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Crude Palm Oil as a Source of Carotenoids and Tocopherols to Enhance Reproductive Potential in Pearlspot *Etroplus suratensis* (Bloch)

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Abstract

Crude palm oil which is rich in carotenoids and tocopherols (700 and 800 ppm, respectively) was incorporated in pelleted feeds fed to pearlspot *Etroplus suratensis* reared in brackishwater earthen ponds. The fish were cultured for 11 months (average stocking size 4.30 cm, 1.70 g) on three supplementary feeds in three separate ponds; a fourth pond in which no feed was given served as the control. Three isoproteinaeceous feeds (about 33% of protein by dry weight) of varying composition were formulated with locally available ingredients: ORT (containing groundnut oilcake, rice bran and tapioca), P-ORT (containing prawn waste, groundnut oilcake, rice bran and tapioca) and oil P-ORT (P-ORT sprayed with a mixture of crude palm oil and fish liver oil in the ratio of 4:1). From observations made on the percentage occurrence of ripe fish, gonadosomatic index, egg volume and histological studies, it could be inferred that oil P-ORT had a positive influence on ovarian development and maturation. However, proximate composition analyses of ovary showed no significant difference between treatments.

Introduction

As much as nutrition affects somatic growth in fish, its impact on gonadal growth and development is also well documented. This effect of nutrition on the reproductive potential of fish was reviewed by Watanabe (1985). Proteins, in terms of both quantity and quality, are known to influence reproductive performance in fish (Takeuchi et al 1981a; Watanabe et al. 1984a; Santiago et al. 1985, 1988; Wee and Tuan 1987; Cumaratunga and Thabrew 1989; De Silva and Radampola 1990; Matty 1990a). Similarly, fats, particularly PUFA, play a major role in ovarian development. Together with the supply of these macronutrients in optimal ratios, a suitable supply of several micronutrients, namely vitamins A, D, E, ascorbic acid and trace elements are indispensable for gonadal development in fish (Takeuchi et al. 1981a, 1981b; Watanabe et al. 1981; Cowey et al. 1983; Sandnes et al. 1984; Soliman et al. 1986; Eskelinen 1989; Matty 1990b).

Within the realm of vitamins, there is evidence that carotenoids and tocopherols play a relatively more important and vital role than the rest in the reproductive physiology of fish, whereby, their inclusion in diets improves reproductive potential. In this study, crude palm oil (being a rich and cheap source of carotenoids and tocopherols) was used as a feed ingredient to study its efficacy as a potential broodstock feed component. The pearlspot *Etroplus suratensis* was selected for its easy availability, commercial importance and its ability to breed in close pond water systems. The reproductive performance of female pearlspot was assessed based on the percentage occurrence of different maturity stages, gonadosomatic index (GSI), fecundity, egg size and proximate composition and histology of ovary.

Materials and Methods

The experiment was conducted for 315 d (7 September 1989 - 18 July 1990) in four brackishwater earthen ponds (50 m^2) and 1 m depth at the Aquarius Fish Farm Complex at Quilon, Kerala, India. Pearlspot juveniles (average size 4.30 cm and 1.70 g) collected from the Ashtamudi Estuary, were stocked 175 to a pond. The ponds designated as P1, P2 and P3 were provided with the feeds ORT, P-ORT and oil P-ORT. The ingredients and proximate composition of the feeds are given in Table 1. Previously weighed out rations were provided once daily at 5% body weight for the first five months and then reduced gradually to 2%. Pond P4 served as the control in which no supplementary feed was given.

Monthly random samples of 12-15 fish were taken from each pond from the third month of stocking. Female fish were identified by dissection and total length and weight of individual fish recorded. The color, shape, size and state of maturity of the ovaries were noted and classified according to the I.C.E.S. scale (Wood 1930). The weight of the ovary expressed as percentage of body weight, was defined as the gonadosomatic index (GSI). Fecundity was determined by the gravimetric method. The total number of yolked eggs was counted (five sub samples) and multiplied by the factor which expressed the ratio between weight of sample and ovary. This gave the total fecundity of the fish. Relative fecundity was calculated as number of eggs per gram body weight of fish. To compare the size of ripe eggs, egg dimensions were measured using an ocular micrometer. The volume of each egg was calculated by the formula $1 \cdot ab^{-2}$ where a=long axis (mm) and b=short axis (mm), as adopted by Rana (1985) to study elliptical-shaped eggs. To study ovarian histology, immature ovaries were fixed in Bouin's fluid, and yolk containing ovaries in Smith's formol dichromate solution. Sections (4-6 μ) were stained with Harris hematoxylin, and eosin was used as the counter stain. The development of oocytes was divided into nine stages as adopted by Yamamoto (1956). The percentage occurrence of the different oocyte stages in the different ovarian stages was recorded.

