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Influence of Dietary Vitamin E on the Content of Vitamin E in Blood Plasma, Ovary Tissue and on Hemolysis of Cultured Female Grey Mullet (*Mugil cephalus*)

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ABSTRACT

Commercial diets containing α-tocopherol (90mg/kg) were supplememted with o, 100 and 200 mg/kg α-form vitamin E acetate respectively and fed to one-year-old (1+) grey mullet (*Mugil cephalus*)for 12 months. Plasma vitamin E increased as dietary vitamin E increased, and showed seasonal changes. It decreased from May to Novemder, then gradually increased until the following February. Vitamin E content in ovary was the lowest (296-376μg/g lipid) in October and the highest (602-694μg/g lipid) in November, then decreased to 408-453μg/g lipid in December. A negative relationship between vitamin E contents in plasma and in ovary was observed. Grey mullet erythrocytes appeared ellipsoidal in shape with a major axis of 9.24 to 9.88μm and a minor axis of 6.33 to 6.67 μm. No significant difference was found in shape and size of erythrocytes from grey mullet fed with diets of different vitamin E levels. Spontaneous hemolysis was 7.55-7.71% in an 8 h test, regardless of the dietary vitamin E intake of the fish. Body and ovary weights were significantly lower for mullet fed on commercial diets than fed on diets supplemented with vitamin E to 190 and 290 mg/kg.

Key words: Mullet, Vitamin E, Plasma, Ovary, Hemolysis

INTRODUCTION

Mullet culture has been successful in Taiwan for many years [1]. Fingerlings are collected from the sea and cultured in fresh water or salt water for meat production. Due to the reduction in ocean catch of wild mullet[2-4], cultured mullet are also being considered as a source of roe. Dried and salted roe sac product costs about USD 35-40 per pair. However, mullet roes from cultured source are generally smaller than those from the wild[5-6].

Vitamin E or α-tocopherol has been reported to increase growth and gonad development and to reduce hemolysis in fish[7-10]. The objectives of this study were to determine the influence of dietary vitamin E on body and ovary weights, blood hemolysis and on the vitamin E level in ovary tissue and blood plasma during maturation of grey mullet. The results may help to establish vitamin E requirements for optimal development and yield of ovary tissue.

Cultured female grey mullet of over 1 year old (1+) matured in the months of October

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through December[6]. In November, the gonadosomatic index reached maximal, being 7.27-11.62%[11]. During these 3 months, the vitamin E contents in blood plasma and ovary tissue were compared with the dietary vitamin E contents.

MATERIALS AND METHODS

Pond Design

An experimental pond of 9400 m^2 was divided with nylon net into compartments of 25m x 8m x 2.1 m, at the Experimental Station of Hanaqua International Corp., Tainan, Taiwan. The culture density was 0.5 mullet/m^2. Salinity was controlled at 11 ± 2‰.

At the start of the feeding experiment, about 100 one-year-old grey mullets (450g per fish) were put into each pond compartment.

Experimental Diet

A commercial feed, (Hanaqua International Corp.) composed of fish meal, full-fat soybean meal, peanut meal and linseed meal as protein sources; wheat bran, wheat flour middling and cassava flour as carbohydrate sources and binder; and rice bran as filler were mixed and controlled to 3200 kcal/kg. Three levels of vitamin E (α-tocopherol acetate, O, 100, and 200mg/kg) were used to supplement the basal diets. Ethoxyquin 0.02% was added to the diet to inhibit the oxidation of lipid and α-tocopherol. Diets were mechanically mixed to ensure homogeneity and pelleted by Hanaqua Corp., then fed to fish throughout the 12 month period. Proximate composition and vitamin E of the diets (Table 1) were analyzed with AOAC methods[12] and with the method[13] of National Laboratories of Food and Drug.

Experimental Duration

The experiment began March 11, 1991, and ran to February, 1992. Water temperature and salinity were recorded through the 12 month period. Five mullets were sampled from each compartment periodically.

Measurement of gonadosomatic index (GSI)

GSI = (Gonad weight (g)/ Body weight (g)) × 100

Blood

Ten ml blood samples were taken from the caudal vein of each fish and immediately mixed with 143 U.S.P units of sodium heparin powder (Sigma Chemical Co. St. Louis, Mo), and kept in ice and analyzed within a half hour.

For serum preparation, 5 ml blood samples in seperate tubes were frozen at -20℃ for later use.

