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The effect of n-6/n-3 fatty acid ratios on broiler breeder performance, hatchability, fatty acid profile and reproduction

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Summary

This experiment was conducted to study the effect of dietary omega6 (n-6) to omega3 (n-3) fatty acid (FA) ratios on performance and reproduction of broiler breeders. In experiment 1, 400 females and 40 males (30 week age) of Ross 308 broiler breeder (20 females and two males in each pen) were randomly assigned to one of the four diets with n-6/n-3 FA ratios of 4, 6, 8 and 16 (control). As a measure of hatchability, fertility of eggs and general incubation traits, 1,200 eggs (60 eggs from each pen) were collected and incubated for 21 days and embryo liver and brain fatty acid profile in 14 and 21 days were determined. In experiment 2, 48 males (three males in each pen) randomly assigned to one of the four diets with n-6/n-3 FA ratios of 4, 6, 8 and 16 (control). Semen was collected twice weekly, and semen volume, spermatozoa concentration and motility and alive and dead spermatozoa were estimated. Egg production and egg mass were decreased by n-6/n-3 FA ratios of 4:1 and 6:1 ($p < .05$). There were no significant differences between treatments on breeder's body weight, eggs fertility and hatchability, embryonic mortality and semen features. Linolenic acid, eicosapentaenoic acid, docosahexaenoic acid and total n-3 of egg yolk, semen, testis and liver and brain of embryo and day-old chicken were increased while concentration of linoleic acid, arachidonic acid and docosatetraenoic acid of mentioned tissues were decreased by increasing n-6/n-3 FA ratios ($p > .05$). In conclusion, absolute amount of n-3 and n-6 FAs in broiler breeder diet may be more important than n-6/n-3 FA ratios and to consider reproductive and performance traits of breeders, it is necessary to supply higher levels of n-3 and n-6 FA with respect to n-6/n-3 FA ratios.

KEYWORDS

broiler breeder, embryonic mortality, hatchability, n-6/n-3 ratio, semen

1 | INTRODUCTION

Reproduction and hatchability is a critical part of poultry production, especially the hatching rate of settable eggs and male and female's reproductive performance. Dietary omega-3 (n-3) and omega-6 (n-6) fatty acids (FAs) have a great impact on avian metabolism, abdominal fat deposition and hatchability (Cherian & Sim, 1997), fatty acid profile of egg yolk (Cherian, 2008; Koppenol, Buyse et al., 2014; Koppenol, Delezie et al., 2014), lipid metabolism in the chick (Ajuyah, Wang, Sunwoo, Cherian, & Sim, 2003; Koppenol et al., 2015). On the other hand, one of the greatest challenges is the reduction in n-3 and increase in n-6 FAs in broiler breeder diets. So it is hypothesized that manipulation of these n-3 to n-6 ratio in broiler breeder diets can improve egg yolk fatty acid (FA) profile, transition of n-3 and n-6 FA from egg to embryonic tissues and offspring and incubation parameter. Less researches are performed regarding the enrichment

of broiler breeder egg by n-3 FAs. The yolk n-3 FAs have unique role in modulation of progeny lipid and eicosanoid metabolism as n-3 FAs are preferentially taken up from yolk sac lipids and are incorporated into cell membrane phospholipids of the developing embryo during avian embryogenesis and post-hatch growth (Koppenol, Delezie et al., 2014; Koppenol et al., 2015).

Previous studies reported that the tissue FA composition of the embryo and the subsequent hatched chick reflected the FA profile of the egg yolk (Cherian & Sim, 1991) and recently researcher described that adding DHA and EPA at a ratio of 1/1, 1/2 and 2/1 (or n6:n3 ratio of 11 to 5.4) resulted in similar ratios of these FA in the yolk, embryonic tissues and offspring. Also, it was reported that maternal supplementation of n-3 FA, EPA and DHA was incorporated in the yolk and transferred via the residual yolk to become available for the developing embryo and resulted in elevated EPA and DHA concentrations in the liver of the offspring at hatch. (Koppenol, Buyse et al., 2014).

To improve male fertility and because of economic reason, nutritionist focused on dietary manipulation of sperm FA profile. There is evidence that the FA compositions of chicken sperm play important roles in maintaining semen quality (Cerolini et al., 1997). Avian semen is characterized by comparatively low proportions of n-3 FA such as docosahexaenoic acid (DHA, 22:6 n-3) and high proportions of n-6 FA such as docosatetraenoic acid (DTA, 22:4n-6) in their phospholipids (Surai, Noble, Sparks, & Speake, 2000).

The n-3 and n-6 FAs and their ratio have different effects, either negative or positive, on avian semen characteristics. The DHA is incharge of maintaining fluidity and flexibility of spermatozoa membranes, and there was positive correlation between fertility and concentration of arachidonic acid (ARA) and DHA in male broiler breeder sperm (Cerolini et al., 1997). Studies showed that n-3 FAs have no effect on avian semen volume, motile spermatozoa and spermatozoa concentration (Cerolini et al., 1997) or negative effect on sperm concentration (Cerolini, Maldjian, Pizzi, & Gliozzi, 2001). On the other hand, n-6 FA-rich diets have positive effect on semen volume and total spermatozoa number (Surai et al., 2000) and a negative effect on spermatozoa concentration (Cerolini et al., 2003) and these discrepant effects of n-3 and n-6 FA on male broiler breeder performance and sexual ability may be due to their competition. There are little-published data on effect of n-3/n-6 FA ratios on broiler breeder reproduction. Previous studies have reported that n-3/n-6 FA ratio from 1 to 4.15 had no significant effect on the testis index while spermatogonial development and germ cell layers were increased (Feng et al., 2015). Although some research has been carried out on n-3/n-6 fatty acid ratio, no studies have been found which fully characterized in relation to egg quality, hatchability and fertility in broiler breeders. Because it is known that the mechanisms involved in lipid metabolism and transition of FAs during incubation are influenced by many factors such as amount and ratio of n-3 and n-6 FAs, the potential effect of n-6/n-3 ratios on the broiler breeder performance, male and female reproduction and FA transfer is taken into account by setting up this study.

