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# GROWTH RESPONSE OF NILE TILAPIA FINGERLINGS (OREOCHROMIS NILOTICUS) FED DIETS CONTAINING DIFFERENT LEVELS OF CLOVE OIL

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**Key words:** Growth, Nile tilapia, *Oreochromis niloticus*, Fingerlings, feeding, Clove oil.

## ABSTRACT

A 16 week feeding experiment was conducted in fiberglass tanks with Nile tilapia ( *Oreochromis niloticus* ) fingerlings of average weight ( 23.2 ± 0.1 g ) to examine the effect of supplemental clove oil in prepared diet on its growth . Four isonitrogenous (34.3± o.6 % crude protein) and isocaloric ( 4720 ± 91 Kcal/kg gross energy ) diets were formulated according to a high quality commercial tilapia diet, containing 10 % fish meal , 20 % meat meal and 24 % soybean meal. Diets B, C, D was supplemented with various percentage of clove oil (4, 8, 12) mg/100 g diets respectively. After 16 weeks, the results showed that individual fish weight, length , feed conversion ratio , feed efficiency ratio , protein effeciency ratio and feed intake were significantly different ( $p \leq 0.01$ ) among treatments and diet (C) showed high value with clove oil supplemented with 8mg/100g diet . Fish flesh composition was also significantly different ($p<0.01$) and fish fed on diet (C) had high value of protein and fat content. This suggests that clove oil appears to meat criteria used as antioxidant and stimulant for fish growth.

## INTRODUCTION

Clove oil is derived from the stem, leaves and buds of the *Eugenia caryophyllata* tree and its active ingredient eugenol (4 '- allyl 2 methoxyphenol), comprises 70-90 % by weight of the oil of cloves (Isaacs, 1983) . Eugenol has many properties that make it useful in a wide variety of applications,: as an antioxidant (Kramer, 1985; Nagababu & Lakshmaiah , 1992; Pulla & Lokesh, 1992; Rajakumar & Rao, 1993), as antifungal agent ( Bullerman, *et al.* 1977 ; Karapmar & Aktug, 1987; Briozzo, *et al.* 1989; Moleyar & Narasimham, 1992 ), as an additive in Kretec cigarettes ( La Voie, *et al.* 1986 ;Council on Scientific Affairs , 1988; Guidotti , 1989) , and as an analgesic and local anaethetic in dentistry (Paffenbarger & Rupp, 1972 ; Curtis , 1990 ). It is stimulant for growth and has antioxidant properties which are of great interest in the study of eugenol and its effect on fish.

The present study was designed to determine if clove oil when added to the diets with its ingredient, eugenol is effective as stimulant and as an antioxidant for fish. Also, if it could be used safely, efficiently and economically in aquaculture and aquatic research.

## MATERIAL AND METHODS

**Diet preparation :**

The practical diets were formulated to contain 34.5 % crude protein and 12.1 % crude fat ( Table 1 ) . All diets were prepared to be similar to high quality tilapia fish feed. The diets B, C, D have various concentrations (4,8,12 mg / 100g of diet ) of supplemental clove oil respectively . Amino acid composition of the diets were calculated from tabular values provided for diet ingredients (NRC, 1993). All diets were formulated to be isonitrogenous and isocaloric ( 4720 Kcal /kg diet ) .

In preparing diets, dry ingredients were first ground to small particle size in wiley mill. Ingredients were thoroughly mixed, the clove oil was dissolved in 10 ml ethyl alcohol then added to diets . Also the

control diet was supplemented with 10 ml ethyl alcohol without clove oil and adding water to obtain 30 % moisture level. Diets were passed through a mincer with 0.4 mm diameter forming spaghetti like strands and were dried under sun for 8 hours. Percentage of protein of diets was determined by micro kjeldahl, percentage of fat by ether extract, moisture by drying (100°C) until constant weight, crude fiber and ash (Muffle burning), using the procedure of the Association of official Analytical Chemists (1990). The calorific values of the diets were calculated using the gross calorific equivalents of 5.65 kcal /g protein, 9.45 kcal/g fat, and 4.1 Kcal /g Carbohydrate (Brett ,1973).