Blood smears were prepared and dried with methanol, stained with Giemsa then observed with microscope, for determination of erythrocyte size.[14]

Spontaneous Hemolysis

Spontaneous hemolysis was determined using a modified Draper and Csallany method[15]. A

Table 1. Proximate composition of experimental diets for cultured 1 year grey mullet.

Vitamin E added (mg/kg)	Moisture (%)	Crude Protein (%)	Crude fat (%)	Ash (%)	Vitamin E (mg/kg)
0	11.45	30.30	9.84	9.73	90 ± 3
100	11.46	32.95	10.11	10.71	190 ± 7
200	12.68	33.80	10.50	10.64	290 ± 11

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0.2 ml blood sample was mixed with 10 ml isotonic saline phosphate buffer pH 7.4 in a sterile test tube and then centrifuged at 500 x g for 10 min. The supernatant was discarded then the precipitate was suspended in 10 ml saline phosphate buffer and centrifuged at 500 x g for 10 min. This operation was repeated at 2 hr intervals at 20℃. The A_{415nms} of the supernatant was measured spectrophotometrically (Hitachi U-2000) as $(A_{415nm})^s$. To obtain complete hemolysis, distilled water was used to replace the isotonic saline buffer. The above procedures were repeated to measure the $(A415nm)_d$.

$$\text{Spontaneous Hemolysis (\%)} = \frac{(A415nm)_s}{(A415nm)_d}$$

α-tcopherol content in diets, plasma and gonad

α-tocopherol content in the experimental diets was analyzed according to the method of National Laboratories of Food and Drugs[13].

The α-tocopherol content in plasma was measured with Emmerie and Engel's method modified by Augustinet al.[16] using 0.3-0.6ml plasma. The method is based on the oxidation of xylene-extracted tocopherols of plasma by ferric chloride. The pink complex of ferrous ions with bathophenathroline is a measuredcolor imetrically at 536 nm using dl-α-tocopherol (Wako Pure Chemical Industries, Japan) as a standard.

Lipid was extracted from 10 g gonad by the method of Folch et al..[17] Accurately weighed lipid was diluted with n-hexane to 10 ml and filtered with 0.45 μm millipore, then analyzed for α-tocopherol content[18], with HPLC (Hitachi F-1050) equipped with a fluorescence spectrophotometer (Model: 050-0702) and Shimadzu integrator C-R5A.

An HPLC chromatographic column was Spheris S5ODS2 stainless steel, 150 x 4.6 mm id, methanol:water (99:1 v/v) was mobile phase at a flow rate of 2.0 ml/min. Excitation and emission wavelengths were 290 nm, and 330 nm respectively. dl-α-tocopherol was used as a standard.

Statistical Analysis

A test of variation among treatments was done by the Krusskal -Wallis one way analysis.[19] If the variations were significant, Krukal-Wallis multiple comparison was further tested.

RESULTS AND DISCUSSION

Dietary Vitamin E supplementation and plasma α-tocopherol

The α-tocopherol in blood plasma from fish fed 3 diets from May 1991, to Feburary 1992, is shown in Fig. 1. As the gonads maturated, α-tocopherol in blood plasma decreased and reached minimum in November, when the GSI was maximal[6]. α-tocopherol in blood plasma started to increase afterwards when gonads began to show atresia, then, completely disappeared in Feburary[6].

Seasonal changes in α-tocopherol of blood plasma drawn from grey mullet fed 90 mg/kg vitamin E (control group) were also shown in Fig. 1. In May, after the mullet were on basal diet for 2 mos, the α-tocopherol concentration in mullet blood was 32.1 μg/ml. In November, it was 2.5μg/ml, then gradually increased to 10.1μg/ml during Feburary.

The decreased α-tocopherol in blood plasma during gonad maturation may indicate that α-tocopherol is required for ovary development, that the minimum requirement of α-tocopherol for mullet growth may vary at different times of the year.

Ovary α-tocopherol Content

Ovary α-tocopherol from mullet sampled in October ranged between 296-376μg/g lipid, increasing to 602-696 μg/g lipid in November, then decreasing to 408-453μg/g lipid in December (Fig 2). Effects of vitamin intake on the α-tocopherol

