Journal of Animal and Veterinary Advances 13 (19): 1120-1125, 2014
ISSN: 1680-5593

The Effects of Vitamin A and E Supplementation into the Female Broodstock Diets of Rainbow Trout (*Oncorhyinchus mykiss*, W.) on the Fecundity and Egg Quality Parameters

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Abstract: This study investigated the effects of different levels of vitamins A and E inclusion in diets on fecundity, egg fertilization and hatching rate in female brood fish of rainbow trout (*Oncorhynchus mykiss*, W.). In this respects 7 diets including control diets (no vitamin A and E) containing two fixed amount of vitamin A with increasing doses of vitamin E (50, 100 ve 150 mg kg^{-1} in diets containing fixed amount of vitamin A). Total number of 350 female and 70 male brood fish that had average mean body weight of 2-3 kg and 3+ aged stock were employed in this investigation. A total of 50 female and 10 male brood fish was stocked into each experimental concrete ponds. Investigation lasted in 70 days and randomly selected 15 female brood fish were spawned for eggs at the end of the study. There were significant ($p<0.05$) differences in terms of spawning performance among fish fed with different dietary treatments. However, it was evident that vitamin levels in diets did not affect the egg diameters but mean egg weights were significantly different in fish fed diets containing increased amount of vitamin E ($p<0.05$). Fertilized eggs were placed into egg trays and inspected throughout the investigation. It was also evident that diets containing increasing amount of vitamin E had significant ($p<0.05$) effect on the hatching rate of fertilized eggs and the survival of larvae during the incubation period. Findings of this study implied that it might be necessary to include sufficient amount of vitamin A and E in diets for rainbow trout for a successful fingerling production.

Key words: Rainbow trout, vitamin A, vitamin E, brood stock, hatching rate, fecundity, yolk sac larvae

INTRODUCTION

Early development in fish is mostly dependent on the essential nutrients available in the egg. However, the nutrient composition of eggs is predominantly determined by the maternal diet prior to and during oogenesis (Lavens *et al*., 1999). Of all the essential nutrients required for successful reproductive performance in fish, dietary fatty acids are one of the strongest determinants of this process as it was demonstrated that enhanced reproductive fitness was achieved by increasing dietary lipids (Izquierdo *et al*., 2001). Fish eggs are known to contain relatively high concentrations of a particular class of lipids which is comprised of mostly by MUFA and PUFA's. PUFA are essential to normal development of fish and are incorporated into cellular and subcellular membranes, helping to maintain the fluidity of those membranes. They are also the precursors of many biologically active compounds like eicosonoids.

The primary non-enzymatic antioxidants in fish eggs are vitamins E and A as well as provitamin A that is known as carotenoids. Content of these lipid-soluble vitamins in fish eggs has been suggested to permit larger initial egg size which in turn has been correlated with larger larval size and better early survival (Lavens *et al*., 1999). Vitamin E is by far the most important physiologically bioactive antioxidant in most vertebrates (Packer, 1991). In fact vitamin E plays an important role in protecting eggs during early development. Vitamin A and carotenoids are however, less bioactive antioxidants compared to vitamin E.

Salmonid fish generally require 30-60 mg of vitamin E per kg of dry diet (King, 1985). Dietary concentrations of selenium, sulphur containing amino acids, other antioxidants, iron, vitamin A and C, choline and quinines can also affect the rate of vitamin E supplementation required in the diet of fishes (Draper, 1980). However, minimum dietary concentrations of vitamin E are to be regarded as a tenuous guideline because the level of vitamin E required is highly dependent on the amount of fatty acids and PUFA lipids in the diet and the degree of their unsaturation (Baker and Davies, 1996). In the case of very high concentrations of PUFA and insufficient vitamin E content, yolk sac hypertrophy and decreased survival can result, presumably from oxidative stress type lesions. Fernandez-Palacios *et al*. (1998) showed that hypertrophy and larval mortality could be reduced in gilthead sea bream (Sparusauratus) fed high PUFA

