini et al., 1997). This raises the possibility of improving male fertility in the chicken by increasing the provision of 22:4n-6 to the developing sperm.

The testis is the site at which polyunsaturated fatty acids are incorporated into the membrane phospholipid of the developing sperm (Retterstol et al., 1998). The aim of this study was to determine the responsiveness of the fatty acid profile of the testicular lipids to dietary manipulation and to compare any changes with those occurring in the liver. The effects of two different oils, both of which contain polyunsaturated fatty acids which can act as metabolic precursors for the biosynthesis of 22:4n-6, were investigated. Arasco Oil (a product of Scotia Pharmaceuticals Ltd, Carlisle, UK) is a rich source of 20:4n-6 which can be converted to 22:4n-6 by a single elongation step. Evening Primrose Oil contains γ-linolenic acid (18:3n-6) which requires three successive enzymic reactions (elongation/desaturation/elongation) to be converted to 22:4n-6. Both these precursors enable the rate-limiting 6-desaturase step for the conversion of linoleic acid (18:2n-6) to 18:3n-6 to be bypassed, potentially increasing the capacity of the tissues for the biosynthesis of 22:4n-6. The details of the metabolic routes for the interconversion of polyunsaturated fatty acids in birds have been described in several review articles (Watkins and Kratzer, 1987; Watkins, 1995; Walzem, 1996).

MATERIALS AND METHODS

Male chickens (Ross broiler-breeder strain) were purchased at 21 weeks of age from a commercial poultry supplier and were housed individually in cages

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in a controlled environment with a photoperiod of 13 h light:11 h dark. The birds were provided with a standard wheat-based diet containing 15.5% crude protein, 3% fat and 11.5 MJ ME/kg and were randomly allocated into 3 experimental groups (10 birds per group) supplemented with either maize oil (rich in 18:2n-6), Evening Primrose Oil (rich in 18:3n-6) or Arasco Oil (rich in 20:4n-6), each present at 5% of feed. The total lipid content of the diets was 8%. The oils were a gift from Scotia Pharmaceuticals Ltd, Carlisle, UK. Vitamin E was included in the diets at a concentration of 200 mg/kg. The amount of feed given each day was adjusted according to body weight as recommended by the Ross Management Manual (Ross Poultry, Inverurie, UK). On reaching 60 weeks of age, the birds were slaughtered and the livers and testes were obtained. The duration of the dietary supplementation was 39 weeks (i.e. from 21 to 60 weeks of age).

Total lipid was extracted from the testis and liver samples after homogenization in a suitable excess of chloroform:methanol (2:1). The total lipid was separated into the major classes (triacylglycerol, phospholipid, cholesteryl ester) by thin-layer chromatography on silica gel G using a solvent system of hexane:diethyl ether:formic acid (80:20:1). The bands corresponding to the lipid classes were visualized and scraped from the plate, the lipid classes were eluted from the silica and subjected to transmethylation to produce fatty acid methyl esters for analysis by gas-liquid chromatography as previously described (Kelso et al., 1996). The fatty acid methyl esters were analysed by injecting on to a Carbowax capillary column 30 m×0.25 mm, with a film thickness of 0.25 μ (Alltech Ltd, Carnforth, UK), fitted within a Chrompack CP9001 instrument (Chrompack Ltd, Middleburg, NL). Following injection of the sample, the column temperature was maintained at 185℃ for 2 min, then increased at a rate of 5℃/min to a temperature of 230℃, and maintained at 230℃ for a further 24 min. The fatty acid compositions (wt %) were derived by integrating the peaks using an EZ Chrom data handling system (Speck Analytical Ltd, Alloa, UK). The amounts of the lipid classes were calculated from the fatty acid content by the EZ Chrom data system using an internal standard of pentadecaenoic acid. The identity of the peaks was confirmed by comparison with the retention times derived from standard mixtures of fatty acid methyl esters (Sigma Ltd, Poole, UK). The fatty acid data, expressed as percentages, were arcsin transformed to make the variance independent of the means (Snedecor and Cochrane, 1967) prior to statistical analysis by ANOVA.