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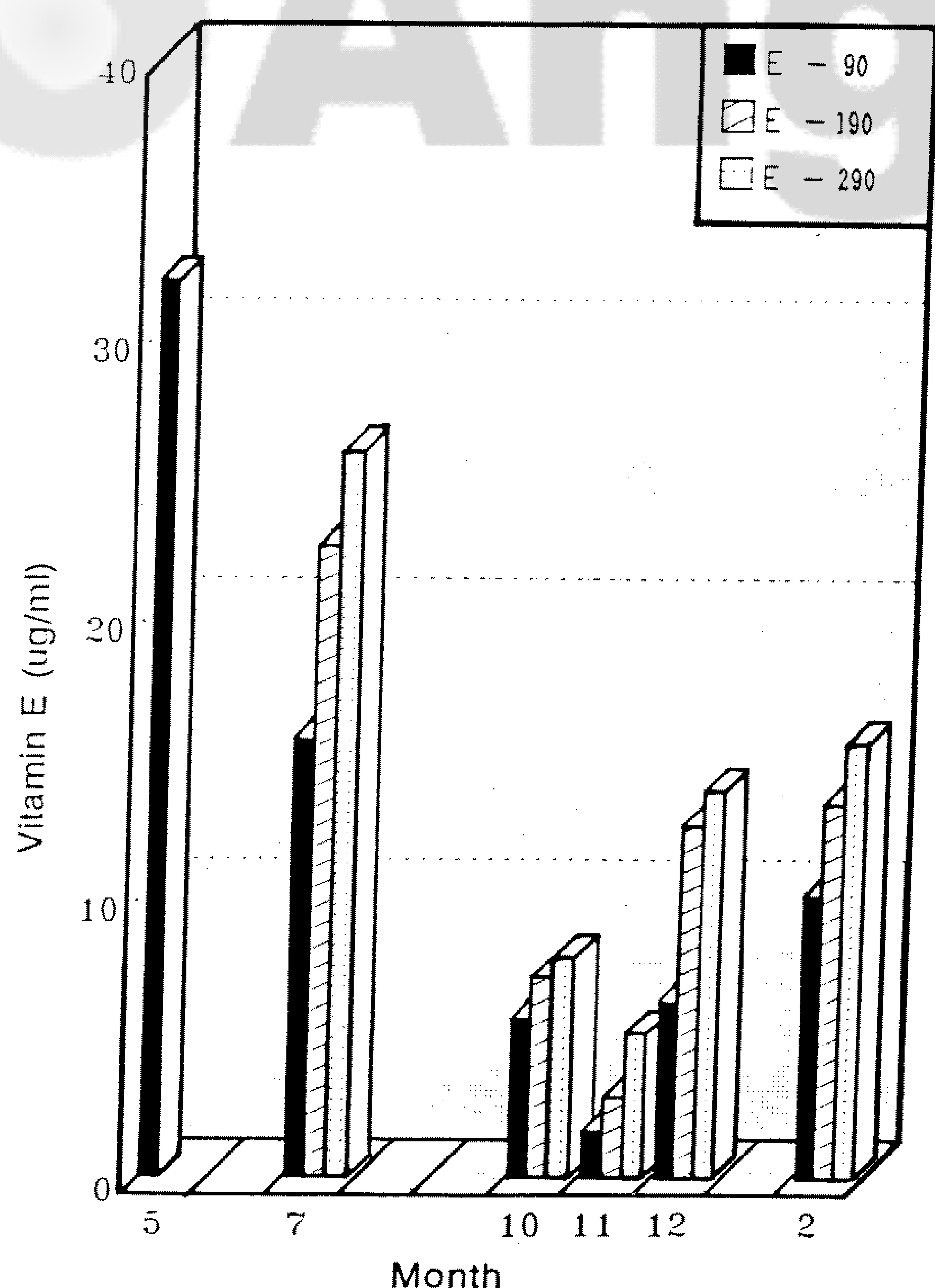

Figure1. Seasonal changes of vitamin E in blood plasma of cultured 1 + female grey mullet fed with vitamin E supplemented diet.