2 | MATERIALS AND METHODS

2.1 | Experiment 1

This experiment was performed during peak egg production (30–40 week of age) in Simorgh Company. Using completely randomized design, 400 females and 40 males (20 females and two males in 20 pens of 3.74 m^2 floor spaces [1.7 m × 2.2 m]) were assigned to four dietary n-6/n-3 FA ratio. Fish and soybean oils were added to the diets to achieve n-6/n-3 FA ratio as 4, 6, 8 and 16 (control or no added fish oil; Table 1). All corn–soybean mash diets for either sex were formulated to be isocaloric and isonitrogenous. The level of linoleic acid (LA) was maintained at 1.2% in all diets to prevent the reduction in egg size. Amounts of feed were adjusted weekly to maintain body weight and optimum hen-day egg production as recommended by Ross broiler breeder (Aviagen 2013). Housing and management of birds during breeder are based on the recommendations of the breeder company (Aviagen 2013).[1] Separate sex feeding was accomplished by feeding females trough grill, and males trough-specific feeder in each pen and 15.5-hr light periods (15.5L:8.5D) was provided for them. Males were housed 2 days before females to allow males more time to adjust to their feeders. A two-tier, 10-hole nest box unit was provided in each pen. Meanwhile lay initiation, feed allotments were given for the maintenance of optimum hen-day egg production as further recommended by Ross Parent Stock Management Manual, 2013 and collection of laid eggs was carried out manually and five times daily. Egg production per cent and egg mass were recorded weekly. Egg mass was calculated by following formula:

$$\text{Egg mass (g)} = \text{average weekly egg production(\%)} \times \text{egg weight (g)}.$$

Body weight and feed intake of all males and females in each pen were recorded weekly. All birds were in healthy conditions, and no mortality and clinical sign of diseases were observed within the total period of the experiment.

2.2 | Hatchability measurement

One thousand and two hundred eggs (60 eggs from each pen) collected and incubated in setter machine (Petersime 576, analogue) at 34 week of age. Hatchability was calculated as the number of chicks being hatched and expressed as percentage of incubated fertile eggs. All of the eggs were candled in 8th day of incubation and infertile eggs removed also, and fertility of eggs was determined. The eggs were transferred to the analogue hatcher incubators model Petersime 192, in 19 days of incubation. Non-hatched eggs bricked after hatching and steps of general incubation traits such as membrane, embryo, ring, feathered, turned, beak to the air sac and second grade chicks were determined. Also, embryonic mortality in the first, second and third week of incubation was calculated. Gender of hatched chicks was determined by feather sexing method, and the ratio of male to female chicks was calculated.

[1]Ross broiler Breeder male and female feeding and management guide, 2013.

TABLE 1 Experimental diet with different ratio of n-6/n-3 fatty acids ratio (g/kg)

	Females diets (n-6/n-3 ratio)				Males diets (n-6/n-3 ratio)			
	4	6	8	16	4	6	8	16
Ingredients								
Corn	443.4	461.8	471.0	456.0	343.3	382.8	403.5	462.0
Wheat	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Soybean meal (44% CP)	219.8	216.4	214.7	219.8	96.4	96.8	97.0	100.0
Barley	0.0	0.0	0.0	0.0	151.1	115.8	97.0	40.0
Wheat bran	0.0	0.0	0.0	0.0	150.0	150.0	150.0	150.0
Carbonate calcium	66.1	66.1	66.1	66.1	19.5	19.5	19.4	19.4
Di-calcium phosphate	14.5	14.5	14.5	14.5	15.9	15.9	15.9	16.0
Fish oil (Kilka fish Oil)	14.0	8.0	5.0	0.0	12.2	7.5	5.0	0.0
Soybean oil	2.9	2.7	2.6	12.8	0.0	0.0	0.0	0.0
Salt	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Breeder supplement[a]	5.2	5.2	5.2	5.2	6.0	6.0	6.0	6.0
Vitamin E	1.0	1.0	1.0	1.0	2.0	2.0	2.0	2.0
Vitamin B	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
DL-Methionine	1.6	1.6	1.6	1.6	0.4	0.4	0.4	0.4
Antioxidant	1.0	1.0	1.0	1.0	2.0	2.0	2.0	2.0
Zeolite (Sodium bentonite)	26.2	17.4	13.0	17.6	0.0	0.0	0.0	0.0
Calculated nutrients (g/kg)								
AME (Kcal/Kg)	2750.0	2750.0	2750.0	2753.0	2740.0	2740.0	2740.0	2748.0
Crude protein	15.00	15.40	15.40	15.50	12.90	12.90	12.90	12.95
Methionine	0.42	0.42	0.42	0.41	0.26	0.26	0.26	0.34
Meth + Cys	0.65	0.65	0.65	0.65	0.05	0.05	0.05	0.05
Lysine	0.80	0.79	0.79	0.80	0.56	0.56	0.55	0.55
Threonine	0.58	0.58	0.58	0.58	0.00	0.00	0.00	0.00
Tryptophan	0.19	0.19	0.19	0.19	0.00	0.00	0.00	0.00
Crude fat	3.82	3.28	3.00	3.42	3.71	3.33	3.13	2.70
Crude fibre	3.17	3.17	3.18	3.00	1.13	4.61	4.55	4.30
Choline	0.09	0.09	0.09	0.09	0.09	0.08	0.08	0.08
Total phosphorous	0.63	0.63	0.63	0.63	0.77	0.77	0.77	0.77
Ave. phosphorous	0.35	0.35	0.35	0.35	0.40	0.40	0.40	0.40
Calcium	3.00	3.00	3.00	3.00	1.20	1.20	1.20	1.20
Sodium	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.15

[a]Each kg of broiler breeder diet contains: vitamin A, 11,000 mg; vitamin D3, 35,000; vitamin K3, 5 mg; vitamin E, 100 mg; vitamin B1, 0.25 mg; vitamin B2, 12 mg; vitamin B5, 15 mg; vitamin B6, 4 mg; vitamin B9, 2 mg; vitamin B12, 0.03 mg; choline chloride, 1,000 mg; Iron, 50 mg; Zn, 100 mg; Mn, 120 mg; Cu, 10 mg; Se, 0.3 mg; antioxidant, 1,000 mg.

Day-old chicks were weighed, and the ratio of chicks to settable eggs was calculated.

2.3 | Egg and tissue collection

Three eggs from each pen were randomly collected at 33 week of age, and the albumen and yolk part of eggs were separated and weighed. The three separated yolks were blended, and yolk samples (20 gr) were stored at −20°C until fatty acid analysis. To consider the alteration of fatty acid composition during incubation, three eggs from each incubated groups randomly removed, by the 14 days of incubation. The embryos were killed by cervical dislocation method; then, liver and brain of embryos manually harvested and stored at −20°C until fatty acid analysis. This procedure was repeated for 1-day-old chicks of each dietary n-6/n-3 FA ratio treatment; then, liver and brain of chicks were removed and stored at −20°C for further fatty acid analysis.