containing diets that were supplemented with vitamin E. Watanabe *et al*. (1985) recommended at least 100 mg kg^{-1} of α-tocopherol should be supplemented to feed, the performance of their fish was adequate because they may have been able to mobilise stores of vitamin E to the gonads that were present in peripheral tissues before the feeding trial ever began. The rate of deposition of vitamin E to eggs was greatest between October and December in fish fed the vitamin E sufficient diet. Furthermore, if vitamin E was removed from the diet after October, it had little effect on egg content of the vitamin. This means that vitamin E in the diet is most important for rainbow trout at least 3 months prior to spawning. Lie *et al*. (1994) also postulated that remobilization of vitamin E stores (from July to November) is only possible when Atlantic salmon (Salmosalar) reaches to gonadal maturation. Furthermore, these authors found that female fish fed diets containing high levels of vitamin E (60 vs. 270 mg kg^{-1} feed) had higher vitamin E content in their eggs. Several studies have examined the relationship between vitamin E concentrations in fish eggs and larvae and measures of reproductive success (King, 1985; Ciarcia *et al*., 2000).

Vitamin A is present in fish in many forms including the free alcohol form retinol (vitamin A1) and the closely related dehydroretinol (vitamin A2) form which differs from vitamin A1 by only one double bond. It has been recommended that carotenoids, primarily astaxanthin and canthaxanthin be included in the feed of broodstock at a level of 10 mg kg^{-1} dry weight. Toxic levels of vitamin A in the diet range from 40 IU g^{-1} for tilapia (*Oreochromis niloticus*) to 2000 IU g^{-1} for fingerling rainbow trout (*Oncorhynchus mykiss*) (Furuita *et al*., 2001). Vitamin A is required for growth, reproduction, maintenance of epithelial tissues and embryonic development of fish. It is present in many forms with at least some of those forms being potent morphogens in the developing embryo. Like vitamin E, vitamin A and carotenoids cannot be synthesised by fish and the amount of each deposited into the egg is a critical factor in determining reproductive fitness. Fish cannot synthesise either of the vitamins, so the maternal dietary content of each prior to oogenesis is an important determinant of reproductive fitness.

This study aimed at demonstrating the effect of adding 3 different levels of vitamin E to the diets containing 2 levels of vitamin A on the spawning performance and egg quality parameters like egg diameter, fertilization rate and hatching rate in female rainbow trout brood stock.

MATERIALS AND METHODS

Fish and diets: Adult 350 female and 70 male rainbow trout (*Oncorhynchus mykiss*, W.), varying between 2000 and 3000 g mean live weight and 3+ years of age were used in the study. There were 50 females and 10 males in each pond (n = 60 brood stock per treatment).

The diets consisted of fish meal, bone-meat meal, soybean meal, wheat and corn gluten, wheat middling, oil and some feed additives like mineral premix, BHT, DL-methionine and vitamin premix containing several doses of vitamin A and E (C: control feed no containg vit. A and E; D1: 0 IU vit. A, 50 mg vit. E; D2: 15000 IU, 50 mg vit. E; D3: 0 IU vit. A, 100 mg vit. E; D4; 15000 IU vit. A, 100 mg vit. E; D5: 0 IU vit. A, 150 mg vit. E; D6: 15000 IU, 150 mg vit. E (Table 1). The whole vitamins using in the premixes was supplied by BASF Co. and the premixes was prepared in the laboratory. Diets were prepared as isonitrogenous (47% CP) and isocaloric (13.32 MJ DE kg^{-1} feed (Table 2). The ingredients were ground to medium fine size (0.6 mm) before pelleted. Pellet size was 6 mm diameter and 8 mm in length. Table 2 shows proximate composition of the diets. The vitamin A and E content of the diets are also shown in Table 3.