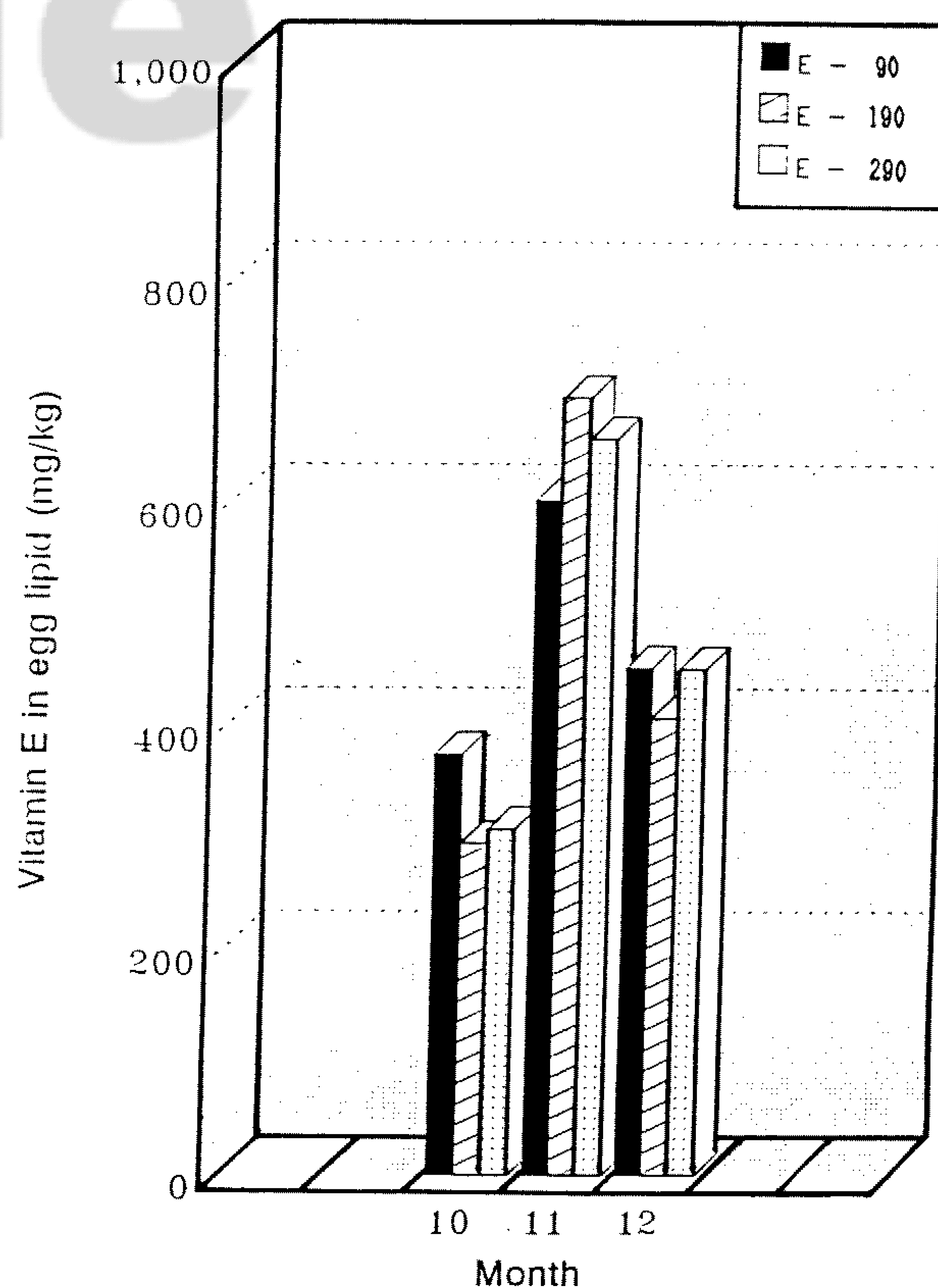

Figure 2. Vitamin E in eggs of cultured 1 + female grey mullet fed with vitamin E supplemented diet.

concentration in ovary lipid was not consistent. However, the body and the gonad weight or GSI of grey mullet fed 190 and 290 mg/kg of vitamin E respectively for 8 mo. sampled in November were significantly higher $p < (0.05)$ than those fed 90 mg/kg (Table 2). But no significant differences were observed between the dietary intake at 190 and 290 mg/kg levels. The α-tocopherol content per pair of ovary also showed the same pattern.

Therefore, an increase in vitamin E supplementation to 190 mg/kg is beneficial to growth and to maturation of grey mullet. Rainbow trout also require a higher level of vitamin E supplementation during maturation, when liver and pancreas α-tocopherol is transported to ovary via blood[20,21].

Although the findings with grey mullet showed no significant difference between 190 and 290 mg/kg vitamin E intake at a dietary lipid content of $10.09 \pm 0.15\%$, changes in dietary lipid content and fatty acid composition [22] may result in different vitamin E requirments for grey mulllet during maturation.

Size and Hemolysis of Erythrocytes

Erythrocytes of female grey mullet are elliptical in shape and have a nucleus in the center (Figure 3). Human erythrocytes are donut-like in shape and have no nucleus.

The erythrocytes of female grey mullet sampled in July have major axis of 9.42 – 9.58 μm, minor axis of 6.33 – 6.67 μm and longitudinal area of 48.2 – 50.1 μm^2 calculated according to Ikeda, et al.[14] No significant differences ($p < 0.05$) in erythrocytes morphology were found between the 3 levels of vitamin E supplementation (Table 3).