2.4 | Experiment 2

Ross broiler breeder males were purchased in 36 week from a Simorgh commercial poultry supplier. In a completely randomized design, 48 males were assigned to 16 pens with 1-m^2 floor space (three males in each). Fish and soybean oils were added to the diets to achieve n-6/n-3 FA ratio as 4, 6, 8 and 16 (control or no added fish oil; Table 1). Diet was based on mash corn–soybean meal. The experimental protocols were reviewed and approved by the Animal Care Committee of Ferdowsi University of Mashhad.

2.5 | Semen collection

The males were adapted to the diets and were trained for semen collection within 36 weeks. The semen was collected twice a week using abdominal massage method (Burrows & Quinn, 1937). The semen volume was determined by reading the scale on the tube, which was graduated from 0 to 15 ml with a precision of 0.1 ml. Pooled semen samples were diluted 1:1 (v/v) with TUR-2 diluent (Wishart & Wilson, 1997). The motility was determined according to (Wishart & Wilson, 1997). To evaluate the sperm concentration, 20 μl of pure sperm was diluted in 19.98 ml (dilution 1:1000) of 1% saline formol buffer. After homogenizing the mixture, an aliquot was removed to the neubauer layer. The viability (live to dead spermatozoa ratio) was performed using eosin–nigrosin smears under light microscope (Bakst & Cecil, 1997). Ten ml semen from each pen was collected and stored at −20°C until FA analysis. At 39 weeks of age, one male from each pen was killed by cervical dislocation and then, liver and both testis samples were manually collected and stored at −20°C for FA analysis. The experimental protocols were reviewed and approved by the Animal Care Committee of Ferdowsi University of Mashhad.

2.6 | Fatty acid analysis

Total lipids of the males and females diets ingredients (soxhlet method), egg yolk, chick and embryo liver and brain (experiment 1), males liver, testis and semen (experiment 2) by the method of Folch, Lees, & Stanley (1957) were extracted. The mass of total lipid content was determined gravimetrically. Extracted lipids of ingredients, yolk, tissues and added oils (fish and soybean oil) were methylated using low-temperature direct methylation method with methanolic KOH (Dugan, McGinnis, & Vadehra, 1966). Fatty acid analysis was performed with a Varian 3,800 gas chromatograph equipped with flame ionization detector and SP-2330 fused silica capillary column (30 mm × 0.25 mm i.d.), and fatty acid profile of diets and n-6/n-3 ratios of diets were calculated (Table 2).

2.7 | Statistical analysis

All measured criteria on the effect of dietary n-6/n-3 ratio on broiler breeder performance and reproductive characteristics were

TABLE 2 Fatty acid profile of experimental diets (% of total fat)

	Female diet (n-6/n-3 ratio)				Male diet (n-6/n-3 ratio)			
	4	6	8	16	4	6	8	16
Fatty Acid								
C16:0	16.176	14.730	13.667	11.974	16.843	15.170	14.077	12.684
C16:1	2.440	1.716	1.191	0.265	2.790	1.962	1.422	0.739
C18:0	3.354	3.013	2.756	2.843	3.299	2.878	2.803	2.266
C18:1 n-9	30.175	29.105	29.386	25.345	30.287	31.194	32.881	28.594
C18:2 n-6	35.234	41.693	45.481	55.537	34.167	38.959	41.289	51.185
C18:3 n-3	1.922	1.693	1.934	3.470	0.883	1.003	1.182	1.220
C20:4	0.223	0.194	0.167	0.279	0.184	0.138	0.109	0.070
C20:5 n-3	1.801	1.603	0.945	0.014	1.865	1.313	0.946	0.483
C22:6 n-3	4.947	3.703	2.822	0.004	5.806	4.165	3.028	1.493
∑ other[a]	3.357	2.348	1.616	0.269	3.865	2.716	1.965	1.015
Calculated ratio								
∑ Saturated	22.608	19.936	17.969	15.345	23.607	20.492	18.658	15.883
∑ MUFA	33.118	31.171	30.815	25.630	33.662	33.567	34.599	29.485
∑ PUFA	43.903	48.693	51.181	59.025	42.722	45.441	46.445	54.381
∑ n-3	8.669	6.999	5.701	3.488	8.555	6.482	5.156	3.195
∑ n-6	35.234	41.693	45.481	55.537	34.167	38.959	41.289	51.185
n-6/n-3	4.064	5.957	7.978	15.922	3.994	6.010	8.007	16.018
Unsaturated/ Saturated	3.407	4.006	4.563	5.517	3.236	3.856	4.344	5.280

[a]∑ other fatty acids including C14:0, C17:0, C17:1, C20:0, C20:1 n-9, C21:0 and C23:0.

TABLE 4 Effect of n-6/n-3 ratio on female broiler breeder production

Age	n-6/n-3 ratio 4	6	8	16	MSE	*p*-Value
Egg weight (g)						
30	58.82	58.79	58.42	58.65	4.26	.13
31	57.89	57.08	59.29	57.77	5.69	.71
32	58.36	59.13	58.02	57.93	1.47	.64
33	59.23	59.23	59.84	60.09	1.81	.24
34	60.25	59.21	58.99	58.69	5.01	.71
35	58.77	60.03	59.41	59.88	0.72	.07
Egg mass (g/bird per day)						
30	50.94	51.31	50.96	51.26	3.371	.99
31	52.68	52.44	54.04	53.61	1.433	.68
32	52.43	54.06	52.79	52.28	0.859	.43
33	50.90[b]	54.49[a]	54.55[a]	55.70[a]	0.971	<.05
34	52.40	52.46	51.43	52.55	1.839	.90
35	48.04[b]	50.58[b]	51.25[ab]	54.65[a]	1.721	<.05

[a–d]Means within a row with no common superscript are significantly different ($p < .05$).

analysed by one-way ANOVA using GLM of SAS (2001) with diet as the main effects. Duncan's multiple range tests were used to compare means ($p < .05$). A repeated measurement analysis was used to compare breeder groups for their performance over time (age), as well as their response patterns over age. Repeated measures ANOVA was generally used when independent samples were taken on the same animals at different times (for example, body weight, egg production and egg mass) were analysed by repeated measurements procedure using mstatc software (de Wreede, Fiocco, & Putter, 2011). Diversity between treatments was considered using Tukey comparisons.