**Experimental organisms :**

A set of 120 Nile tilapia (*Oreochromis niloticus*) fingerlings (average fish weight 23.18 ± 0.86 g and average length 11.96 ± 0.4 cm) were used in the feeding trial. The experiment was carried out in Shebin El - Kom , Faculty of Agriculture Menofyia University .Ten fingerlings were randomly stocked into each fiberglass tank with three replications per treatment. They were maintained in well aerated water at a temperature of 28.4 ± 0.8 °C, pH 7.5 ± 0.1 , dissolved oxygen of 6.0 ± 0.2 mg/L , total ammonia 0.2± 0.1 mg/ l , nitrite , 0.07 ± 0.02 mg/l and total alkalinity 180 = 6 mg/l according to the method of Golterman (1977).

**Experimental design:**

The feeding trial was conducted in 12- fiberglass tanks, each containing 1 $m^3$ of tap water. About one third of the water volume, in each tank, was daily replaced by aerated fresh water after cleaning and removing accumulated excreta. All tanks were supplied with compressed air for oxygen requirement, continuous illumination was supplied by fluorescent ceiling lights.

All fish were fed the quantity of food they could consume, twice daily (at 0800 and 1600 h) for 16 week. At the start and end of the feeding trial, 15 and 4 fish per tank respectively, were killed by decapitation, then fish flesh was homogenized in a blender, stored in polyethylene bags and frozen for subsequent protein, fat, moisture and ash analysis according to AOAC (1990) .

**Growth parameters:**

Growth performance and feed conversion were measured in terms of final individual fish weight (g), total length (mm), survival (%), feed conversion ratio (FCR), protein efficiency ratio (PER), feed intake (g/fish). Growth response parameters were calculated as follows: FCR=live weight gain (g)/ dry food given (g) X100, PER = live weight gain (g) / protein given n (g), feed intake = total dry feed consumed (g) /fish (Richardson, *et al.*, 1985).

**Blood analysis :**

The blood was obtained by arterial puncture at the end of experiment. Coagulation was prevented by the use of heparine . For erythrocyte counts, saline solution (0.7 %. Nacl and Handrich's diluting fluid (1952 ) were used . Shaw's solution (1930) was used for white cell count , Giemsa stain was found suitable . The microhaematocrit method was used to measure the packed cell volume. Blood mixed with anticoagulant was collected in duplicate standard capillary tubes and centrifuged for 15 minutes at 500 r.p.m. Haemoglobin levels were measured by the alkali haematin methods ( Oser *et al.* , 1965) .

**Statistical analysis :**

Data were analyzed by analysis of variance (ANOVA) using the SAS ANOVA procedure (Statistical Analysis System, 1988). Duncan's multiple range test was used to compare differences among individual means. Treatment effects were considered significant at $P \leq 0.01$ .All

percentage and ratio were transformed to arcsin values prior to analysis ( Zar , 1984) .

## RESULTS

### Weight gain:

Fish fed on the control (diet A) showed no change in direction and avoiding the source of stimulus. By increasing the level of clove oil in the diets, increased the respond of fish to different levels of clove oil . Also fish exhibited a slight loss of reactivity to direction and this phenomenon increased by increasing the level of clove oil. This response appeared on increasing fish weight and length. Fish fed on diet (C) were significantly ($p \leq 0.01$) longer ($17.5 \pm 0.16$ cm) than fish fed on other diets. However, fish fed diet A,B,D were not statistically different (Table 2 ). Also the final fish weight of Nile tilapia fed on diet (c) supplemented with 80 mg cloves oil per kg diets significantly showed higher difference ($p \leq 0.01$ ) when compared with other treatment diets( A,B, D) .

Percentage weight gain , and fish final weight , showed the same trend as before , where diet ( C) was superior to other diets.

### Feed consumption:

Dietary clove oil concentration had a marked significant effect ($p \leq 0.01$) on feed conversion ratio. Feed consumption increased with increasing dietary clove oil up to 80 mg /kg. diet (C) then feed consumption decreased but still higher than control . The highest protein efficiency ratio (PER) was observed when fish were fed on diet (C) containing 80 mg / kg of diet (Table 3) . Feed efficiency ratio has been found to reach its maximum when using clove's oil up to 80 mg /kg (diet C) (Table3 ).

**Body composition :**

Chemical analysis of fish flesh at the end of the feeding trial resulted in significant differences ( $p \leq 0.01$ ) in moisture fat, protein and ash in weight due to the effect of clove oil .