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Table 2. Body weight, ovary weight, gonadosomatic index (GSI) and total vitamin E content in Ovary tissue of cultured 1+ female grey mullet fed with vitamin E (sampled in November).
1+ means 1-year-old grey mullet.

Dietary vitamin E (mg/kg)		Body weight (g)	Ovary weight (g/pair)	GSI * (%)	α-tocopherol (mg/pair)
added	total				
0	90	737 ± 73[a]	53.6 ± 5.3[c]	7.3 ± 0.7[e]	5.2 ± 0.5g
100	190	1089 ± 47[b]	126.5 ± 5.5[d]	11.6 ± 0.4f	14.1 ± 2.6h
200	290	1147 ± 123[b]	128.0 ± 13.7[d]	10.5 ± 2.4[f]	13.3 ± 1.4[h]

[a-h] Figures in the same column having different superscripts are significantly different (P(0.05).

$$* \mathrm{GSI} = \left(\frac{\text{gonad (ovary) weight}}{\text{body weight}}\right) \times 100$$

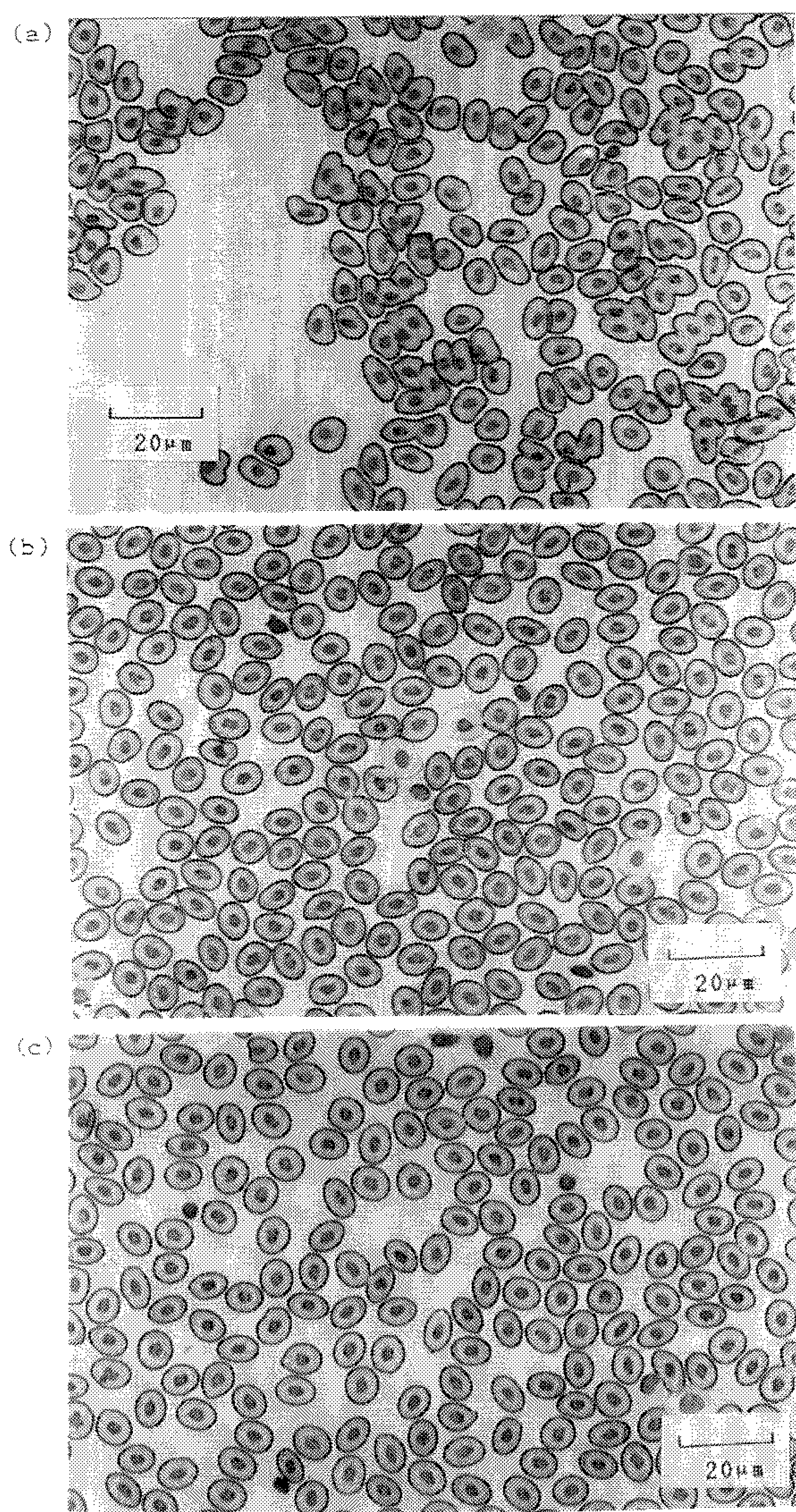

Figure 3. Blood smear of cultured 1+ female grey mullet fed with (a) 90 mg/kg vitamin E, (b) 190 mg/kg vitamin E, (c) 290 mg/kg vitamin E(Sampled in November).

Spontaneous hemolysis fo whole blood during incubation at 20℃ is shown in Figure 4. Immediately after mixing with a saline-phosphate buffer, the whole blood from grey mullet showed that spontaneous hemolysis ranged 1.04-2.88%, and increased to 7.55-7.71% in 8 hrs of incubation. The basal diet (vitamin E 90mg/kg) of fed mullet showed slightly higher spontaneous hemolysis than those fed with 190 and 290 mg/kg.