TABLE 3 Effect of n-6/n-3 fatty acids ratio on female and male broiler breeder body weight (kg)

Age	n-6/n-3 ratio 4	6	8	16	MSE	*p*-Value
Females						
30	3.56	3.49	3.53	3.50	0.016	.39
31	3.66	3.63	3.57	3.63	0.017	.36
32	3.69	3.73	3.63	3.70	0.027	.70
33	3.64	3.61	3.60	3.61	0.025	.13
34	3.71	3.70	3.64	3.62	0.027	.23
35	3.74	3.73	3.69	3.65	0.032	.47
Males						
30	3.80	3.74	3.72	4.01	0.061	.12
31	3.83	3.60	3.75	4.00	0.057	.08
32	3.87	3.75	3.75	3.95	0.056	.54
33	3.88	3.67	3.85	3.91	0.059	.51
34	3.90	3.77	3.76	3.98	0.054	.42
35	3.92	3.76	3.79	3.94	0.063	.34

[a–d]Means within a row with no common superscript are significantly different ($p < .05$).

3 | RESULTS

3.1 | Production and hatchability

Repeated measurement statistical analysis was used to evaluate the effect of n6/n3 FA ratios effects on male and female broiler breeder body weights during 30–35 weeks. It can be seen from the data in Table 3 that no significant differences were observed between dietary n-6/n-3 FA ratios on the females and males body weight from 30 to 35 weeks of age. The first set of analysis examined the impact of n6/n3 FA ratios on production performance of broiler breeders. As shown in Table 4, there were no significant differences between dietary n-6/n-3 FA ratios on egg weight (g) and egg mass (g/bird per day) during the 30–32 weeks while egg mass of 33 and 35 weeks was increased by dietary n-6/n-3 FA ratio of 16:1. Egg production of n-6/n-3 FA ratio of 4:1 was significantly lower than those of the rest during the 33 and 35 weeks (Table 5). Interestingly, the hens subjected to diet containing n-6/n-3 FA ratio of 8:1 and 16:1 had lower egg yolk and higher egg white percentage as compared to those received 4:1 and 6:1 n-6/n-3 FA ratio.

Effect of dietary n-6/n-3 FA ratios on incubation parameters of broiler breeders is presented in Tables 6. It is apparent that there were no significant differences between dietary n-6/n-3 FA ratios on general incubation traits such as eggs fertility, hatchability, kinds of mortality, embryonic mortality in the first, second and third week of incubation, male and female per cent and chick to egg percentage. The results of experiments 1 and 2 were indicated that n-6/n-3 FA ratios did not affect eggs hatchability, embryonic mortality and male's semen quality. The next question asked is about the effects

TABLE 5 Effect of n-6/n-3 ratio on broiler breeder egg production and egg albumen and yolk percentage

	n-6/n-3 ratio					
	4	6	8	16	MSE	*p*-Value
Age						
30	86.57	87.28	87.143	87.43	0.902	.99
31	91.00	91.86	91.143	92.86	0.616	.73
32	89.86	91.44	91.00	90.28	0.683	.87
33	86.00[b]	92.00[a]	91.14[a]	92.71[a]	0.884	<.05
34	87.00	88.57	87.14	89.57	0.701	.54
35	81.75[b]	84.25[b]	86.25[ab]	91.25[a]	0.154	<.05
Egg parts (%)						
Yolk	25.26[b]	24.57[b]	27.14[a]	26.45[ab]	1.16	<.05
White	67.71[a]	68.00[a]	65.40[b]	66.16[b]	1.14	<.05

[a–d]Means within a row with no common superscript are significantly different ($p < .05$).

TABLE 6 Effect of n-6/n-3 fatty acids ratio on general incubation traits (% of settable eggs)

	n-6/n-3 ratio					
	4	6	8	16	MSE	*p*-Value
Parameter						
Fertility	99.30	98.00	98.33	99.33	0.36	.48
Hatchability	90.79	91.19	87.77	90.29	1.12	.66
Early embryonic mortality	3.38	2.04	4.10	4.71	0.54	.35
Mid-embryonic mortality	2.74	2.72	4.39	2.67	0.48	.54
Late embryonic mortality	2.39	2.04	2.07	1.66	0.46	.96
Kind of mortality during incubation						
Membrane	1.00	0.33	2.00	1.67	0.67	.33
Ring	1.01	1.35	2.67	3.00	0.68	.36
Eye	1.68	3.71	2.00	0.67	0.81	.11
Feathered	0.33[b]	0.68[b]	2.33[a]	1.00[ab]	0.47	.04
Turned	0.34	0.00	0.68	1.34	0.55	.39
Beak to air sac	0.67	0.35	0.68	0.69	0.48	.95
Second grade hatched chick	0.35	0.67	1.67	0.68	0.56	.41
Sum	6.38	7.09	12.00	9.00	1.88	.09

[a–d]Means within a row with no common superscript are significantly different ($p < .05$).

of n-6/n-3 FA ratios on male's semen qualitative and quantitative features such as testis weight, semen volume, sperm motility score and live, death and total spermatozoa percentage. Using fish oil and increasing n-6/n-3 FA ratio from 4 to 16 did not affect the semen traits (data not shown).

3.2 | Fatty acid profile

The effects of dietary treatments on fatty acid profiles of egg yolk, male's liver, testis and semen are presented in Tables 7–9. Dietary n-6/n-3 FA ratios did not have significant effect on total lipids of these tissues (data not shown). In the subject of fatty acid profile alteration, Table 7 compares the effects of n-6/n- FA ratios on yolk fatty acid profile. A positive correlation was found between dietary n-6/n- FA ratio and yolk n-3 FA so that n-6/n-3 FA ratio of 4:1 increased DHA, EPA, LNA and total n-3 FAs in egg yolk and decreased n-6/n-3 FA ratio of yolk lipid whereas no increase or decrease in n-6 FAs was detected ($p < .05$).