Table 1. Fatty acid composition of the diets supplemented with different dietary oils

Fatty acids (wt %)	Dietary oil		
	Maize	Arasco	Evening Primrose
16:0	12.5	8.7	9.8
18:0	1.8	6.0	1.9
18:1n-9	25.0	30.6	13.3
18:2n-6	53.6	16.0	63.4
18:3n-6	0.0	0.0	5.3
18:3n-3	2.6	1.7	1.6
20:4n-6	0.0	28.0	0.0

RESULTS

The fatty acid compositions of the feeds supplemented with the different oils (table 1) confirm that the major polyunsaturates provided by the maize oil and Arasco Oil diets were repectively 18:2n-6 and 20:4n-6. Although 18:2n-6 was the predominant fatty acid of the diet supplemented with Evening Primrose Oil, this diet also provided a significant amount of 18:3n-6, a fatty acid which was absent from the other two diets.

Phospholipid formed 55.3±2.2, 46.7±2.2 and 58.1 ±1.8 % (by weight) of the total lipid of the testis for birds on the maize oil, Arasco Oil and Evening Primrose Oil diets respectively. There were a number of significant differences in the fatty acid compositions of the phospholipid of the testis as a result of the different diets (table 2). The Arasco Oil diet produced increases in the proportions of both 20:4n-6 and 22:4n-6 and a decreased level of 18:2n-6 in this tissue lipid class when compared with the effects of the maize oil diet. By contrast, the Evening Primrose Oil had no significant effect on this fatty acid composition when compared with the maize oil diet.

Triacylglycerol formed 3.5±0.3, 3.6±1.1 and 4.8

Table 2. Effect of dietary polyunsaturated oils on the fatty acid composition of testis phospholipid

Fatty acids (wt %)	Dietary oil		
	Maize	Arasco	Evening Primrose
16:0	22.4 ±0.4^{a}	24.4 ±1.6^{a}	23.4 ±0.5^{a}
18:0	16.4 ±0.1^{a}	11.9 ±1.7^{b}	16.2 ±0.4^{a}
18:1n-9	9.2 ±0.1^{a}	10.2 ±0.6^{a}	8.9 ±0.5^{a}
18:1n-7	2.4 ±0.1^{a}	2.9 ±0.1^{b}	2.4 ±0.2^{ab}
18:2n-6	3.5 ±0.4^{a}	0.5 ±0.1^{b}	3.5 ±0.1^{a}
20:1n-9	3.0 ±0.1^{a}	3.8 ±0.5^{a}	2.7 ±0.2^{a}
20:4n-6	16.7 ±0.4^{a}	20.4 ±1.2^{b}	17.6 ±0.2^{ab}
22:4n-6	15.6 ±0.6^{a}	18.0 ±0.1^{b}	15.5 ±0.1^{a}
22:6n-3	1.2 ±0.2^{a}	1.8 ±0.1^{a}	1.1 ±0.1^{a}

^{a,b,c} Values in a row without a common superscript are significantly ($p<0.05$) different.

±1.0% (by weight) of the total lipid of the testis for birds on the maize oil, Arasco Oil and Evening Primrose Oil diets respectively. There were no significant differences in the proportions of the various polyunsaturates in triacylglycerol as a result of the three diets (table 3), although the proportion of oleic acid (18:1n-9) was much higher in the case of the Arasco-supplemented birds.

Cholesteryl ester formed 18.4±2.8, 24.0±1.0 and 14.7±0.1% (by weight) of the total lipid of the testis for birds on the maize oil, Arasco Oil and Evening Primrose Oil diets respectively. The main differences in fatty acid composition (table 4) were that Arasco Oil feeding increased the proportions of 18:1n-9 and 20:1n-9 but did not affect the level of 20:4n-6 in comparison with the maize oil diet. The proportion of 22:4n-6 was very low in this lipid class and was not detected in the Arasco group. Supplementation with Evening Primrose Oil did not alter the fatty acid profile of testis cholesteryl ester when compared with the maize oil group.