Rainbow trout on a vitamin E deficient diet showed hemolysis greater than 60%[23]. In vitro spontaneous hemolysis was greater than 85% for rats on vitamin E free diets, and reduced to less than 10% with supplementaion at 10 ppm level in tested rats[24]. α-tocopherol in red blood cell membrane prevents hemolysis in vitro in the presence of oxidizing agents[25].

A free radical initiator induced hemolysis when it was incubated with erythrocytes extracted from vitamin E -supplemented or deficient huuman subjects[26]. Humans on diets supplemented with α-tocopherol for 3 weeks increased resistence to oxidation of low density lipoprotein[27]. Dietary supplementation of α-tocopherol up to 800 u/d for 6 weeks resulted in 3.3 fold increase in plasma α-tocopherol and reducd susceptibility of LDL to oxidation[28].

Therefore, vitamin E functions as a free rad-

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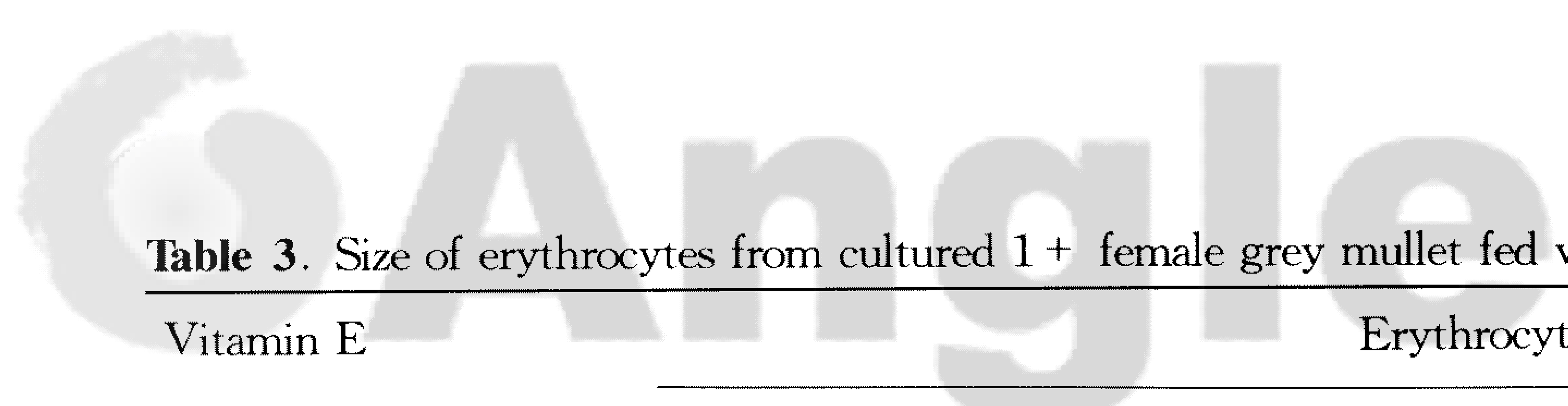

Table 3. Size of erythrocytes from cultured 1 + female grey mullet fed with vitamin E.

