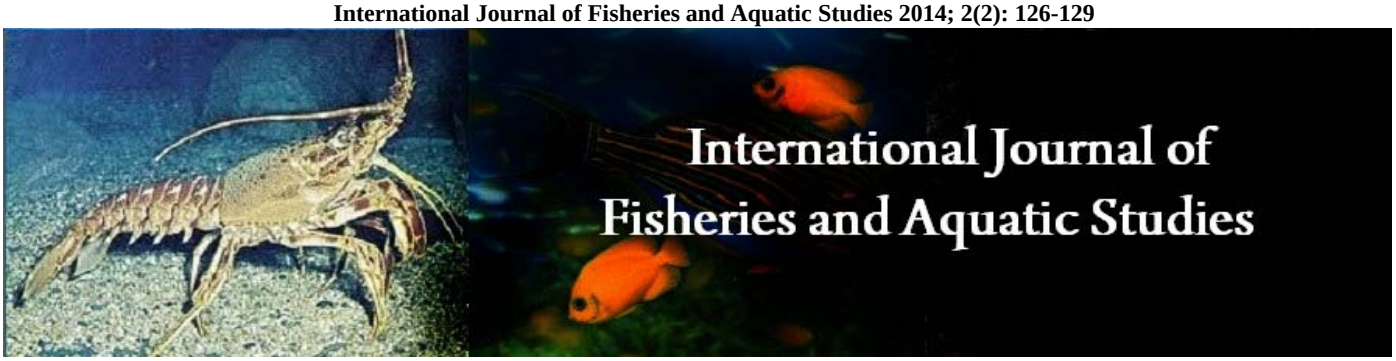


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# Enrichment of artificial feed with vitamin E for gonadal maturation of Mali Fish (*Labeobarbus festivus*)

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**Abstract**
The aim the research is to increase the reproductive potential of *L. festivus* female that is time matured gonads, somatic ovi index, fecundity, egg diameter, hatching rate and time to hatching in the control group (feed without enrichment of vitamin E) and exposed to 150 mg/kg, 300 mg/kg, 450 mg/kg feed of vitamin E. Treatment of vitamin E levesls were significant ($p<0.05$) different with respect to time ripe gonads, ovi somatic index, fecundity and egg diameter. Females exposed to 300 µg/kg feed of vitamin E can increased time matured gonads 67 days, somatic ovi index 14.95%, absolute fecundity 13547 eggs/spaw, relative fecundity 98 eggs/g gonado weight and egg diameter 1.16 mm. But hatching rate and time to hatching did not different between the treatments ($p>0.05$).

**Keywords:** *Labeobarbus festivus*, Feed, Vitamin E, 0 Reproduction, Hatching rate.

## 1. Introduction
Kampar Kanan River is one of the streams contained in Riau Province, which contributed significantly to support the activities of the people who live in the surrounding area. Activity on the river border are oil palm plantations and industry, whereas activity in the river water body is the transportation, sand mining and fish catching is not selective. All these activities are indirectly or directly affect fish biota [1].

The *L. festivus* is one of the native species that live in Kampar Kanan River [2], as a source of income for rural community around the area Kampar Kanan River and important fisheries commodity in Kampar Region with the price of 35,000 IDR /kg in off sea-son [3]. The problem is now are *L. festivus* populations already scarce in the Kampar Kanan River [2] and in Koto Panjang Reservoir, because the catching were conducted continuously with a variety of non-selective fishing gear [3]. It is therefore important conservation efforts began in insitu is *L. festivus* by performing domestication in order to produce larval mass.

Continuity fingerling is one of the limiting factors in aquaculture activities, so that the larvae can be produced continuously in proper amount and quality, then the fish can feed enriched with vitamin E [4, 5, 6]. Vitamin E can accelerate the achievement of mature gonads time and increase the number of eggs [5, 7, 8]. Fernandez *et al*., [9] stated that fish are deficient in vitamin E in the feed will affect gonadal development, fecundity and egg hatchability. The need for vitamin E in the feed of fish for reproduction process varies. The *Pangasius sutchi* needed to 190 mg/kg of feed [10], *Mystus nemurus* is 150 mg/kg of feed [4], *Tor douronensis* was 439.29 mg/kg feed [8]. Enrichment of vitamin E to the diet at different doses in order to improve the reproduction of fish larval of *L. fastivus* important to do so can be mass produced.