Phospholipid formed 60.6±1.0, 66.6±1.7 and 67.4 ±2.3% (by weight) of the total lipid of the liver for birds on the maize oil, Arasco Oil and Evening Primrose Oil diets respectively. In comparison with the maize oil diet, the Arasco-supplemented diet resulted in major increases in the proportions of 20:4n-6 and 22:4n-6 in this tissue lipid class, commensurate with a dramatic decrease in the level of 18:2n-6 (table 5). Large increases in the proportions of 20:4n-6 and 22:4n-6, and also of dihomo--linolenic acid (20:3n-6), were observed as a result of feeding Evening Primrose Oil.

Triacylglycerol formed 13.9±2.3, 7.5±1.0 and 9.0 ±1.0% (by weight) of total liver lipid for birds on the maize oil, Arasco Oil and Evening Primrose oil respectively. The main effect of feeding Arasco Oil (table 6) was a dramatic increase in the proportion of 20:4n-6 and a marked decrease in the level of 18:2n-6 in this lipid class, when compared to the maize oil group. Liver triacylglycerol contained a detectable amount of 20:3n-6 in the case of the Evening Primrose group but not in the other two dietary groups. No 22:4n-6 was detected in the case of the maize oil group, but was present in the other two groups.

Cholesteryl ester formed 7.8±0.9, 7.5±0.6 and 8.1 ±1.0% (by weight) of total liver lipid for birds on the maize oil, Arasco Oil and Evening Primrose Oil diets respectively. The main effects of the Arasco Oil were to greatly increase the proportion of 20:4n-6 and to decrease that of 18:2n-6 when compared with the maize oil group (table 7). Supplementation with Evening Primrose Oil did not affect the proportion of 20:4n-6. No 22:4n-6 was detected in this lipid class for birds on the three dietary regimes.

The fresh weights (g) of the testis were 23.9±5.2, 40.6±4.6 and 43.8±6.4 for birds on the maize oil,

Table 4. Effect of dietary polyunsaturated oils on the fatty acid composition of testis cholesteryl ester

Fatty acids (wt %)	Dietary oil		
	Maize	Arasco	Evening Primrose
16:0	6.9 ± 0.5^{ab}	5.8 ± 0.2^{b}	7.5 ± 0.5^{a}
18:0	12.8 ± 0.7^{a}	10.5 ± 0.1^{b}	13.1 ± 0.5^{a}
18:1n-9	54.7 ± 3.3^{a}	65.0 ± 0.2^{b}	52.1 ± 3.8^{a}
18:1n-7	2.3 ± 0.1^{a}	2.8 ± 0.1^{b}	2.6 ± 0.3^{ab}
18:2n-6	7.0 ± 1.5^{a}	0.0^{b}	6.1 ± 1.6^{a}
20:1n-9	2.6 ± 1.2^{a}	7.8 ± 0.7^{b}	4.1 ± 1.3^{a}
20:4n-6	2.6 ± 0.5^{a}	2.1 ± 0.3^{a}	2.7 ± 0.7^{a}
22:4n-6	1.3 ± 0.2^{a}	0.0^{b}	1.1 ± 0.5^{a}

^{a,b,c} Values in a row without a common superscript are significantly ($p<0.05$) different.

Table 3. Effect of dietary polyunsaturated oils on the fatty acid composition of testis triacylglycerol

Fatty acids (wt %)	Dietary oil		
	Maize	Arasco	Evening Primrose
16:0	19.6±0.7^{a}	16.4±2.4^{a}	22.7±1.8^{a}
18:0	16.9±0.8^{a}	15.6±0.8^{a}	16.1±0.1^{a}
18:1n-9	12.2±0.2^{a}	28.5±6.1^{b}	11.6±0.1^{c}
18:1n-7	2.3±0.1^{a}	2.4±0.2^{a}	2.2±0.2^{a}
18:2n-6	3.8±0.7^{a}	2.1±0.1^{a}	3.6±0.2^{a}
20:1n-9	8.8±0.9^{a}	7.5±0.6^{a}	7.7±0.4^{a}
20:4n-6	6.0±0.7^{a}	7.9±1.6^{a}	5.0±0.8^{a}
22:4n-6	14.6±2.1^{a}	10.3±1.8^{a}	14.2±1.4^{a}

^{a,b,c} Values in a row without a common superscript are significantly ($p<0.05$) different.