The effects of dietary n-6/n-3 FA ratios on males' liver, testis and semen fatty acid profile were tabulated in Tables 8 and 9. As dietary n-3/n-6 were decreased from 16 to 4, the EPA, DHA and total n-3 of males liver were increased while total n-6, ARA, total PUFA and n-6/n-3

TABLE 7 Effect of n-6/n-3 fatty acids ratio on egg yolk fatty acid profile (% of total fat)

	n-6/n-3 ratio					
Egg yolk	4	6	8	16	MSE	*p*-Value
C18:1n-9	8.16	8.56	8.31	8.95	0.526	<.05
C18:2n-6	15.1^{c}	15.66^{b}	16.3^{a}	16.55^{a}	0.231	<.05
C18:3n-3	1.45^{a}	0.75^{bc}	0.7^{c}	0.97^{b}	0.084	<.05
C20:4n-6	0.85^{c}	1.33^{b}	1.81^{a}	1.85^{a}	0.007	<.05
C20:5n-3	0.79^{a}	0.64^{a}	0.39^{b}	0.14^{c}	0.14	<.05
C22:5n-3	0.06^{ab}	0.04^{b}	0.13^{a}	0.12^{a}	0.02	<.05
C22:6n-3	2.45^{a}	2.18^{b}	1.57^{c}	1.16^{d}	0.100	<.05
∑PUFA	20.20	20.10	19.90	19.80	0.080	.21
∑ n-6	15.95^{b}	16.99^{ab}	18.11^{a}	18.40^{a}	0.009	.37
∑ n-3	4.75^{a}	3.61^{b}	2.79^{c}	2.4^{c}	0.073	<.05
n-6/n-3	3.75^{c}	3.72^{c}	6.49^{cb}	7.67^{a}	0.023	<.05

$^{a-d}$Means within a row with no common superscript are significantly different ($p < .05$).

TABLE 8 Effect of n-6/n-3 fatty acids ratio on fatty acid profile of males liver (% of total fat)

	n-6/n-3 ratio					
Fatty acid	4	6	8	16	MSE	*p*-Value
C18:2n-6	16.64	17.53	17.32	18.34	1.42	.89
C18:3n-3	0.26	0.26	0.14	0.44	0.11	.51
C20:4n-6	4.42^{c}	6.58^{b}	7.45^{ab}	8.63^{a}	0.37	<.05
C20:5n-3	0.54^{a}	0.32^{b}	0.32^{b}	0.29^{b}	0.067	<.05
C22:5n-3	0.17	0.18	0.17	0.15	0.611	.54
C22:6 n-3	3.23^{a}	3.02^{a}	2.16^{ab}	1.96^{b}	0.285	<.05
∑ PUFA	25.26^{b}	27.89^{a}	27.56^{a}	29.81^{a}	1.13	<.05
∑ n-6	21.06^{b}	24.11^{ab}	24.77^{ab}	26.97^{a}	0.86	<.05
∑ n-3	3.98^{a}	3.78^{a}	3.01^{b}	2.84^{b}	0.057	<.05
n-6/n-3	5.29^{b}	6.38^{b}	8.23^{a}	9.50^{a}	0.49	<.05

$^{a-d}$Means within a row with no common superscript are significantly different (P < 0.05).

FA ratio were decreased ($p < .05$). Interestingly, for those subjects with n-6/n-3 FA ratio of 4:1, addition of fish oil significantly decreased DTA and n-6/n-3 FA ratio of semen and testis FAs whereas DHA and total n-3 FAs were increased as compared to other groups. The n-6/n-3 FA ratio of semen and testis was significantly influenced by dietary n-6/n-3 FA ratios although the semen qualitative and quantitative features were not affected by dietary treatments ($p > .05$).

3.3 | Fatty acid changes during incubation

The effect of n-6/n-3 FA ratios on embryo and day-old chickens' liver and brain fatty acid changes during incubation were displayed in Tables 10 and 11. The results showed that dietary n-6/n-3 FA ratios significantly influenced embryo and chicken liver and brain fatty acid profile ($p < .05$). As dietary n-6/n-3 FA ratios decreased from 16 to 4, embryo brain and liver DHA, total n-3 was significantly increased but LNA, total n-6 and n-6/n-3 FA ratio were decreased. Comparing the fatty acid profile of embryo and day-old chicks showed that during the last week of incubation in all of the dietary treatments, EPA, DHA and total n-3 FAs of chick liver elevated and LA, ARA, total n-6 and n-6/n-3 FA ratio were decreased (Table 10). Comparing the fatty acid profile of embryo and day-old chicks showed that during the last week of incubation in all of the dietary treatments, significant increase was observed in LA, LNA, ARA, DHA, total PUFA and total n-3 FAs of chick brain increased and EPA and n-6/n-3 FA ratio were decreased (Table 11). The n-6/n-3 FA ratios in the brain of embryo and newly hatched chicks were lower than those measured in the liver of embryos. These results indicated that liver PUFAs were decreased and brain PUFAs were increased during last week of incubation.

4 | DISCUSSION

4.1 | Production and hatchability

The current study found that body weight of breeders was not affected by dietary n-6/n-3 FA ratio. These results are in agreement with recent studies indicating that feeding diets with different

TABLE 9 Effect of n-6/n-3 fatty acids ratio on fatty acid profile of testis and semen (% of total fat)

	n-6/n-3 ratio					
	4	6	8	16	MSE	*p*-Value
Testis fatty acid						
C18:2n-6	6.83	5.1	5.25	5.94	0.43	.11
C18:3n-3	1.27	1.24	1.15	0.95	0.09	.43
C20:4n-6	3.89	4.17	3.97	3.52	0.44	.19
C20:5n-3	0.48	0.43	0.46	0.41	0.08	.64
C22:4n-6	10.61^{c}	12.92^{b}	13.68^{a}	14.28^{a}	0.37	<.05
C22:5n-6	0.14	0.12	0.13	0.14	0.08	.35
C22:6 n-3	3.82^{a}	3.46^{a}	2.85^{b}	1.7^{c}	0.62	<.05
∑ PUFA	27.04	27.44	27.49	26.93	0.42	.40
∑ n-6	21.47	22.31	23.03	23.88	0.49	.12
∑ n-3	5.57^{a}	5.13^{a}	4.46^{a}	3.05^{b}	0.47	<.05
n-6/n-3	3.85^{c}	4.35^{b}	5.16^{b}	7.83^{a}	0.07	<.05
Semen fatty acid						
C18:2n-6	12.32	12.55	12.72	12.85	0.063	.23
C18:3n-3	nd	nd	nd	nd	nd	.87
C20:4n-6	8.06	8.15	8.55	9.26	0.09	.09
C22:4n-6	7.05^{c}	8.14^{b}	9.86^{a}	10.80^{a}	0.350	<.05
C22:5n-6	1.18	1.26	1.12	1.04	0.071	<.05
C22:6n-3	3.16^{a}	2.50^{b}	2.33^{b}	1.56^{c}	0.62	<.05
∑ PUFA	31.97	32.60	34.58	35.51	1.97	.09
∑ n-6	28.61	30.10	32.25	33.95	1.57	.08
∑ n-3	3.16^{a}	2.50^{ab}	2.33^{ab}	1.56^{b}	0.54	<.05
n-6/n-3	9.12^{c}	12.04^{b}	13.84^{ab}	21.76^{a}	0.62	<.05

