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EFFECT OF 3, 5, 3'-TRIIODOTHYRONINE (T_3) HORMONE ON NUCLEIC ACID AND PROTEIN CONTENT OF THE MUSCLE AND THE GROWTH OF GIANT GOURAMY, *OSPHRONEMUS GOURAMY LAC.*

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ABSTRACT

This experiment was conducted to study the effect of 3, 5, 3'-triiodothyroninc (Tj) hormone on nucleic acid and protein content of the muscle and the growth of giant gouramy, *Osphronemus gouramy* Lac. Five experimental diets, which contain isocaloric diets, but different in T_3 hormone level were used in this experiment (0.0, 2.5, 5.0, 7.5, and 10.0 mg T_3 hormone/kg diet). The experimental diets were tested to three different groups offish for sixty days feeding trial. Fish body weight in groups I, II, and III were 0.39-0.42 g/fish; 19.11 -21.99 g/fish, and 37.52-40.79 g/fish, respectively.

The results showed that the highest RNA, DNA concentration and RNA/DNA ratio of the muscle were produced by 10.0 mg T_3 hormone/kg diet for group I and II; and 2.5 mg T_3 hormone/kg diet for group III. Similar results also were found for the protein content of the whole body, protein retention, and the daily growth rate of the fish.

Key words : *Osphronemus gouramy/Ti* hormone/nucleic acid/protcin/growth

INTRODUCTION

Giant gouramy is well known as a slow growing fish that has been traditionally cultured for a long time in West Java, Indonesia. Intensification of the culture of this fish has been started recently. However, our studies indicated that the growth of the fish could be enhanced by fulfilling their nutrient requirements (Mokoginta *et al.* 1994, 1995a & b, 1996). In addition to fulfilling their nutrient requirements, growth could be enhanced by hormone treatment as suggested by Matty (1985).

Some studies on thyroid hormone treatment revealed that the hormone plays an important role on somatic growth of fish (Donaldson *et al.* 1979; Fagerlund *et al.* 1984; Matty *et al.* 1982). It was assumed that thyroid hormone could activate protein synthesis in liver and muscle cells (Jackim and La Roche 1973; Narayansingh and Bales 1975; Medda and Ray 1979; Matty *et al.* 1982) by increasing RNA and DNA concentration of liver and muscle cells. Woo *et al.* (1991) showed that administration of 3, 5, 3'- triiodothyronine (T_3) hormone in diet

of red sea bream increased the growth rate, feed efficiency, digestive enzymes activity in the intestine and activities of other key enzymes in various pathways of carbohydrate metabolism.

T3 affects protein synthesis by biphasic mode i.e., anabolic at lower dose, but catabolic at higher dose (Matty and Lone 1985; Medda and Ray 1979). Medda and Ray (1979) showed that the administration of thyroxine (T_4) hormone at the dose of 2 or 4 mg/g body decreased protein accumulation and RNA content in the liver and brain, whereas dosage of 1 mg/g body weight increased protein accumulation and RNA content in liver and brain.

Therefore, in this experiment, we study the effect of the administration of *Tj* hormone in diet on nucleic acid and protein content of the muscle and the growth of giant gouramy.

MATERIALS AND METHODS

Fish

Experimental fish were obtained from a fish farmer in Parung. The fish were divided into three groups based on body weight. Group I was of 0.39 - 0.42 g body weight, group II was of 19.11 - 21.99 g body weight and group III was of 37.52 -40.73 g body weight. Fish were reared in aquaria (50 x 30 x 30 cm, 20 1 volume) with ten fish in each aquarium (Group I), while for fish of group II and III with one fish in each aquarium.

Fish were fed three times daily to satiation for 60 days. To maintain water quality, 70 - 80 % of total water volume was replaced and feces were siphoned out every day. Aquaria were cleaned and water was totally replaced once a week. This experiment was conducted at water temperature of 27 - 30°C; pH 7.5 - 8.0; DO 6.28 - 7.2 ppm; and ammonia 0.08 - 0.13 ppm.

Diets

Semi-purified diets were used in this study. The fish were fed with a diet of 43 % protein level (Group I), while the other fish (groups II and III) were fed with 32 % protein level. All diets have protein-energy ratio of 8 kcal DE/g protein. The different protein levels between groups were based on the protein requirement of giant gouramy (Mokoginta *et al.* 1994,1995 a & b, 1996).

The diet was supplemented with 0, 2.5, 5.0, 7.5, and 10 mg of T_3/kg. Before mixing, T_3 was dissolved in 70 % alkaline alcohol (33 ml 90% ethanol - 12 ml 0.1 N NaOH) and the solution was sprayed on the diet. The control diet was sprayed with alkaline ethanol without T_3. Following supplementation of T_3, the diets were air dried and then stored in a freezer (-20°C) until feeding.