Table 5. Effect of dietary polyunsaturated oils on the fatty acid composition of liver phospholipid

Fatty acids (wt %)	Dietary oil		
	Maize	Arasco	Evening Primrose
16:0	19.4 ± 0.2^{a}	19.0 ± 0.6^{a}	15.1 ± 0.2^{b}
18:0	25.5 ± 0.4^{a}	25.9 ± 0.3^{a}	29.4 ± 0.2^{b}
18:1n-9	5.3 ± 0.3^{a}	4.2 ± 0.3^{b}	2.7 ± 0.3^{c}
18:2n-6	22.5 ± 0.7^{a}	6.7 ± 0.2^{b}	22.9 ± 0.4^{a}
20:3n-6	1.4 ± 0.1^{a}	0.0^{b}	2.8 ± 0.2^{c}
20:4n-6	10.6 ± 1.1^{a}	25.6 ± 2.0^{b}	17.3 ± 0.2^{c}
22:4n-6	1.0 ± 0.2^{a}	3.9 ± 0.6^{b}	2.4 ± 0.1^{c}
22:6n-3	8.5 ± 0.5^{a}	10.2 ± 2.9^{a}	2.0 ± 0.1^{b}

^{a,b,c} Values in a row without a common superscript are significantly ($p<0.05$) different.

Table 6. Effect of dietary polyunsaturated oils on the fatty acid composition of liver triacylglycerol

Fatty acids (wt %)	Dietary oil		
	Maize	Arasco	Evening Primrose
16:0	19.6 ± 1.1[a]	13.5 ± 0.1[b]	19.6 ± 0.8[a]
18:0	16.2 ± 0.9[a]	14.8 ± 0.9[a]	17.1 ± 0.9[a]
18:1n-9	20.8 ± 1.7[a]	25.4 ± 0.6[b]	15.3 ± 1.1[c]
18:2n-6	30.9 ± 2.8[a]	9.8 ± 0.3[b]	34.8 ± 1.6[a]
20:3n-6	0.0[a]	0.0[a]	1.4 ± 0.1[b]
20:4n-6	2.9 ± 0.6[a]	22.9 ± 0.7[b]	3.9 ± 0.2[a]
22:4n-6	0.0[a]	3.7 ± 0.4[b]	1.2 ± 0.1[c]

[a,b,c] Values in a row without a common superscript are significantly ($p<0.05$) different.

Table 7. Effect of dietary polyunsaturated oils on the fatty acid composition of liver cholesteryl ester

Fatty acids (wt %)	Dietary oil		
	Maize	Arasco	Evening Primrose
16:0	27.6 ± 2.6[ab]	22.9 ± 1.0[a]	32.7 ± 1.1[b]
18:0	20.2 ± 1.0[a]	19.9 ± 1.6[a]	23.1 ± 1.1[a]
18:1n-9	22.9 ± 2.5[a]	35.0 ± 2.9[b]	15.1 ± 0.4[c]
18:2n-6	20.9 ± 1.6[a]	7.1 ± 0.3[b]	20.1 ± 1.2[a]
18:3n-3	1.7 ± 0.1[a]	0.0[b]	0.0[b]
20:4n-6	1.1 ± 0.2[a]	11.6 ± 0.6[b]	1.5 ± 0.1[a]

[a,b,c] Values in a row without a common superscript are significantly ($p<0.05$) different.

Arasco Oil and Evening Primrose Oil diets respectively. The increased testis weights resulting from supplementation with Arasco Oil or Evening Primrose Oil were statistically significant ($p<0.05$). The fresh weights (g) of the livers, at 36.8 ± 1.8, 37.0 ± 1.9 and 37.3 ± 2.1 for birds on the maize oil, Arasco Oil and Evening Primrose Oil diets respectively, did not differ significantly between the three groups.

DISCUSSION

The high content of 20:4n-6 in Arasco Oil results in dramatic increases in the proportions of this polyunsaturate in the lipid classes of the liver when this oil is fed to chickens. Since 20:4n-6 can be converted to 22:4n-6 by a single enzymatic step, the feeding of Arasco Oil also results in increased proportions of 22:4n-6 in the phospholipid and triacylglycerol of the liver. However, 22:4n-6 remains a relatively minor fatty acid in the liver lipids compared with the much larger amounts of 20:4n-6. Both 20:4n-6 and especially 22:4n-6 are more predominant in the lipids of the testis than in those of the liver for birds on the maize oil diet. The feeding of Arasco Oil results in significant but relatively modest increases in the proportions of these polyunsaturates in testis phospholipid. Thus although greater amounts of 20:4n-6 and 22:4n-6 are potentially available for the synthesis of sperm phospholipid as a result of the Arasco Oil diet, the effect is not dramatic.