Vitamin E (mg/kg)	Month	Erythrocyte Major axis (um)	Erythrocyte Minor axis (um)	Erythrocyte Longitudinal area** (um^2)
90	Jul	9.26±0.12*	6.32±0.20	46.0±1.2
90	Nov	9.44±0.23	6.52±0.16	48.2±1.0
190	Nov	9.50±0.27	6.67±0.16	50.1±0.7
290	Nov	9.47±0.39	6.56±0.10	48.8±2.5

*Figures in the same column are not significantly different ($P>0.05$).
**Longitudina area = $\pi/4$ (Major axis x Minor axis)(池田等 1986)[24].

ical scavenger in blood to inhibit oxidation of lipoprotein and membrane; at the same time, it prevents hemolysis in humans, rats and fish. The commercial feed being used as basal diet that contains 90mg/kg of vitamin E is sufficient to prevent hemolysis in grey mullet.

CONCLUSION

The discovery by Rose and György[29] that erythrocytes of vitamin E -deficent rats hemolyze in the presence of mild oxidizing agents has been used to estimate the vitamin E requirements of animals and man[25]. When hemolysis is used as an indicator of vitamin E status of grey mullet, the basal diet appears to be sufficient. However, when gonad development is the determinant, then vitamin E supplementation to 190mg/kg is suggested based on the fact that plasma vitamin E was minimal in November when the size of ovary was maximal. In addition , body and ovary weight were significantly increased when the basal diet was supplemented with 100mg/kg vitamin E. Therefore it can be concluded that limited vitamin E intake prevents erythrocyte hemolysis . Additional vitamin E is needed to accelerate body growth and gonad development.

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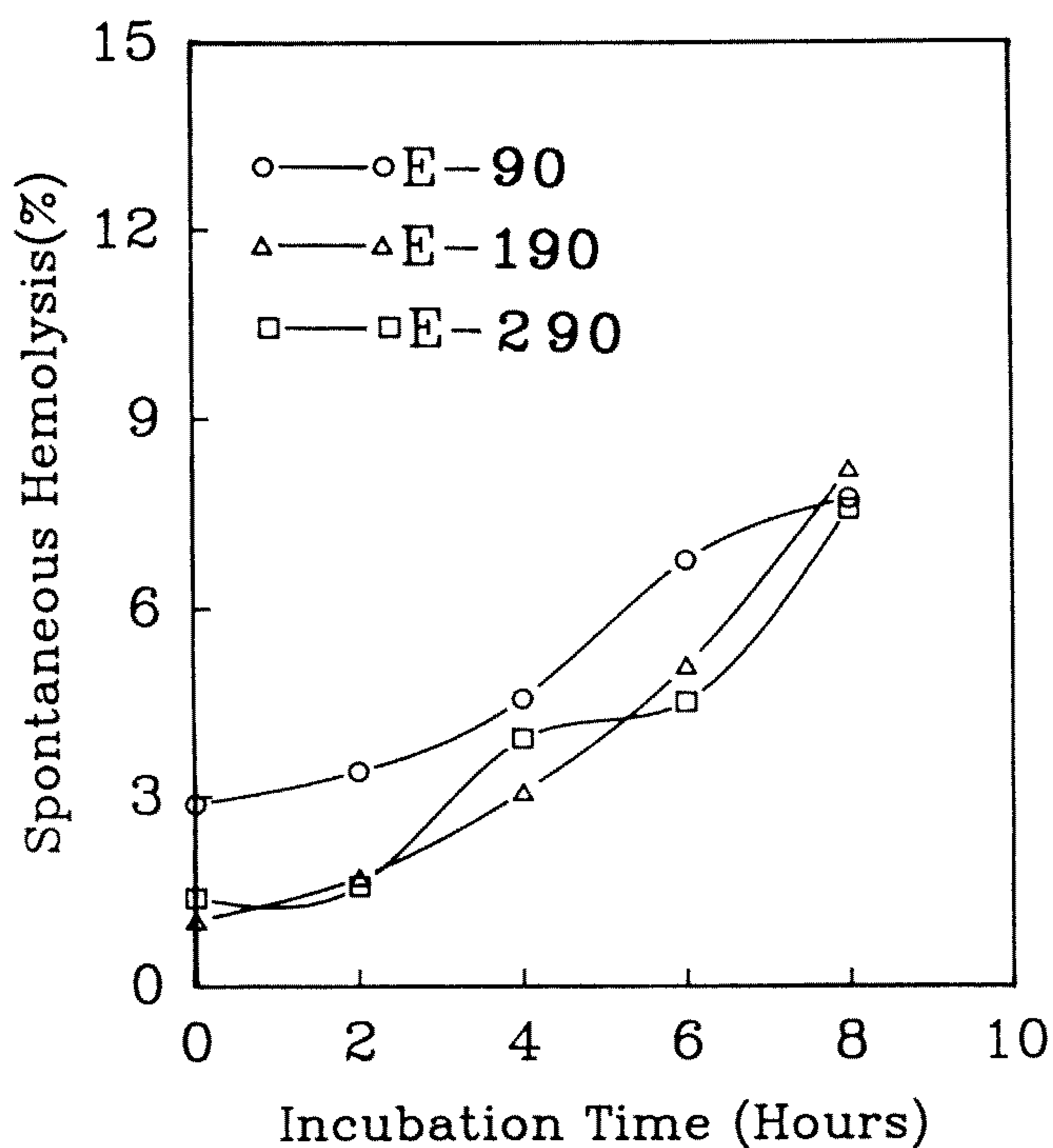