A general tendency of fat increase was observed in all treatments with their growth rate and age. This increase was remarkably higher in group (C) fed on diet supplemented with 80mg clove oil. Similarly, the results on wet weight basis, protein percentage increased with increasing clove oil level, especially in tilapia fed on diet C (Table 4). Statistical differences are observed in the body composition (on wet weight bases) of *Oreochromis niloticus*. Erythrocyte count increased markedly by increasing dose of clove oil and reached maximum at 80 mg / kg of diet then decreased on diet (D) containing 120 mg clove oil / kg of diet.

There are remarkable variations in erythrocyte volume. On using the volume of erythrocyte, diet (A) was high, while decreased in case of diet (C) as shown in table (5).

**Haemoglobin content:**

There are considerable changes in haemoglobin content of fish fed on clove oil , and these changes increased on increasing the content of clove oil in diet to level of 80 mg / kg diet. It seems that it is the only erythrocytic property which is highly affected by clove level.

**White blood corpuscles:**

Changes in the total number of leucocytes and their differential counts in *Oreochromis niloticus* fed on different diets containing different levels of clove oil at the end of the experiment, was observed with a very slight increase in case of fish fed on diet (c).

## DISCUSSION

The results suggest that clove oil is acting as stimulant for growth due to its effect as antioxidant, which prevent oxidative rancidity or lipid per oxidation, that can cause reduction of the nutritional value of fat, certain vitamins and other feed components. Care should be taken to

include natural antioxidant e.g clove oil and vitamin E or synthetic antioxidants and safe margins for the labile vitamins as a matter of course in diet formulation (NRC, 1993). This information, coupled with its long use as a local antioxidant (Kramer, 1985; Nagababu & lakshmaiah 1992; Pulla Reddy, Lokesh, 1992 and Rajakumar & Rao, 1993), suggest that clove oil can be regarded as a true antioxidant used with B.H.T. (butylated hydroxytoluene) and ethoxyquin (1.2 dihydro 6 ethoxy 2,2,4 trimethylquinoline) which are effective synthetic antioxidants commonly used in animal feeds( Rumsey , 1980) .

Dietary fats that have undergone peroxidation may have detrimental effects on fish growth. The process can either be prevented or halted by adding ample levels of clove oil. Koenig (1981) indicated that antioxidants, prooxidants, polyunsaturated fatty acids and selenium are closely related in the nutrition of animals and that all should be carefully considered for optimum nutrition, health and economy.

The regulation of lipid peroxide oxidation is at least important as the inactivation of oxygen free radical, because with age, the accumulated lipid peroxides accelerate the organism senescence. Also lipid peroxides inhibit the cell division and retard thereby the healing of damaged tissues (Stroeve, 1989). Environmental factors (ionizing and ultraviolet radiation and metals) elicit formation of fatty acid radicals and peroxide radicals deleterious to the membrane function. Stroeve (1989) reported that compounds that activate peroxide oxidation (prooxidants) are (1) vitamins A and D. and naphthoquinones (2) Reductants, NAD.H and lipoic acid (3) Free- radical metabolites as produced by the action of the prooxidants.

Rajakumar and Rao (1993) reported that clove oil containing dehydrozingerone and isoeugenol act on interrupting process of prooxidants of lipid due to the presence of free radical scavengers which inhibit oxygen free radicals.

Also the stimulant effect of clove oil result from the inhibition of prostaglandin H synthase (PHS) by eugenol (Dewhirst & Goodson, 1974; Thompson & Eling, 1989; Karapmar, 1990, Pongprayoon, *et al.*, 1991). The major area of entry and excretion of egunol in fish is through the gills and the rate of passage through the gills depends largely on its degree of ionization and lipid solubility (Locke, 1969; Brandenburger, *et al.*, 1972; Hunn & Allen, 1974; Ferreira, *et al.*, 1984)