To determine the proximate composition, ovaries were dried overnight at 60°C and stored in the dessicator until analysis. Protein was determined by the Micro-Kjeldahl method as N x 6.25. Lipid was extracted by the

Table 1. Ingredient composition and proximate composition of supplementary feeds (% dry weight).

	ORT	P-ORT	Oil P-ORT
Prawn waste	-	15.00	15.00
Groundnut oil cake	65.00	55.00	55.00
Rice bran	25.00	20.00	20.00
Tapioca flour	10.00	10.00	10.00
Fish liver oil	-	8-	1.00
Crude palm oil[2]	-	-	4.00
Vitamin and mineral mix[3]	00.25[1]	0.25[1]	0.25[1]
Moisture	5.98	6.15	6.15
Crude protein	33.42	33.22	33.22
Crude lipid	5.79	4.83	9.80
Ash	11.37	14.57	14.57
Crude fiber	9.20	12.06	12.06
NFE	34.24	29.17	24.20
Caloric value ($Kcal \cdot g^{-1}$)	3.81	3.52	3.99

[1]Added in excess of 100%.
[2]Chemical characteristics of crude palm oil: free fatty acids 0.9%, moisture and impurities 0.22%, iodine value 52, saponification value 195, unsaponificable matter 0.56%, carotene 700 ppm, tocopherols 800 ppm, peroxide value nil, ansidine value 0.14, diene value (E253 1%) 0.17, triene value (E269 1%) 0.15; iron 4.0 ppm, copper 0.5 ppm, C14:0 1.22%, C16:0 42.44%, C18:0 5.17%, C18:1 37.01%, C18:2 11.71% (source: Arumughan et al. 1989).
[3]Composition of vitamin-mineral mix (Supplivite-M), each 250 g provides: vitamin A 500,000 IU; vitamin D3 10,000 IU, vitamin B2 0.2 g; vitamin E 75 units, vitamin K 0.1 g, calcium pantothenate 0.25 g, nicotinamide 1.0 g, vitamin B12 0.6 g.
Choline chloride 15 g, calcium 75 g, manganese 2.75 g, iodine 0.1 g, iron 0.75 g, zinc 1.5 g, copper 0.25 g.

chloroform-methanol method (Gradwohl 1963). Ashing was done in a muffle furnace at 500°C for 5 h. Due to nonavailability or scarcity of spent ovary samples, the analyses of the same could not be done.

The mean value and standard deviations were calculated for each observation, and differences between means were compared by way of the Student's 't' test.

Results

Due to lack of pond facilities, the treatments could not be replicated. However, the clear trends observed are thought to be indicative of biological validity of the results.

Total Percentage Occurrence of Different Maturity Stages of Fish (Table 2)

In P1, maturing specimens were noticed from March onwards, and ripe fish (1.70%) in June. However, no spent fish were observed. In P2, maturing specimens occurred from April onwards, while ripe fish (3.28%) and spent fish (1.64%) were observed in June and July, respectively. In P3, maturing specimens were noticed as early as January and ripe fish (8.07%) were encountered in June and July. Spent fish (1.61%) were obtained only in July. In P4, maturing fish were observed in June alone and no ripe fish were obtained.

Morphological Observations

The highest range in GSI values was recorded for P3 from 0.186±0.06 in Dec. 1989 to 2.47±1.5 in July 1990 (Table 2). The differences in fecundity observed between the feeds were not significant (P>0.05). The largest eggs in terms of volume were recorded in P3 (1.82±0.21) which differed significantly (P<0.001) compared with those in P1, P2 and P4.

Table 2. Recorded parameters of fish in the four culture ponds[1].