Figure 4. Hemolysis of erythrocytes from cultrued 1 + female grey mullet fed with vitamin E fortified diet in saline-phosphate buffer at 20℃ (sampled in November, five replicates at each level).

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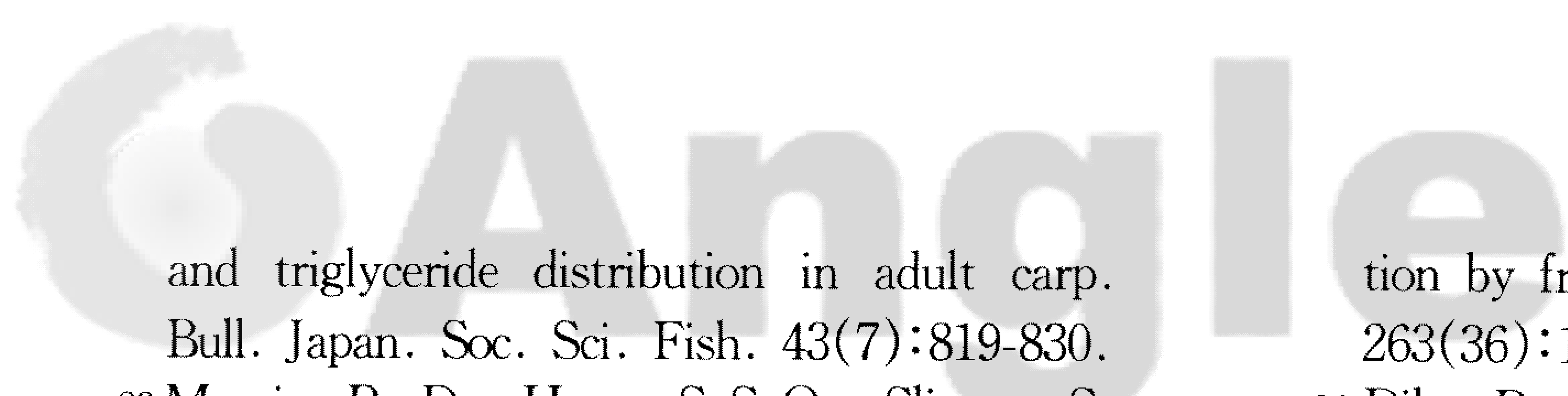

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養殖雌烏維生素 E 攝食量與血漿及卵中維生素 E 含量及溶血之關係

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摘　要

為探討養殖雌烏攝食維生素 E 對其血漿及卵中維生素 E 含量與成熟時紅血球之影響，以已含 90mg/kg α 型維生素 E 之基本飼料，添加 0、100、200mg/kg α 型維生素 E 投餵魚齡為一年之烏魚，飼養八個月。血漿維生素 E 濃度隨攝食維生素 E 量的增加而增加，隨生殖週期的接近而下降，至十一月時為最低，後上升。卵巢脂質中維生素 E 含量不因攝食 90-290μmg/kg 維生素 E 而異，但隨生殖週期而變化，以十月最低 296-376μg/g lipid，十一月最高 602-696μg/g lipid，十二月再下降至 408-453μg/g lipid，與血漿之維生素 E 濃度有互為消長的關係。烏魚的紅血球帶核，呈橢圓形，長軸大小介於 9.42-9.58μm 之間，短軸介於 6.33-6.67μm 之間，不因攝食維生素 E 量不同而明顯差異。紅血球經靜置 8 小時後，溶血率為 7.55-7.71%，溶血情形不因攝食維生素 E 量 90-290mg/kg 不同，而有明顯不同。但烏魚之體重及卵巢重則以攝食基本飼料者顯著低於攝食添加維生素至 190-290mg/kg 者(P〉0.05)。