Feeding trial: Experiment were performed in a commercial trout farm near Bozuyuk-Bilecik, Turkey. Fish were divided into seven groups. They were stocked in rectangular concrete ponds (dimension: 3×15×1 m) in October. Values of pH (D-51 Horiba), dissolved oxygen and water temperature (OM-51 Portable Dissolved Oxygen meter) measured periodically and detected as 7.52-7.67; 11.3-12.2; 7.8-8.6, respectively and water flow rate was 10 L sec^{-1} during the trial. The female brood stocks were observed by breeding behaviours and controlled by compressing of abdomen. The 15 females were selected for spawning in each experimental groups.

Brood stock fish were fed qualitative (40% CP and 11.5 MJ DE kg^{-1} feed) and quantitative (as 0.3-0.5% ratio of mean live weight 1 meal per day between pawning seasons) restricted feeding regime but fish were fed daily to apparent satiation with the diets containing different doses of vitamin A and E throughout the trial period (Table 1). The trial continued for 70 days and special feeding were ended before 3 days of spawning time.

The chemical compositions of feed ingredients and diets were measured according to AOAC Methods (Anonymous, 1995). Dietary vitamin A and E contents were conducted using the reversed phase HPLC procedures as described by Bieri *et al*. (1979).

Table 1: Composition of experimental fish diets (DM%)

	Diets						
Ingredients	C[a]	D1[b]	D2[c]	D3[b]	D4[c]	D5[b]	D6[c]
Fish meal	27.5	27.5	27.5	27.5	27.5	27.5	27.5
Bone-meat meal	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Corn gluten	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Soybean meal	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Wheat gluten	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Wheat middling	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Sunflower seed oil	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Vit. premix (vit. A and E free)	0.8	0.0	0.0	0.0	0.0	0.0	0.0
Test vit. premix[b, c]	0.0	0.8	0.8	0.8	0.8	0.8	0.8
Vit. A (IU) (Added into premix 2)	-	0.0	150000.0	0.0	150000.0	0.0	150000.0
Vit. E (g) (Added into premix 3)	-	4.5	4.5	11.0	11.0	17.0	17.0
Mineral complex[d]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	0.3	0.3	0.3	0.3	0.3	0.3	0.3
DL-methionine[e]	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Lignobond[f]	0.8	0.8	0.8	0.8	0.8	0.8	0.8
ButilHydroxiToluen[g]	0.4	0.4	0.4	0.4	0.4	0.4	0.4

[a]Vitamin premix 1 (mg or IU/kg of DM)of control feed: thiamine 40 mg, riboflavin 50 mg, pyridoxine 40 mg, calcium pantothenate 117 mg, nicotinic acid 200 mg, biotin 1 mg, folic acid 10 mg, cyanocobalamin 0.5 mg, choline chloride 2700 mg, inositol 600 mg, ascorbic acid 5000 mg, menadione 20 mg, cholecalciferol 2500 IU and α-cellulose was used as a carrier. All the vitamins was supplied by BASF Co. [b]Vitamin premix 2 (mg or IU/kg of DM)of experimental diets: thiamine 40 mg, riboflavin 50 mg, pyridoxine 40 mg, calcium pantothenate 117 mg, nicotinic acid 200 mg, biotin 1 mg, folic acid 10 mg, cyanocobalamin 0.5 mg, choline chloride 2700 mg, inositol 600 mg, ascorbic acid 5000 mg, menadione 20 mg, cholecalciferol 2000 IU, α tocopherylacetate 4.5, 11 and 17 g, respectively and α-cellulose was used as a carrier. All the vitamins was supplied by BASF Co. [c]Vitamin premix 3 (mg or IU/kg of DM) of experimental diets: thiamine 40 mg, riboflavin 50 mg, pyridoxine 40 mg, calcium pantothenate 117 mg, nicotinic acid 200 mg, biotin 1 mg, folic acid 10 mg, cyanocobalamin 0.5 mg, choline chloride 2700 mg, inositol 600 mg, ascorbic acid 5000 mg, menadione 20 mg, cholecalciferol 2000 IU, retinyl acetate 150000 IU, α tocopherylacetate 4.5, 11 and 17 g, respectively and α-cellulose was used as a carrier. All the vitamins was supplied by BASF Co. [d]Mineral premix (% of DM): calcium orthophosphate 1.80 g, calcium carbonate 5 g, ferrous sulphate 1.7 g, magnesium sulphate 1.8 g, potassium phosphate 3.0 g, sodium phosphate 1 g, aluminium sulphate 0.02 g, zinc sulphate 0.24 g, copper sulphate 0.20 g, manganese sulphate 0.08 g, potassium iodate 0.02 g. α-cellulose was used as carrier. [e]These additives were obtained by Sigma Co. Tfiis commercial product is used as pellet binder and [g]Antioxidant powder