The composition of diets for fish in group I, II and III are shown in Tables 1 and 2, respectively.

Table 1. The proximate composition of the experimental diets for fish in group I

Proximate composition	T_3 levels (mg/kg diet)				
	A (0)	B (2.5)	C (5)	D (7.5)	E (10)
Protein	43.35	43.28	43.17	43.35	43.03
Lipid	16.08	16.12	16.06	16.10	16.11
Ash	4.91	4.97	5.43	5.13	5.42
NFE	33.80	33.82	33.53	33.63	33.63
Crude fiber	1.86	1.81	1.81	1.79	1.82

Table 2. The proximate composition of the experimental diets for fish in groups II and III

Proximate composition	T_3 levels (mg/kg diet)				
	A (0)	B (2.5)	C (5)	D (7.5)	E (10)
Protein	32.08	32,29	32.27	32.24	32.24
Lipid	1.99	1.95	1.97	1.98	1.96
Ash	5.70	5.53	5.67	5.73	5.69
NFE	57.73	57.63	57.54	57.64	57.68
Crude fiber	2.50	2.60	2.55	2.41	2.43

Data collection and chemical analysis

Each group of fish was weighed and taken for proximate analysis at the beginning and the end of the experiment. RNA and DNA concentrations were determined at the end of the experiment.

Proximate analyses were determined using methods as described by Takeuchi (1988). Analysis of RNA concentration was determined by the orcinol method, while for DNA concentration by diphenilamin method (Plummer 1979).

Calculation and statistical analysis

Daily growth rate (DGR) was calculated according to equation of Huisman (1976) : $Wt = (Wo + 0{,}01\ a)^1$ where Wo and Wt are initial and final mean of body weight, respectively, t is the number of days, and a is daily growth rate (%/day).

Protein retention was calculated according to the equation of Takeuchi (1988): Protein retention (%) =(total protein content of body (final) - total protein content of body (initial)) (g)/ total protein consumed (g) X 100.

The study was designed using complete randomized design with five treatments and three replications. The effect of T_3 hormone on protein retention, RNA and DNA concentration, and RNA/DNA ratio and growth rate were evaluated by using analysis of variance followed by polynomial orthogonal test (Steel and Torrie 1993).

RESULTS AND DISCUSSION

RNA and DNA concentrations and RNA/DNA ratio in muscle are shown in Table 3. The Table shows that RNA/DNA ratio increased following T_3 levels supplemented into the diet. Increasing T_3 level from 2.5 mg T_3/kg diet to 10 mg T_3/kg diet increased RNA/DNA ratio in groups I and II. The highest level of T_3 hormone (10 mg T_3/kg diet) caused an increase in RNA/DNA ratio to 10.8 % and 4.4 % over control in groups I and II, respectively. In group III, increasing RNA/DNA ratio (2.3% over control) was obtained at the level of 2.5 mg T_3/kg diet, but the

Table 3. RNA and DNA concentration, and RNA/DNA ratio of the muscle from fish in groups I, II, and III.

T_3 level (mg T_3/kg diet)	RNA (mg/g of muscle)	DNA (mg/g of muscle)	RNA/DNA Ratio
1. Group I (0.39 – 0.42 g/fish)			
A (0.0)	400.30 ± 6.89	270.39 ± 6.02	1.48 ± 0.03
B (2.5)	430.65 ± 5.83 (7.6 %)*	289.92 ± 1.35 (7.2 %)*	1.49 ± 0.01 (0.68 %)*
C (5.0)	442.10 ± 0.00 (10.4 %)*	290.59 ± 2.24 (7.5 %)*	1.52 ± 0.01 (2.7 %)*
D (7.5)	468.21 ± 2.69 (17 %)*	295.13 ± 0.00 (9.2 %)*	1.59 ± 0.01 (7.4 %)*
E (10.0)	506.97 ± 6.24 (26.6 %)*	309.31 ± 1.77 (14.4 %)*	1.64 ± 0.08 (10.8 %)*
2. Group II (19.11 – 21.99 g/fish)			
A (0.0)	517.65 ± 1.07	379.83 ± 0.00	1.36 ± 0.00
B (2.5)	529.07 ± 12.14 (2.2 %)*	385.42 ± 7.52 (1.5 %)*	1.37 ± 0.01 (0.7 %)*
C (5.0)	544.45 ± 1.81 (5.2 %)*	388.41 ± 7.71 (2.3 %)*	1.40 ± 0.02 (2.9 %)*
D (7.5)	562.42 ± 10.71 (8.7 %)*	398.51 ± 8.59 (4.9%)*	1.41 ± 0.06 (3.7 %)*
E (10.0)	567.17 ± 11.21 (9.6 %)*	398.64 ± 3.27 (4.95 %)*	1.42 ± 0.09 (4.4 %)*
3. Group III (37.52 – 40.79 g/fish)			
A (0.0)	563.90 ± 0.00	428.79 ± 0.16	1.32 ± 0.00
B (2.5)	591.92 ± 3.54 (5 %)*	439.70 ± 1.46 (2.5 %)*	1.35 ± 0.00 (2 .3%)*
C (5.0)	588.28 ± 2.19 (4.3 %)*	442.12 ± 2.23 (3.1 %)*	1.33 ± 0.02 (0.76 %)*
D (7.5)	522.93 ± 3.45 (- 7.3 %)*	426.71 ± 0.00 (-0.49%)*	1.23 ± 0.01 (-6.8 %)*
E (10.0)	508.05 ± 3.55 (- 9.9 %)*	426.39 ± 1.87 (-0.56 %)*	1.19 ± 0.00 (-9.85 %)*