$^{a-d}$Means within a row with no common superscript are significantly different ($p < .05$).
nd = not detected.

n-6/n-3 FA ratio from 1:1 to 1:11 to broiler chickens (Mi, Me, Ma, & Ah, 2014) and laying hens diets containing up to 3% menhaden oil for 4 week (Trebunova et al., 2007). Broiler breeders have manually and controlled feed allocation and possible explanation for broiler breeder performance is that because the females were received approximately constant amount of PUFA, so their performance were not affected by dietary treatments.

Increased supply of PUFA interferes with the synthesis of very low-density lipoprotein (VLDL), and it is precursor for egg yolk lipids, so the first explain for broiler performance in our study may be the mechanism that resulted in eggs with smaller yolks, smaller eggs and consequently led to smaller-sized embryos (Pappas, Acamovic, Sparks, Surai, & McDevitt, 2006), and it was in agreement with others who include fish oil to broiler breeder diet to obtain different EPA/DHA ratios of 1/1 (EPA = DHA), 1/2 (DHA) or 2/1 (EPA) and reported that all n-3 treatments led to lower egg weight, (Koppenol, Delezie et al., 2014).

A strong relationship between egg weight and yolk weight has been reported in the literatures, and it was mentioned that the lipids contained the biggest part of the yolk. Yolk weight of the females was fed diets with n-6/n-3 FA ratios of 1:4 and 1:6 were decreased and their eggs albumen weight was increased and consequently influenced egg mass while in agreement with our results, by inclusion of fish oil to broiler breeder diet to obtain different EPA/DHA ratios of 1/1 (EPA = DHA), 1/2 (DHA) or 2/1 (EPA), researchers reported that all n-3 treatments led to lower absolute and relative yolk weight of eggs while absolute and proportional albumen weight and absolute and proportional shell weight did not differ between treatment groups (Koppenol, Delezie et al., 2014). There is reduction in lipid synthesis in the liver of females were fed fish oil diet. Acyl-co-A synthetase is the rate-controlling enzyme in fatty acid biosynthesis in liver, and its activity was decreased by dietary n-3 FAs. A possible explanation for our results might be that long-chain n-3 FAs consumption causes a decrease in serum triglycerides in females; hence, a decrease in amount of lipids available for yolk formation and in some cases the lower egg weight may relate to the fact that less feed is being consumed (Elswyk, Dawson, & Sams, 1995). Furthermore, n-3 FAs may influence circulating estradiol, affecting liver lipid metabolism.

Studies have shown that alteration of yolk fatty acid composition can affect fertility, hatchability and embryonic survival of chicks. Regarding the plausible experimental limitation, artificial insemination

TABLE 10 Effect of n-6/n-3 PUFA ratios on liver fatty acid changes during last week of incubation (% of total fat)

	n-6/n-3 ratio					
	4	6	8	16	MSE	*p*-Value
Embryo liver fatty acid						
C18:2n-6	13.85^{a}	13.64^{b}	13.44^{b}	14.03^{a}	0.328	<.05
C18:3n-3	0.36	0.24	0.24	0.19	0.081	.12
C20:4n-6	6.28^{b}	6.99^{a}	6.88^{ab}	7.02^{a}	0.117	<.05
C20:5n-3	0.16^{a}	0.18^{a}	0.15^{a}	0.10^{b}	0.009	<.05
C22:6n-3	2.36^{a}	1.28^{b}	0.83^{c}	0.14^{d}	0.244	<.05
∑ PUFA	23.01^{a}	22.33^{b}	21.54^{b}	21.48^{b}	1.120	<.05
∑ n-6	20.13^{b}	20.63^{b}	20.32^{b}	21.05^{a}	0.045	<.05
∑ n-3	2.88^{a}	1.70^{b}	1.22^{c}	0.43^{d}	0.003	<.05
n-6:n-3	6.99^{c}	12.14^{c}	16.66^{b}	48.95^{a}	0.340	<.05
Chicken liver fatty acid						
C18:2n-6	13.80^{a}	12.50^{c}	13.00^{b}	13.17^{b}	0.144	<.05
C18:3n-3	0.75^{a}	0.67^{ab}	0.58^{b}	0.75^{a}	0.025	.19
C20:4n-6	3.23^{b}	3.54^{ab}	3.70^{a}	3.33^{b}	0.065	.09
C20:5n-3	0.06^{b}	0.05^{c}	0.06^{b}	0.08^{a}	0.003	<.05
C22:6n-3	2.59^{a}	2.38^{b}	1.15^{c}	0.97^{c}	0.045	<.05
∑ PUFA	20.43^{a}	19.14^{b}	18.49^{b}	18.30^{b}	1.121	<.05
∑ n-6	16.03^{b}	16.04^{b}	16.70^{a}	16.85^{a}	0.014	<.05
∑ n-3	3.40^{a}	3.10^{a}	1.79^{b}	1.81^{b}	0.006	<.05
n-6/n-3	5.01^{b}	5.17^{b}	9.33^{a}	9.17^{a}	0.090	<.05

$^{a-d}$Means within a row with no common superscript are significantly different ($P < .05$).