Table 2: Proximate composition of experimental diets (g/100 g DM)

Ingredients	Test diets (g/100 g feed)
Dry matter	92.20±0.10
Crude protein	47.10±0.45
Crude oil	10.60±0.82
Crude fibre	2.80±0.64
Nitrogen free extract	22.77±1.23
Ash	8.93±0.37
Digestible energy (MJ kg^{-1})	13.32±0.12

Table 3: Vitamin A and E content of experimental diets

Diets	Vitamin A (IU kg^{-1} feed)	Vitamin E (mg kg^{-1} feed)
C	1350±0.04	15±0.01
D1	1350±0.05	50±0.04[b]
D2	2500±0.04[a]	50±0.03
D3	1350±0.03	100±0.02
D4	2500±0.04	100±0.03
D5	1350±0.04	150±0.03
D6	2500±0.02	150±0.02

[a, b]Minimum vitamin A and E requirements of rainbow trouts (Hardy, 2002)

Sample collection: The 15 females that had similar weight were selected for spawning behaviour in each experimental groups. Spawning procedure continued for 2 days. All female brood fish were spawned and fertilized with milt from two males of the same group by dry method. In this feeding trials, the weights of fish at spawning, total spawned eggs and average egg numbers were recorded to calculate absolute and relative fecundity (number of eggs/female and number of eggs/kg female, respectively). The fertilization rates (total number of developing eggs/total number of eggs) of each batch of eggs (per female) were assessed on the 2nd day after hatching. Hatching rate was calculated as the number of hatched larvae to total incubated eggs. The total number of eggs per individual fish was estimated by taking total weight of eggs and the mean of sub samples containing 100 eggs into account after leaching treatment. Egg diameters were measured on a profile projector using digital calliper after fertilization and swelling during 2 days. Viable and dead eggs were separated and counted after fertilization.

After weighing and determining of egg diameters, some water were added over eggs and eggs were kept 5 min until swelling. These eggs for each treatment group were placed into 5 egg trays (50×40 cm) and incubated at 12.1°C water temperature. The 30000 (6000×5) egg from each treatment group were stocked into 5 trays. These trays were observed and unfertilized or damaged eggs were taken out, counted and recorded throughout hatching period.

Statistical analyses: Among treatments "Randomized Block Design Model" was performed to observe the differences. All statistical analyses were performed using the statistical package, SPSS 17.0 for Windows. The significance of treatment effects on the different parameters measured were determined by one-way

ANOVA followed by Tukey's multiple comparison test where appropriate. Differences were reported as significant if $p<0.05$. Results presented in Table 4 are reported results as means±SD for female adults (n = 15) and egg diameter (n = 50), viable rate and hatching rate (n = 5 for every treatment groups).

RESULTS AND DISCUSSION

Spawning performance of rainbow trout broodstock: Females were mature in early January corresponding to week 10 of feeding trial, the number of females that began spawning during week 10 was similar compared to all dietary groups. However, females fed with diets D3-D6 displayed better absolute and relative fecundities than females fed with diets C, D1 and D2 (4450 vs. 3154 eggs/female and 1926 vs. 1495 eggs kg^{-1} female, respectively $p<0.05$ (Table 4).