Although Evening Primrose Oil is especially rich in 18:2n-6, this oil is also a source of 18:3n-6. This latter polyunsaturate can be converted to 20:4n-6 and 22:4n-6 by 2 and 3 enzymatic steps respectively; a process which bypasses the rate-limiting 6-desaturase reaction. As a consequence, the feeding of this oil to chickens produces considerable increases in the proportions of 20:4n-6 and 22:4n-6 in liver phospholipid compared with the feeding of maize oil. The effect is, however, less marked than that of Arasco Oil, presumably because this latter oil provides large amounts of ready-formed 20:4n-6 which only requires one enzymatic step for conversion to 22:4n-6. Dietary supplementation with Evening Primrose Oil also increases the proportion of 20:3n-6 in phospholipid and triacylglycerol of the liver. This is consistent with the role of 20:3n-6 as an intermediate in the synthesis of 20:4n-6 from 18:3n-6 (Watkins and Kratzer, 1987). The feeding of Evening Primrose Oil did not, however, produce any significant elevation of the levels of 20:4n-6 and 22:4n-6 in the lipids of the testis in comparison with the maize oil diet.

The results indicate that, in terms of fatty acid profile, the lipids of the testis are far less amenable to dietary manipulation than are those of the liver. However, the observation that supplementation with an oil enriched in 20:4n-6 can significantly elevate the proportion of both 20:4n-6 and 22:4n-6 in testis phospholipid, raises the potential for optimizing the fatty acid composition of spermatozoa with the possibility of improving fertility (Cerolini et al., 1997). Further work is needed to test this possibility. Other studies have shown that the feeding of linseed oil (Kelso et al., 1997) or fish oil (Blesbois et al., 1997), which alter the n-6/n-3 ratio of polyunsaturates in spermatozoa, can also improve the fertility of the male chicken although the mechanism of this effect is not clear.

Possibly the most dramatic finding of the present study was the increase in testis weight as a result of dietary supplementation with either Arasco Oil or Evening Primrose Oil. This could potentially result from the increased availability of 20:4n-6 and 22:4n-6 promoting spermatogenesis and causing increased testis mass due to the greater number of developing sperm in the tissue. Although this explanation is consistent with the effect of the Arasco Oil diet in enhancing the content of these polyunsaturates in the testicular

lipids, it is unlikely to pertain to the feeding of Evening Primrose Oil which did not affect the amounts of these fatty acids in this tissue. An alternative explanation could be based on the effects of the different dietary oils on the amounts and types of eicosanoids synthesized in the tissues of the chicken. Thus the high level of 20:4n-6 provided by the Arasco Oil serves as the substrate for the synthesis of type 2 prostaglandins and type 4 leukotrienes. By contrast, 20:3n-6 derived from the 18:3n-6 of the Evening Primrose Oil is the precursor of type 1 prostaglandins and type 3 leukotrienes (Fischer, 1989). The possibility that eicosanoids could promote spermatogenesis in the chicken is worthy of future investigation. Although the prostaglandins present in seminal plasma have been shown to regulate sperm function in mammals (Gottlieb and Bygdeman, 1988), it should be noted that several prostaglandins actually inhibit spermatogenesis in rodents (Abbatiello et al., 1976). An alternative explanation could be that the maize oil that was present in the control diet somehow caused a reduction in testis mass relative to that which would have been observed in birds maintained on a normal commercial diet. This is unlikely, firstly because commercial diets normally contain 18:2n-6 as the main polyunsaturate and secondly because the testis mass of the birds supplemented with maize oil was in the normal range that has previously reported (De Reviers and Williams, 1984). Thus, we conclude that the feeding of oils rich in 18:3n-6 or 20:4n-6 results in an increased testis mass by 60 weeks of age, when compared with diets which contain 18:2n-6 as the main polyunsaturate.

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