	P1 (ORT)	P2 (P-ORT)	P3 (Oil P-ORT)	P4 (Control)
Individual observations (No. of females)	59	61	62	63
Total occurrence of the different maturity stages (%)				
Stage I	69.49	73.77	56.45	80.95
Stage II	23.73	18.03	20.97	17.46
Stage III	5.08	3.28	12.90	1.59
Stage IV	1.70	3.28	8.07	-
Stage V	-	1.64	1.61	-
G.S.I	1.45 ± 1.30	2.39 ± 1.44	2.43 ± 1.26	1.36 ± 1.25
Total fecundity (mean ± SD)	782.35^a ± 212.55	$1,017.80^a$ ± 457.30	932.94^a ± 170.54	630.41^a ± 75.59
Relative fecundity (mean ± SD)	19.67^a ± 5.11	19.30^a ± 9.88	15.44^a ± 2.35	15.69^a ± 1.88
Egg volume (mean ± SD)	1.39^a ± 0.21	1.32^a ± 0.21	1.82^b ± 0.21	1.53^c ± 0.12

[1]Values in a row with the same superscript are not significantly different (P>0.05).

Histological Observations

DIMENSIONS OF OOCYTES

In the perinucleolus stage, the mean diameters in P1, P2 and P3 were significantly (P<0.05) higher than in P4 (Table 3). However, in the ripe egg stage, the largest eggs for both long and short axes were encountered in P3 (1,204.20 ± 198.46 μ and 699.00 ± 102.24 μ, respectively).

PERCENTAGE OCCURRENCE OF DIFFERENT OOCYTE STAGES

The percentage occurrence of the different oocyte stages in stages I, II and III ovaries showed no significant differences between the four ponds (Fig. 1). In stage IV ovary, variations in the percentage occurrence of chromatin nucleolus and ripe egg stages between ponds was obvious. P3 recorded a minimum of chromatin nucleolus stage (13.64%) and maximum of ripe egg stage (50%). Stage IV ovaries were not obtained in P4.

Table 3. Dimensions of different oocyte stages of fish in the four culture ponds (μ)[1].

Ponds (nucleolus)	Chromatin nucleolus stage (diameter)	Peri-nucleolus stage (diameter)	Yolk vesicle stage (diameter)	Yolk stage (diameter)	Migratory nucleus stage		Ripe egg stage	
					Long axis	Short axis	Long axis	Short axis
P1 (ORT)	52.20^{a} ± 15.77	173.40^{a} ± 43.01	378.00^{a} ± 71.04	446.40^{a} ± 71.66	747.00^{a} ± 155.44	584.40^{a} ± 122.62	994.80^{a} ± 250.42	592.20^{a} ± 117.04
P2 (P-ORT)	52.20^{a} ± 14.34	167.40^{a} ± 28.26	358.80^{ab} ± 79.77	528.00^{b} ± 93.57	898.00^{b} ± 155.95	575.40^{a} ± 70.74	1,088.40^{ab} ± 208.26	691.80^{b} ± 72.03
P3 (Oil P-ORT)	58.80^{a} ± 19.31	171.00^{a} ± 36.57	352.80^{ab} ± 67.50	525.60^{b} ± 90.80	793.80^{a} ± 160.34	504.60^{b} ± 93.30	1,204.20^{b} ± 198.46	699.00^{b} ± 102.24
P4 (Control)	54.00^{a} ± 18.00	144.60^{b} ± 24.37	316.80^{b} ± 62.75	441.00^{c} ± 100.39	718.20^{a} ± 159.35	428.40^{c} ± 86.21	1,096.80^{ab} ± 225.15	716.40^{b} ± 121.67

[1]Mean ± SD values in the same column having different superscripts are significantly different ($P<0.05$).