The toxicity (LC50) of eugenol for Nile tilapia fingerlings is in normal level not more 52 - 81 ppm and is similar to that of Marking, (1966). Eugenol has been widely tested to determine its safety for human use and consumption. Clove oil and eugenol are all listed as generally recognized as safe (1500 p.p.m) (USFDA, 1978). Furthermore eugenol and its conjugates and metabolites are rapidly lost from the blood stream and tissues in man (Fischer & Dengler, 1990) and are considered neither toxic nor carcinogenic in man and other animals including rats, mice and Chinese hamesters (Liu & Gibson, 1977, Maura, *et al.*, 1989; Fischer, *et al.*, 1990, Phillips, 1990, Zheng, *et al.*, 1992. This suggests that the levels of eugenol used as stimulant agent for fish may have little negative effect on fish, and little or no subsequent effects on human who consume fish treated with clove oil As clove oil is generally recognized as safe, a withdrawal period may not be necessary for fish exposed to it. The length of recovery time for fish exposed to clove oil (containing eugenol) has little effect on the efficient operation of aquaculture facility especially when no mortalities are observed in fish. Ross & Ross (1984) suggested that clove oil has persistent or latent negative effects upon fish physiology or behaviour.

In conclusion Clove oil appears to meet criteria used to define as an ideal antioxidant and stimulant for fish growth (Kramer, 1985, Nagababu & lakshmaiah, 1997, Pulla Reddy & Lokesh, 1992). Its main advantage is low cost and safe to both fish and human. The recommended

dose of clove oil is 80 mg/kg diet that will produce high fish growth. High levels (>80mg) of clove oil may be used as anesthetic for the purpose of transport.