Egg production: In each vitamin treatment group, mean egg weight remained affected by the level of vitamin A and E in the broodstock diets (Table 4). The best mean egg weights were obtained from the female brood fish fed diet D6 ($p<0.05$). Although, no significant differences in fertilization rate was found among vitamin treatments, a significant decline in survival from the eyed stage onwards was noticeable in the group fed with control diet (no added vitamin A and E) ($p<0.05$). The best survival rates was obtained on the group D6 but differences between the group D1-D5 was not significant. As for egg size, significant differences in mean rainbow trout yolk sac larvae were noticed at hatching (Table 4 and Fig. 1, 2).

The requirement for vitamin A and E in reproduction and embryonic development is well known in terrestrial animals (Zile, 1998; Clagett-Dame and DeLuca, 2002). But there have been few studies on vitamin A and E requirement for broodstock fish. In this study, absolute and relative fecundity were affected by the levels of vitamin A and E. Similarly, Areechon reported that the higher vitamin E (600 mg kg^{-1}) in the diet increased number of spawned fish in Nile tilapia. The results obtained for absolute and relative fecundity in this study were also in line with the study by Fernandez-Palacios *et al.* (1998) that found fish fed with increased amount of vitamin A had increased fecundity in

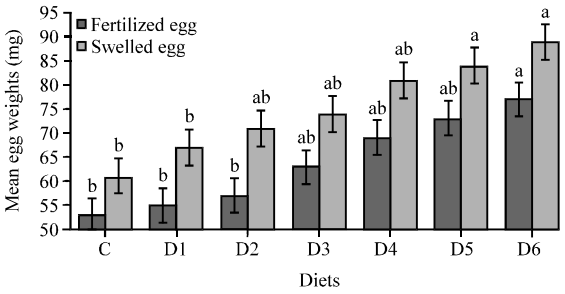

Fig. 1: Mean egg weights of experimental groups. Bars show the means and bars with different letters are different ($p<0.05$)

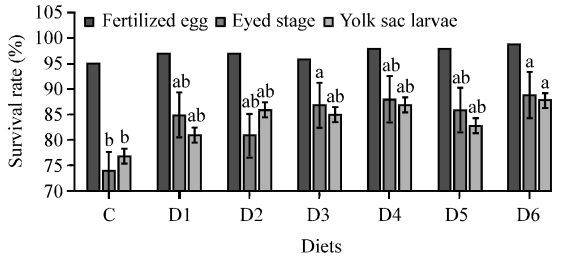

Fig. 2: Survival rates of eggs and larvae of experimental groups. Bars show the means and bars with different letters are different ($p<0.05$)

Table 4: Results of the experiment*

	Diets ($\bar{X}\pm S_{\bar{X}}$)						
Items	C	D1	D2	D3	D4	D5	D6
Trial period (days)	70	70	70	70	70	70	70
Number of inspected fish	15♀8♂	15♀8♂	15♀8♂	15♀8♂	15♀8♂	15♀8♂	15♀8♂
Live weight before spawning (kg) (n = 15)	2.56±0.4	2.74±0.2	2.81±0.4	2.95±0.3	2.91±0.3	2.66±0.3	2.87±0.2
Live weight after spawning (kg) (n = 15)	2.11±0.18	2.22±0.09	2.43±0.19	2.51±0.13	2.33±0.10	2.26±0.08	2.31±0.11
Absolute fecundity	3154 ± 956^{b}	3450 ± 537^{b}	4040 ± 141^{b}	$4370\pm495^{a,b}$	$4100\pm194^{a,b}$	$3984\pm314^{a,b}$	4450 ± 181^{a}
Relative fecundity	1495 ± 172^{b}	1542 ± 207^{b}	1662 ± 160^{b}	$1740\pm118^{a,b}$	$1761\pm174^{a,b}$	$1763\pm171^{a,b}$	1926 ± 133^{a}
Egg diameter (mm) (n = 50)	4.2±0.3	4.1±0.4	4.1±0.2	4.0±0.6	4.3±0.2	4.5 ±0.3	4.7±0.5
Spawn weight (kg)	0.17±0.1	0.19±0.1	0.24±0.1	0.28±0.1	0.29±0.1	0.29 ±0.1	0.35±0.1
Mean egg weight (mg)							
Fertilized	53 ± 11^{b}	55 ± 9^{b}	57 ± 9^{b}	$63\pm10^{a,b}$	$69\pm7^{a,b}$	$73\pm8^{a,b}$	77 ± 9^{a}
Swelled	61 ± 8^{b}	67 ± 9^{b}	$71\pm10^{a,b}$	$74\pm6^{a,b}$	$81\pm8^{a,b}$	84 ± 6^{a}	89 ± 10^{a}
Survival (%)							
Fertilized	95±3	97±3	97±1	96±2	98±2	98±2	99±1
Eyed stage	74 ± 4^{b}	$85\pm8^{a,b}$	$81\pm5^{a,b}$	87 ± 8^{a}	$88\pm11^{a,b}$	$86\pm9^{a,b}$	89 ± 7^{a}
Yolk sac larvae	77 ± 3^{b}	$81\pm13^{a,b}$	$86\pm9^{a,b}$	$85\pm7^{a,b}$	$87\pm9^{a,b}$	$83\pm9^{a,b}$	88 ± 6^{a}