## 2. Material and Methods
### 2.1. Broodstock
Adult female and male of *L. festivus* were obtained from a commercial fisherman in Kampar Kanan River Padang Lawas Village, Kampar Regency, Riau Province and have been kept for > three months in cages, body weight ranged between 132 to 140 / individuals. *L. festivus* has been given feed were distributed into twelve cages (200×60×50 cm) each stocked with ten individual. All cages are placed in Kampar Kanan River with an average water height of two meter. River water temperature ranged from 26 to 28 ºC. Feeding was done twice daily and fish were fed a predetermined ration of 5% body weight day$^{-1}$. The feed is commercial feed. Proximate composition are water content (% dry weight) 12,0%, crude protein 38.0%, lipid 2.0%, carbohydrate 5.6% and crude ash 13.0%.

### 2.2. Checking the oocytes maturation

All fish were individually marked using floy-tags and weighed. Oocytes sampled in vivo were taken from females using the method described by Syandri [11], and were placed in Serra's solution (6:3:1, 70% ethanol, 40% formaldehyde and 99.5% acetic acid). for clarification of the cytoplasm. After 5 min, the position of oocytes nucleus was determined using a four-stage scale:

stage 1 germinal vesicle in central position

stage 2 early migration of germinal vesicle (less than half of radius)

stage 3 late migration of germinal vesicle (more than half of radius)

stage 4 periphery germinal vesicle or germinal vesicle breakdown (GVBD)

### 2.3. Feed treatment

Vitamin E is used for the enrichment of the feed is in the form of soft capsule contains 100 IU d-α tocopherol. Vitamin E is first dissolved in corn oil, then mixed into the feed evenly with doses of 0, 150, 300, 450 mg/kg of feed, then the feed is dried for 15 minutes in an open room with no sunlight. Experiments conducted on *L. festivus* female gonad maturity stage one with four groups and three replications. Control group were fed without vitamin E and three experimental ones. Group two, three and four given a given feed that has been enriched with vitamin E single dose of vitamin E with 150 mg/kg feed, 300 mg/kg feed, 450 mg/kg feed. Feed given as much as 5% of the weight of the biomass with a frequency of 2 times/day. The spawning *L festivus* conducted with GnRHa stimulation with dopamine antagonist at a dose of 0.5 ml / kg body weight. Egg samples from each treatment and replications. Gilson is preserved with a solution consisting of 100 ml, 60% alcohol, 880 ml of distilled water, 15 ml of nitric acid, 18 ml of glacial acetic acid and 20 grams of mercury chloride. Furthermore, the diameter of the eggs was measured with a microscope Olympus CX21 to 30 eggs of from each treatment and replications. Fertilized eggs were collected after ten hours and checked under a stereomicroscope. The eggs were randomly separated into four groups of aquarium (40x20x20 cm, eight liters of water volume), with three replicates for the control and three replicates for each exposure group. The total number of eggs in the control and the exposure groups was 200. The water temperature, dissolved oxygen (DO), and pH levels of the test chambers were regularly monitored.

Time mature gonadal calculated from the time the fish began to vitamin E granted until the fish reaches a mature gonadal (days). Ovi somatic index (IOS) was determined using the expression: IOS = BTO/BW x100%; where BTO: ovulation egg weight and BW: Body weight. Absolute fecundity (AF) =OVA×GW; where OVA: oocyte number per ovary gram and GW: gonadal weight. Relative fecundity was estimated using the formula: RF = AF/BW; where AF: absolute fecundity; BW: Body weight.

### 2.4. Statistical analyzed

Data were analyzed by one-way analysis of variance (ANOVA) and then tested using Dunnett's test. All statistical analyses were performed using SPSS 10.0J (SPSS, Tokyo, Japan). In all tests, the level of significance used was 0.05.