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Sheet1 Chart 2

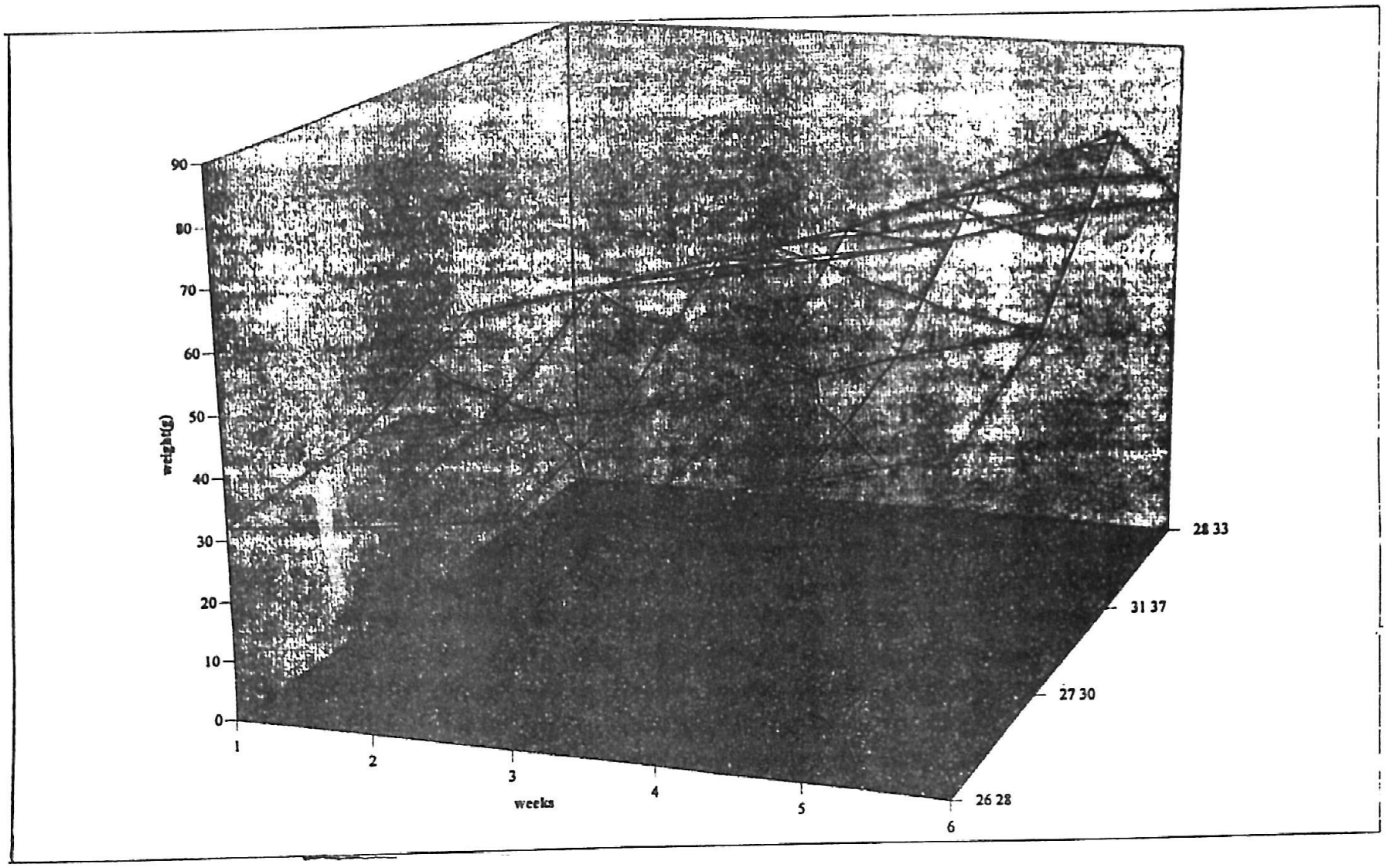


**Fig (1) : Changes in body weight (g ) of Nile tilapia fed different experimental diets ( See material and methods )**

**Table 1. Composition of diets similar to a high quality commercial Nile tilapia diet ( with fish meal ) and experimental diets ( with different levels of clove oil ) fed to Nile tilapia fingerlings**

| | Diets | | | |
|---|---|---|---|---|
| | A | B | C | D |
| * Ingredient (%) | | | | |
| Menhaden fish meal (60 % C.P) | 10 | 10 | 10 | 10 |
| Meat meal (55 % C.P) | 20 | 20 | 20 | 20 |
| Soybean meal (44 % C.P) | 24 | 24 | 24 | 24 |
| Wheat bran | 20 | 20 | 20 | 20 |
| Corn meal | 20 | 20 | 20 | 20 |
| Oil | 5 | 5 | 5 | 5 |
| [1]Premix | 1 | 1 | 1 | 1 |
| Clove oil mg/ 100 g diet | 0 | 4 | 8 | 12 |
| * Nutrient composition ( %) | | | | |
| Moisture | 5.9 | 6.4 | 7.3 | 6.6 |
| Crude protein | 34.7 | 34.1 | 33.5 | 34.7 |
| Crude fat | 12.1 | 12.4 | 12.5 | 14.4 |
| Ash | 12.9 | 10.2 | 9.6 | 9.8 |
| Crude fiber | 5.4 | 5.6 | 5.5 | 5.3 |
| [2]CHO | 29.0 | 31.3 | 31.6 | 29.2 |
| [3]Gross energy Kcal /Kg diet | 4617 .6 | 4722 . 1 | 4706 .4 | 483 7.35 |

1-Premix : supplied the following vitamins and minerals (mg or IU ) Kg of diet : vit A, 8000 IU; vit. D3 , 4000 IU; vit E, 50 IU; vit. $k_3$ 19 IU;vit. $B_2$ 25 mg ; vit. $B_3$ , 69mg, Nicotinic acid , 125 mg ; Thiamin , 10mg, Folic acid , 7mg; Biotin 7mg ; vit.$B_{12}$, 75 mg; cholin , 400 mg ; vit.C, 200mg; 350mg Manganese; 325mg Zinc; 350 mg Iron ; 0. 4 mg Iodine ; 2 mg cobalt, 7 mg copper; 0. 7 mg Selenium ; 0.7 mg B.H.T according to (lovell, 1989 ) .

2. CHO by difference

3. Gross energy , Protein = 5.56 Kcal /g ; Fat = 9.45 Kcal/g; Carbohydrates = 4.1 Kcal /g according to ( Brett, 1973)

**Table 2. Growth parameters and survival rate of Nile tilapia fingerlings fed diets containing different levels of clove oil.**

| Diets | Length (cm) | | weight (g) | | Percentage weight gain | survival (%) |
|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | | |
| A | 11.96± 0.6 | 14.2 ± 1.9$^{b}$ | 231±0.9 | 52.7±1 1.1$^{b}$ | 128.2± 12.1$^{c}$ | 100 |
| B | 11.96±0.4 | 15.8±. 1.6$^{b}$ | 23.1 ± 1.0 | 52.9± 1.8$^{b}$ | 159.3± 14.1$^{b}$ | 100 |
| C | 12.0± 0.5 | 17.5 ± 1.6$^{a}$ | 23.2± 1.0 | 83.2± 1.3$^{a}$ | 261.2±13.9$^{a}$ | 100 |
| D | 11.9 ± 0.7 | 15.3±1.6$^{b}$ | 23.3± 0.8 | 62.7= 1.5$^{b}$ | 169.6± 10.6$^{b}$ | 100 |

Percentage weight gain = (Weight gain (g)/ initial weight) 100

a, b. c means in the same column bearing different letters differ significantly at 0.01 level .