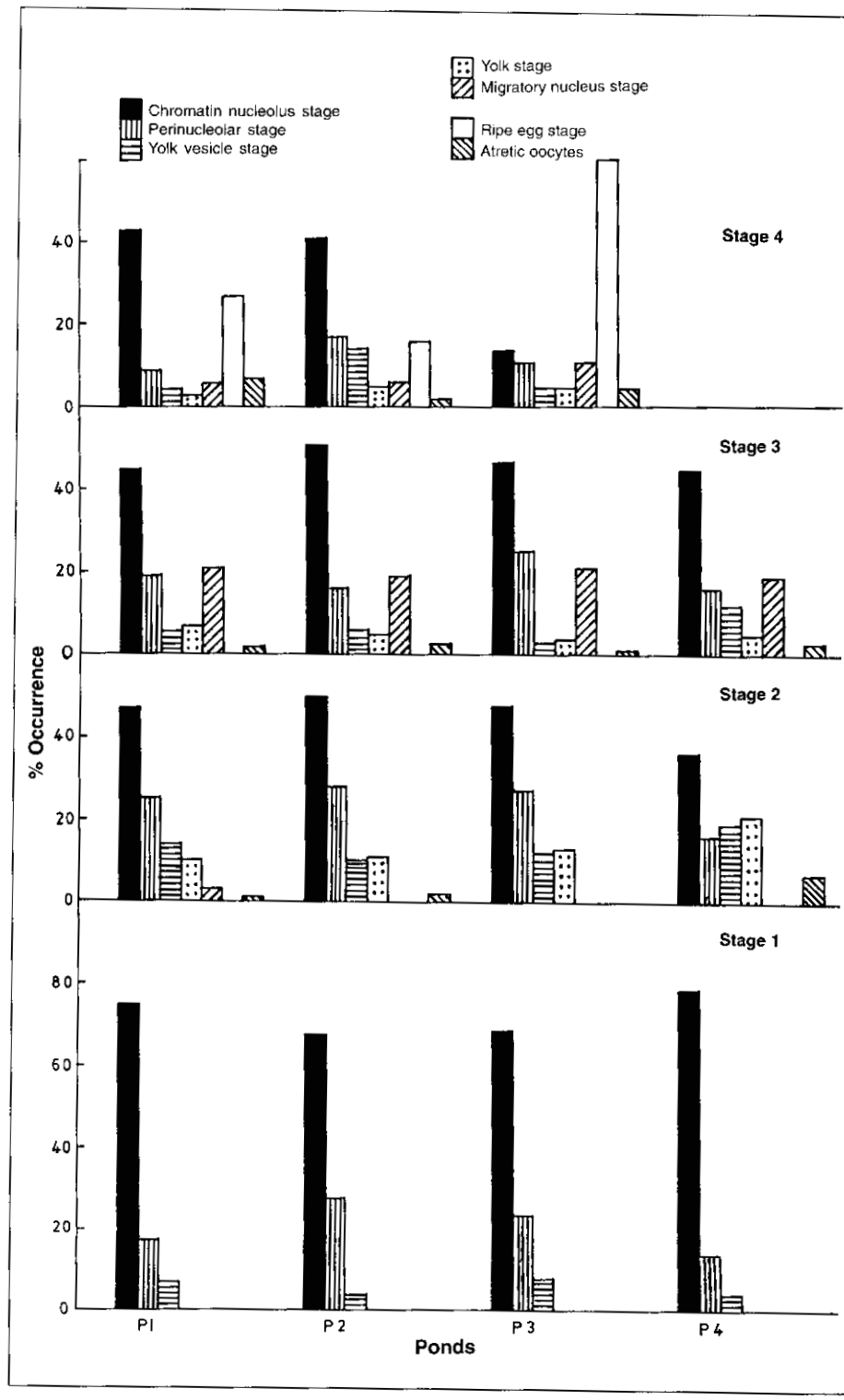

Fig. 1. Percentage occurrence of different oocyte stages in the different maturity stages of fish from culture ponds.

PROXIMATE COMPOSITION OF OVARIES IN DIFFERENT MATURITY STAGES

Moisture content decreased during the course of maturation. P3 recorded the highest lipid, and lowest moisture content in stages III and IV (Table 4). Stage I showed a relatively high protein content ranging from 63.22% in P3 to 70.26% in P2. In stage IV it was maximum in P3 (62.24%) and minimum in P2 and P1 (54.73%). Lipid levels recorded in stage III in P1, P2 and P4 were more or less similar (27.87, 28.27 and 27.39%, respectively), but higher in P3 (34.09%). In stage IV it was 27.35, 35.98 and 29.56% in P1, P2 and P3, respectively.

Table 4. Proximate composition of ovaries in different maturity stages in the four culture ponds.