TABLE 11 Effect of n-6/n-3 PUFA ratios on brain fatty acid changes during last week of incubation (% of total fat)

	n-6/n-3 ratio					
	4	6	8	16	MSE	*p*-Value
Embryo brain fatty acid						
C18:2n-6	4.96^{c}	6.02^{b}	6.88^{b}	8.86^{a}	0.433	<.05
C18:3n-3	0.46^{a}	0.36^{a}	0.10^{b}	0.10^{b}	0.045	<.05
C20:4n-6	5.02	5.32	5.77	6.40	0.57	.36
C20:5n-3	0.49	0.44	0.42	0.33	0.17	.68
C22:6n-3	2.69^{a}	2.56^{a}	1.31^{b}	1.28^{b}	0.052	<.05
∑ PUFA	13.42	14.80	14.48	16.97	1.13	.14
∑ n-6	9.98^{c}	11.34^{c}	12.65^{b}	15.26^{a}	0.36	<.05
∑ n-3	3.64^{a}	3.26^{a}	1.83^{b}	1.71^{b}	0.54	<.05
n-6/n-3	2.74^{c}	3.48^{c}	6.91^{b}	8.92^{a}	0.024	<.05
Chicken brain fatty acid						
C18:2n-6	7.42	8.09	8.87	9.05	1.36	.15
C18:3n-3	0.46	0.43	0.35	0.34	0.09	.39
C20:4n-6	5.88^{b}	6.89^{b}	7.91^{a}	7.85^{a}	0.064	<.05
C20:5n-3	0.07	0.06	0.06	0.05	0.02	.11
C22:6n-3	2.99^{a}	2.52^{a}	1.53^{b}	1.35^{b}	0.82	<.05
∑ PUFA	16.81	17.99	18.72	18.64	1.32	.76
∑ n-6	13.30^{b}	14.98^{a}	16.78^{a}	16.90^{a}	0.42	<.05
∑ n-3	3.52^{a}	3.01^{a}	1.94^{b}	1.74^{b}	0.85	<.05
n-6/n-3	3.79^{b}	4.98^{b}	8.65^{a}	9.71^{a}	1.23	<.05

$^{a-d}$Means within a row with no common superscript are significantly different ($p < .05$).

was not practiced to provide the hatching eggs and they were obtained through the natural mating so it could not be associated with preferential mating because the rate of fertility and hatchability for all of the treatments was above 98% and 87% respectively (Table 6). In this regard, our results did not provide further support for the hypothesis that maternal n-6/n-3 FAs ratios influence hatchability and fertility and in addition increasing egg n-3 and n-6 PUFA content via maternal diet did not have a detrimental effect on the development of the embryo, which is desirable. These results are in accord with some studies (Bautista-Ortega, Goeger, & Cherian, 2009; Cherian, 2008) indicating that addition of fish oil as n-3 sources did not increase breeders hatchability and fertility and with others reported that adding DHA and EPA at a ratio of 1/1, 1/2 and 2/1 (or n6:n3 ratio of 11 to 5.4) had no effect on fertility and hatchability of female broiler breeder, early, mid and late embryonic mortality (Koppenol et al., 2015). A possible explanation for eggs hatchability and embryonic mortality might be related to oleic acid level of incubated eggs. Referring to Table 7, C18:1 fatty acid of laid eggs was not affected by different n-6/n-3 FA ratio and as previously reported, decreases in yolk C18:1 and total monounsaturated FAs with associated increases in saturated FAs attributed high embryonic chick mortality and high level of PUFA and the associated increase in free radicals generated or may simply reflect physical constraints arising from the smaller eggs laid by these hens (Pappas, Acamovic, Sparks et al., 2006).

No evidence of dietary n-6/n-3 FA ratios on embryonic mortality during third wk was detected. Earlier findings indicated that addition of PUFA to broiler breeder diet from 23 to 27 week of age increased embryo livability and hatchability (Pappas, Acamovic, Surai, & McDevitt, 2006). The amount of the added fish and soybean oils to the diets in our experiment were lower than 2%, so it could be concluded that to decrease the embryonic mortality, using low levels of n-3 and n-6 PUFA in the broiler breeder diets is applicable.

Surprisingly, semen characteristics did not affect by dietary treatments while fatty acid composition of semen and testis changed by dietary n-6/n-3 FA ratio of 4:1 and 6:1 in 39 week. These results seem to be consistent with other research which was found that using fish and corn oils did not influence semen volume, sperm concentration and motility of turkey and proportion of viable spermatozoa was increased in the ejaculations collected from fish oil diet fed birds compared to the corn oil diet fed birds (Zaniboni, Rizzi, & Cerolini, 2006). In addition, addition of five different oil sources and five n-6/n-3 FA ratios (sunflower, 126.2; soybean, 13.6; canola, 6.62; fish/soybean, 8.38 and linseed, 0.88) in white leghorn roosters did not influence the semen volume and spermatozoa motility (Zanini et al., 2003). Improvements in fertility were attributed to a reduction in the n-6/n-3 FAs ratio in spermatozoa membrane, which may alter the physical properties of the membrane or its resistance to peroxidation damage.

4.2 | Fatty acid profile

Several studies have shown that proportion of motile spermatozoa in human and chicken semen is positively correlated with DHA (Cerolini, Zaniboni, Maldjian, & Gliozzi, 2006). Some researchers found that n-3 FAs increased spermatozoa motility (Kelso, Cerolini, Noble, Sparks, & Speake, 1997). In contrast, other studies reported no effect of DHA or fish oil supplementation on spermatozoa motility in animals (Blesbois, Douard, Germain, Boniface, & Pellet, 2004; Surai et al., 2000). There is a negative correlation between the level of saturated FAs (C16:0 and C18:0) and sperm motility (Cerolini et al., 1997). It is unclear that how DHA may be involved in regulating sperm motility but it is most likely that the biophysical properties of DHA contribute to the membrane fluidity and flexibility demanded by the motility of the tail (Connor, 1988). Because higher n-6/n-3 FA ratios resulted in higher saturated FAs in spermatozoa and reduced cell membrane flexibility and addition of fish oil lowers n6 fatty acid deposition in the spermatozoa, and probably influencing cell membrane flexibility (Zanini et al., 2003).

Proportion of DTA in spermatozoa phospholipid positively correlated with the proportion of motile spermatozoa, linear movement and overall fertility. There is a resistance to n-3 incorporation in chicken spermatozoa, whereas, the n-6/n-3 FA ratio in broiler breeder spermatozoa reflected the ratio of these FAs in our diets dramatically. As a consequence, the n-6/n-3 FA ratio of testis and spermatozoa was significantly increased from 3.85 to 7.83 and from 9.12 to 21.76 in the males fed n-6/n-3 FA ratio of 4:1 compared to the males fed n-6/n-3 FA ratio of 16:1 respectively. These results are in agreement with (Blesbois, Lessire, Grasseau, Hallouis, & Hermier, 1997; Zaniboni et al., 2006; Zanini et al., 2003). Another possible explanation for discordant results reported on the effect of n-3 feeding on spermatozoa motility might be related to the different used techniques.