## 3. Result

### 3.1. The time sexual maturity and percentage of eggs weight

The mean time to reach the mature gonadal of the control group was 93 days, whereas in vitamin E enriched feed treatment group on average ranged from 67 to 87 days (Table 1). There were significant differences ($p< 0.05$) between treatment groups vitamin E against time reaches mature gonads. Average egg ovulation in the control group was 5,60%, whereas the treatment group ranged from 7.12% to 14.95% (Table 1). There were significant differences ($p<0.05$) between control and treatment groups vitamin E.

**Table 1:** Time matured the gonads and percentage of eggs weight

| Level vitamin E (mg/kg feed) | Time matured the gonadal (days) | Somatic Ovi Index (%)[a] |
|---|---|---|
| Control | 93±6[a] | 4.60±0.89[a] |
| 150 | 87±4[b] | 7.12±1.85[b] |
| 300 | 67±4[c] | 14.95±1.91[b] |
| 450 | 81±7[b] | 12.77±2.37[b] |

[a] Weight of eggs ·100%/female weight.
[abc] Values with the different superscript in each column are significantly different from each other ($P < 0.05$).

### 3.2. Fecundity and eggs diameter

The mean absolute fecundity *L. festivus* of the control group was 8497 eggs/spaw, while that of the exposure groups ranged between 9903 to 13547 eggs/spaw (Table 2). Absolute fecundity tended to increase with increasing level of vitamin E, although the increases occurred at level of 150 and 300 mg/kg feed. At a level of 450 µg/kg body weight has not increase fecundity when compared with a level of 450 mg/kg feed, but larger than the level of 150 mg/kg feed. The absolute fecundity control with dose 150 mg/kg feed no significantly ($p>0.05$), but were significantly ($p<0.05$) dose 300 and 450 mg/kg feed.

**Table 2:** Fecundity and eggs diameter in the control and treatment groups exposure to various levels of vitamin E

| Dose vitamin E (mg/kg feed) | Average body weight (g) | Fecundity (number of eggs per *spawn* ) | Relative fecundity (number of eggs per g gonadal weight ) | Eggs diameter (mm) |
|---|---|---|---|---|
| Control | 140±46 | 8497±1234[a] | 60±2.0[a] | 0.95±0.02[a] (n=50) |
| 150 | 132±25 | 9903±668[a] | 75±2.0[b] | 1.06±0.09[b] (50) |
| 300 | 138±36 | 13547±2166[ab] | 98±5.0[c] | 1.16±0.00[b] (50) |
| 450 | 136±50 | 12462±1865[ab] | 91±6.0[d] | 1.13±0.02[b] (50) |

Values with the different superscript in each column are significantly different from each other ($p< 0.05$).

The mean relative fecundity (number of egss/g gonadal weight) of four vitamin E exposure group (Table 2). Fecundity relative on control group of 60 eggs/g gonadal weight. On the group two, three and four, respectively 75, 98 and 91 eggs/g gonadal weight. The relative fecundity was significant ($p<0,05$) differences between the control and exposure groups. There were no significant changes ($p<0.05$) in egg diameter among the vitamin E levels for all four spawning (Table 2). The eggs diameter on the control group was 0.95 mm, whereas in group vitamin E imposed ranged from 1.06 to 1.16 mm

**Table 3:** Hatching rate and time to hatching of embryos in the control and treatment groups exposure to various levels of vitamin E

| Dose vitamin E (mg/kg feed) | Hatching rate[b] (%) | Time to hatching[b] (hours) |
|---|---|---|
| Control | 34.16±5.13[a] (n = 200) | 24±1.0[a] (n=128) |
| 150 | 36.50±2.29[a] (200) | 23±1.5[a] (133) |
| 300 | 37.83±2.25[a] (200) | 21±1.0[a] (135) |
| 450 | 35.83±5.20[a] (200) | 22±1.5[a] (140) |

[a] Values with the same superscript in each column are not significantly different from each other ($p>0.05$).