Parameter (%)	Pond	Stage I	Stage II	Stage III	Stage IV
Moisture	P1	89.33	77.85	68.60	68.30
	P2	91.80	83.75	72.64	64.93
	P3	92.66	86.84	66.82	63.86
	P4	92.31	76.60	69.56	-
Crude protein[1]	P1	69.19	55.05	59.85	54.73
	P2	70.26	63.89	54.97	54.73
	P3	63.22	55.90	58.33	62.24
	P4	70.00	59.46	61.12	-
Crude lipid[1]	P1	23.81	31.97	27.87	27.35
	P2	35.92	34.35	28.27	35.98
	P3	21.94	34.77	34.09	29.56
	P4	25.53	31.21	27.39	-
Ash[1]	P1	5.18	5.67	4.96	2.36
	P2	1.46	7.74	9.26	4.41
	P3	6.45	6.20	5.09	1.34
	P4	4.93	1.52	6.23	-

[1]Expressed as dry weight percent.

Discussion

The advantage of supplementary feed in P1, P2 and P3 in terms of reproductive efficiency was obvious when compared to P4, as was also observed by Santiago et al. (1985) in *O. niloticus*. However, between the three feeds, oil P-ORT had a desirable effect on oocyte maturation, resulting in a higher range of GSI values, higher percentage of ripe fish and larger eggs. This differential impact of the supplementary feeds on the reproductive potential of female *E. suratensis* may be interpreted in terms of the feed components as relevant to broodstock fish.

The positive influence of oil P-ORT may be attributed to the oil component, i.e., fish liver oil and crude palm oil, in the feed. The impact of EFA, especially PUFA, on fish reproduction has been confirmed by several workers

(Takeuchi et al. 1981a; Watanabe et al. 1984b; Kanazawa 1985; Watanabe 1985). The fish liver oil added in oil P-ORT would have been an effective source of mono- and polyunsaturated fatty acids. The positive effect of crude palm oil may be attributed to its carotenoid and tocopherol content which occur at levels of 700 and 800 ppm, respectively, rather than to its fatty acid profile. Several studies support the inference that this would have aided ovarian maturation of fish in P3. Watanabe and Takashima (1977) observed significantly lower GSI in carp fed on ∝-tocopherol-deficient diet than in those of control fish. Takeuchi et al. (1981b) observed poor spawning in ayu fed diets deficient in vitamin E. This impact of tocopherols on reproduction was further confirmed by Watanabe et al. (1984a) in red sea bream. Carotenoids are known to fulfill some physiological function such as anti-oxidation rather than there being an absolute need for the pigment itself (Miki et al. 1984). β-carotene-, astaxanthin- and canthaxanthin-supplemented diets increased the reproductive efficiency of red sea bream (Watanabe et al. 1984c). Torrisen (1983) inferred a direct relationship of egg and alevin survival in Atlantic salmon to carotenoid levels in eggs. As such, it could be said that the carotenes in crude palm oil would have positively aided ovarian maturation in fish in P3, but its role needs to be further investigated.

The superiority of larger eggs compared to smaller eggs remains controversial. However, according to Bagenal (1969) and Springate et al. (1985), larger eggs produce larger fry and it is possible that under adverse conditions, as for example those experienced in the wild, these larger progeny may show better chances of survival due to the additional material in bigger eggs, and have a selective advantage over the rest.

Proximate composition showed no pronounced difference between treatments and control. Moisture content decreased with maturation. The fact that *E. suratensis* produces demersal eggs which adhere firmly to underwater objects may explain the absence of hydration during oocyte maturation in this fish. Protein levels showed a general increase from stage II to IV. This is related to the accumulation of yolk granules which have been proven to be rich in a neutral polysaccharide-protein complex in *E. suratensis* (Paulraj and Stanley 1988). Furthermore, in non-mammalian vertebrates, vitellogenin, a large lypoglycophospho-protein produced in the liver and released into the blood, is sequestered by developing oocytes, proteolytically cleaved into smaller proteins and stored as egg yolk (Wallace 1985). The fact that peak lipid values occurred at earlier stages of maturation than protein may be related to the sequence of yolk formation observed in most teleosts, where the accumulation of lipid yolk occurs prior to that of protein yolk (Weigand 1982; Mayer et al. 1988).

Finally, it needs to be pointed out that ideally the treatments should have been replicated, and that two additional treatments, a diet with only fish oil sprayed, or other with only palm oil sprayed would have been desirable. The paucity of pond facilities did not permit us to conduct our experiments as above, however.

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