It is also known that added fat to the female's diet can influence the yolk fatty acid profile of fresh eggs and has little or no significant effect on the total lipid content of the egg (Cherian & Sim, 1991). In the present study, the egg yolk and tissue total fat content remained unchanged when the females and males were fed the dietary n-6/n-3 diets (data not shown) and our results are in accord with recent studies indicating that egg yolk n-6 and n-3 FA composition did not affect total lipid content of heart, lung, liver and brain of newly hatched chicks (Bautista-Ortega et al., 2009).

Because the fish oil that used in our experiment was DHA-rich, the fatty acid analysis showed that order of n-3 PUFA in egg yolk, semen and testis was DHA < LNA < EPA and breeder females fed n-6/n-3 FA ratio of 4:1 laid eggs with increased DHA content whereas those fed n-6/n-3 FA ratio of 16:1 laid eggs with increased content of LA fatty acid. Several reports have shown that as compared to n-6-rich oils, addition of fish oil increased n-3 PUFA in the yolk of eggs (Ajuyah et al., 2003; Cherian, 2008). Similar to our results, by inclusion of fish oil to broiler breeder diet to obtain different EPA/DHA ratios of 1/1 (EPA = DHA), 1/2 (DHA) or 2/1 (EPA), researchers reported that all n-3-enriched diets resulted in a lower n-6 and higher n-3 PUFA concentrations in the yolk, resulting in a much lower yolk n-6:n-3 ratio and yolk EPA concentrations highly resembled dietary EPA supplementation (Koppenol, Delezie et al., 2014). In the present study, a significant increase in the n-3 PUFA proportion in the

studied tissues was always associated with a significant reduction in the n-6 PUFA proportion. DHA proportion in eggs laid of females fed diet with n-6/n-3 FA ratio of 16:1 and 8:1 was lower than other ratios, and it is probably due to the fact that conversion of ALA into EPA is limited, and further transformation to DHA is even lower. This is caused by competition for the coxylooxygenase enzymes involved. Although n-3 FAs are the preferred substrates for Δ-6-desaturase, the enzyme also acts on LNA and it is suggested that DHA might be preferentially incorporated into membranes in comparison with EPA (Cherian, 2008). However, the conversion efficiency is affected by several factors. First of all, the presence of high amounts of n-6 PUFA in the diet increases the competition for the desaturase enzymes, causing a decrease in n-3 conversion efficiency. As a consequence, the n-6/n-3 FA ratio of the diet is one of the major influencing factors. Secondly, researchers estimated that hens' age and strain have an effect on the efficiency of n-3 elongation and desaturation (Fredriksson, Elwinger, & Pickova, 2006). It has been postulated that older females have a larger liver, allowing a more effective conversion of ALA into DHA.

4.3 | Fatty acid changes during incubation

During the incubation process, notable changes occur in yolk fatty acid composition. Tissue maturation in the embryo depends on the acquisition of highly characteristic fatty acid profiles by the cell membrane phospholipids (Innis, 2008). The importance of PUFA in avian development is illustrated by the recovery of a high proportion of the yolk's DHA and ARA in the tissue lipids of the newly hatched chick in comparison with the recovery of all the other yolk FAs (Fredriksson et al., 2006).

The loss of lipid from the yolk increases substantially between d 15 and 21 of incubation and is characterized by high secretion rates of corticosterone, a known promoter of lipid metabolism in the newly hatched chick (Latour et al., 1996; Speake, Decrock, Surai, Wood, & Groscolas, 2003). The high levels of long-chain n-3 and n-6 FAs, especially DHA and ARA, reflect a unique role of embryonic liver in supplying long-chain n-3 and n-6 FAs to the developing chick. In the present experiment, proportion of DHA in the brain and liver decreases dramatically during embryonic development and it is suggesting that species of lipids like phospholipids which contain this fatty acid are being utilized preferentially.

During the last week of incubation, the concentration of ARA in embryo liver was dramatically decreased (3.7% vs 7.0%) and by contrast, it was increased in the brain (6.4% vs 7.9%) lipids. Like to our results, inclusion fish oil to broiler breeder diet to obtain different EPA/DHA ratios of 1/1 (EPA = DHA), 1/2 (DHA) or 2/1 (EPA) led to lower n-6/n-3 ratio in the residual yolk, liver at d 1 when breeders were fed an n-3 supplemented diet (EPA = DHA, DHA and EPA) compared with control-fed breeders (Koppenol, Buyse et al., 2014). Maternal supplementation of n-3 FA, EPA and DHA was incorporated in the yolk and transferred via the residual yolk to become available for the developing embryo and resulted in elevated EPA and DHA concentrations in the liver of the offspring at hatch. This altered the n-6/n-3 ratio in the developing embryo, favouring the accumulation of EPA and DHA in tissue, because conversion from LNA to EPA and DHA is more effective when competing amounts of n-6 are lower and the maternal transition of EPA and DHA to the liver is rather important because these FAs are absent in the post-hatch diet (Goyens, Spilker, Zock, Katan, & Mensink, 2006).

Brain phospholipids also display relatively high levels of ARA which performs various regulatory roles in brain development and neurotransmission (Innis, 2008). The fact that lipoprotein lipase is not expressed in the brain of the chick embryo precludes the possibility of direct uptake by this tissue of FAs derived from locally hydrolysed plasma lipoproteins (Speake et al., 2003). Docosahexaenoic acid has been shown to be mainly transported as a component of plasma triacylglycerol in the chicken embryo, whereas ARA is largely presented in plasma phospholipid. Our results indicated a pure mobilization of n-3 and n-6 fatty acid from embryo liver and brain towards embryo and day-old chick brain during last week of incubation.

In conclusion, under the conditions of this study, n-6/n-3 FA ratios in low fish oil added (1.5%) diets did not affect male and female body weight, egg weight, egg mass, hatchability and fertility, embryonic mortality and semen parameters whereas yolk and tissue n-6 FAs were decreased, and yolk and tissue fatty acid n-3 were increased by applying n-6/n-3 FA ratios of 4:1 and 6:1. The dietary n-6/n-3 FA ratios of 4:1 and 6:1 led to decrease n-6/n-3 FAs of egg yolk and n-3 FAs via the residual yolk to become available for the developing embryo and increased mobilization of n-3 fatty acid from embryo liver and brain towards day-old chick brain and liver during last week of incubation. It is suggested that amount of n-3 FAs source (fish oil) and n-6 FAs source (soybean oil) in broiler breeder diet may be more important than n-6/n-3 FAs ratios and it is necessary to supplement higher levels of these oils with mentioned n-6/n-3 FAs ratios to consider the reproductive and performance characteristics of broiler breeders.

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