The mean hatching rate of the control group was 34.16% while that of the exposure groups ranged from 35.83% to 37.83% (Table 3). The mean hatching time in the control group was 24.10 hours while in the exposure groups it each one is 23.0 21.0 and 22.0 hours (Table 3). No significant ($p>0.05$) differences were found between hatching rate and mean hatching time in the control and exposure groups.

## 4. Discussion

The *L.festivus* females group vitamin E exposed can reaches the gonadal mature more rapidly, increased of the index ovi somatic, absolute fecundity and relative fecundity, when compared to with the control group, but had not effective on hacthing rate and time to hatching. The best level vitamin E to rapidly of mature gonadal and increased fecundity was 300 µg/kg feed. While the exposed to levels of 150 µg/kg and 450 mg/kg feed longer time matured gonads and less fecundity. Aryani [5] stated that the enrichment feed of green catfish (*Mystus nemurus*) with vitamin E at a dose of 100 mg/kg of feed time ripe gonads achievement for 73 days, Whereas the *Osphronemus gourami* with a dose of vitamin E was 338.72 mg/kg feed during the time ripe gonads reached 58 days [7], *Tor douronensis* fish fed the enriched with vitamin E at a dose of 438.29 mg/ kg feed, the time ripe gonads achieved during 116 days [8].

The vitamin E was mobilized from peripheral tissues during vitellogenesis although the plasma vitellogenin content was not affected, suggesting that lipoproteins may be involved in the transport of vitamin E during this period[12]. Vitamin E (α-tocopherol) has an important role in the reproductive processes of fish [13]. His main function of vitamin E is as an antioxidant compound that may help prevent the oxidation of unsaturated fatty acids in the cell. Unsaturated fatty acids, especially linoleic fatty acid group and linolenic, phospholipid generally part of the cell membrane [14]. Is suspected the addition of vitamin E in the diet can affect the levels of vitamin E in the fish egg *L. festivus*, but in this study, the authors have not reported the effects of enrichment of vitamin E into the field to the levels of vitamin E in the egg cell. Mokoginta *et al*., [10] states that the feed is enriched with vitamin E at 114 mg/kg of feed obtained levels of vitamin E in eggs of fish *Clarias batrachus* of 12.15 ug/g wet weight of eggs, whereas the enrichment of vitamin E was 308 mg/kg diet of vitamin E found in the egg was 17.12 ug/g wet weight of eggs.

Hatching rate eggs of *L. festivus* ranged from 34.16% to 37.80% and was not significantly different ($p>0.05$) between treatments. Hatching rate eggs of *L. festivus* smaller when compared with the hatchability of eggs of fish *Clarias batrachus* were getting feed enriched with vitamin E doses of 300 and 308 mg/kg of feed, ie respectively 71.87% and 68.48% [10]. The low hatchability of eggs of fish *L. festivus* likely caused by the process of egg fertilization by artificial spawning less well conducted. The *L. festivus* wild fish are fish that live in the waters of the river and new succeeded in domestication is insitu and researcher have yet to find the right technology to produce spawning larvae mass.

In general the exposed to 150 to 450 mg/kg feed of vitamin E can increased ovi somatic index of *L. festivus* ranged between 7.12% to 14.95%, whereas in the control group was 4.60%. According to [6] that of *H. nemurus* exposed to 100 mg/kg feed to produced somatic ovi index 6.50%, whereas of *Tor douronensis* are 9.07% [8] and *Pangasius hypothalamus* are 8.25% [15]. The fish reproduction is influenced by the quality of feed [4], vitamin E levels [8], Increased levels of dietary vitamin E (up to 2000 mg/kg) in red seabream diets improved percentages of buoyant eggs, hatching rates and percentage of normal larvae [16] and environmental factors [17]. In this research the same type of feed given and maintained in the same environment with indicators of water quality parameters is important that is water temperatures ranged from 24 to 27 ºC, pH ranged from 7 to 8 and dissolved oxygen ranged from 6 to 8 $mg.l^{-1}$.

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