**Table 3. Efficiency of feed and protein utilization by Nile tilapia fingerlings fed diets containing different levels of clove oil**

| Diets | Clove oil per 100 g feed | [1]feed conversion ratio | [2]protein efficiency ratio | [3]feed efficiency ratio | Total feed intake/ fish | Feed intake per day |
|---|---|---|---|---|---|---|
| A | - | 3.6± 1.1$^{a}$ | 0.9±0.3$^{b}$ | 30.2±9.2$^{c}$ | 96.7 ± 2.9$^{b}$ | 1.01 |
| B | 4 | 2.9 ± 0.6$^{b}$ | 1.1±0.2$^{b}$ | 36.0± 8.4$^{a}$ | 103.7±3.6$^{b}$ | 1.1 |
| C | 8 | 2.2±0.6$^{c}$ | 1.4 ± 0.5$^{a}$ | 49.0±17.6$^{a}$ | 132.2 ± 6.3$^{a}$ | 1.4 |
| D | 12 | 2.9 ± 0.8$^{b}$ | 1.1 ± 0.3$^{b}$ | 36.8±11.6$^{b}$ | 113.2 ± 4.2$^{b}$ | 1.18 |

1.Feed conversion ratio = dry food consumed (g ) / live weight gain (g ) .

2.Feed efficiency ratio = live weight gain(g)/ dry food given (g) X 100

3. Protein efficiency ratio = live weight gain (g)/ protein intake( g ).

a, b , c , means in the same column bearing different superscripts differ significantly at 0.01 level.

**Table 4. Effect of different levels of clove oil on chemical composition of fish flesh on wet weight basis .**

| Diets | Clove oil mg/100g. diet | Moisture % | Crude protein % | crude fat % | Ash % |
|---|---|---|---|---|---|
| A | - | $78.9 \pm 0.5^{b}$ | $16.6 \pm 50^{b}$ | $2.75 \pm 0.16^{b}$ | $2.6 \pm 0.2^{b}$ |
| B | 4 | $77.9 \pm 0.2^{b}$ | $16.6 \pm 0.1^{b}$ | $2.7 \pm 0.1^{b}$ | $2.9 \pm 0.^{b}$ |
| C | 8 | $74.9 \pm 0.6^{a}$ | $19.4 \pm 0.1^{a}$ | $3.1 \pm 0.1^{a}$ | $2.6 \pm 0.1^{a}$ |
| D | 12 | $77.1 \pm 0.1^{b}$ | $17.3 \pm 0.2^{b}$ | $2.4 \pm 0.3^{b}$ | $3.1 \pm 0.2^{b}$ |

a, b, c means in the same column bearing different superscripts are significantly different

( $P < 0.01$ )

**Table 5. Effect of different levels of clove oil on blood characteristic of *Oreochromis niloticus.***

| Blood characteristic | Level of clove oil in mg/100g of diet | | | |
|---|---|---|---|---|
| | 0 | 4 | 8 | 12 |
| Erythrocyte (million/$mm^3$) | 0.93±0.1 | 1.03± 0.1 | 1.62± 0.2 | 1.13 ± 1.2 |
| Haemeglobin content (g %) | 6.3± 0.7 | 6.7± 1.2 | 7.7± 0.2 | 6.6± 0.0 |
| Hematocrit value (%) | 37.03 ±1.8 | 40.1 ±1.9 | 45.58±3.3. | 38.75± 43 |
| Erythrocite volume ($M^3$) | 407.1±62.3 | 395.7 ±51.8 | 285.6 ±37.7 | 358.65±76.1 |
| Total leucocyte (million /mm3) | 8.504±0.1 | 9.213 ±0.1 | 10.312±0.2 | 8.4641±0.1 |
| Total serum protein (%) | 3.3± 0.2 | 3.5± 0.1 | 3.7±0.2 | 3.4±0.1 |