*Means in the same row that do not share a common superscript letter differ significantly ($p<0.05$)

rainbow trout. However, the results of this study is not in line with the findings by Serezli *et al*. (2010) who reported that relative and total fecundity and egg size were not affected by vitamin E inclusion in diets either positively nor negatively. The differences for egg diameters among groups were also found insignificant in this study. Although not significantly different, the differences observed among treatments could be attributed to the inherent performance ability of individuals possibly acquired from the parents (Onivie *et al*., 2010).

This study also showed that increased levels of vitamin A and E had a positive impact on viability of hatching eggs. Watanabe *et al*. (1991) found that increased levels of dietary vitamin E up to 2000 mg kg^{-1} in red sea bream diets improved percentages of buoyant eggs, hatching rates and percentage of normal larvae. The antioxidant enzyme systems present in the liver and other tissues of adult fish are not active until the late embryonic development stage of larval fish (Palace and Werner, 2006). This makes early antioxidant protection crucial through maternally derived non-enzymatic antioxidant system (Cowey *et al*., 1985; Ciarcia *et al*., 2000). The lowest fertility and larval survival rate was also reported for the eggs from brood stock fed with the lowest dietary levels of α-tocopherol (Izquierdo *et al*., 2001). The function of vitamin E as an inter and intra-cellular antioxidant is to maintain homeostasis of labile metabolites in the cell and tissue plasma and this is well known. The antioxidant function of vitamins C and E could provide an important protective role for the sperm cells during spermatogenesis and until fertilization by reducing the risk of lipid peroxidation which is detrimental for sperm motility (Izquierdo *et al*., 2001).

When hatching survival rate is taken into account, this results were probably due to the fact that yolk sac larvae of broodstock fish fed with the diets containing increasing amount of vitamin A and E displayed better survival rate than to control group. This is in agreement with the results of Fernandez-Palacios *et al*. (1998) who stated that increased dietary α-tocopherol levels from 125-190 mg kg^{-1} prevented the appearance of yolk sac hypertrophy and larval mortality and the positive effect of high vitamin E levels in embryos of gilthead sea bream on their survival.

CONCLUSION

Information on the nutrient requirements of brood stock fish is limited to a few species. Certain nutrients such as essential fatty acids and antioxidant nutrients and vitamins have been shown to be particularly important in brood stock nutrition. The requirements for vitamins as non-enzymatic antioxidant compounds during reproduction is much higher than that of those required for juveniles but deficient or excess amounts of these vitamins or an imbalance between them could also be detrimental for reproduction process. The importance of many nutrients such as vitamin A, vitamin B_6 and folic acid has not yet been established within brood stock feeds correctly and deserves attention for the future investigations.

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