RNA/DNA ratio (Y) – T_3 level (X) correlation:
Group I : $Y_1 = 1.46 + 0.02X_1$; $r^2 = 0.93$
Group II : $Y_2 = 1.36 + 0.01X_2$; $r^2 = 0.96$
Group III : $Y_3 = 1.33 + 0.008X_3 - 0.002X_3^2$; $r^2 = 0.87$

* percentage of increase/decrease in RNA concentration, DNA concentration, RNA/DNA ratio compared to control (treatment A).

Several studies showed that RNA concentration and RNA/DNA ratio were closely related to growth and nutritional status of fish. Therefore, RNA concentration and RNA/DNA ratio could be used as an indicator of fish growth and nutritional status. It is commonly accepted that RNA concentration in liver or muscle of fish may reflect protein synthesis, where increase in RNA concentration was followed by a rise in protein synthesis (Yang and Dick 1993). RNA/DNA ratio is index of metabolic activity and change in RNA/DNA ratio reflects change in protein synthesis (Brafield 1985; Widler and Stanley 1983). Correlation between administration of T_3 and protein synthesis might be explained by the effects of T_3 hormone on the increase of RNA concentration through interactions between T_3 hormone and receptor in nucleus which activated enzyme for RNA synthesis (Matty *etal.* 1982; Djojosoebagio 1990).

This study showed that administration of 10 mg T_3/kg diet in groups I and II increased RNA and DNA concentration, RNA/DNA ratio to 26.6 %, 14.4% and 10.8% over control, respectively, for group I; and 9.6%, 4.95%, and 4.4% over control, respectively, for group II. While in group III, the highest value of RNA/DNA concentrations and its ratio were reached at 2.5 mg T_3/kg diet i.e. 5%, 2.5% and 2.3% over control, respectively (Table 3).

As mentioned above, increasing RNA concentration in muscle was followed by a rise in protein synthesis, RNA/DNA ratio and growth rate of fish. Alien *et al.* (1979) defined growth as increasing mass by two fundamental biological processes; protein accretion and proliferation cell.

This study, clearly showed that administration of 10 mg T_3/kg diet in groups I and II increased RNA concentration (26.6 % and 9.6% over control, respectively) and protein retention (30 % and 16.5 % over control, respectively). The ability to retain the body protein was followed by an increase in growth rate offish (9.3% and 14.4 % over control, respectively) and also reflected by an increase in RNA/DNA ratio (10.8 % and 4.4% over control, respectively) (Tables 3 and 4).

In group III, higher RNA concentration was attained at 2.5 mg T_3/kg diet and followed by increasing body protein level (5 % over control). Furthermore, protein retention and growth attained a higher value, an increase of 10 % and 17.5 % over control, respectively (Tables 3 and 4). The effect of T_3 hormone on protein synthesis is biphasic, i.e. anabolic at low doses and catabolic at higher dose (Medda and Ray 1979). As RNA and protein concentrations in a cell depend upon the synthetic and degradation rate, and as the thyroid hormones increase the turnover rate of this cellular constituents, it is possible that at higher doses the turnover rate becomes much higher and the degradation rate surpasses the synthetic rate (Goldberg *et al.* 1980 *in* Matty *et al.* 1982). In this study, biphasic effect of T_3 was only shown in group III, but not in other groups.

In conclusion, all groups in this study showed that the effect of T_3 hormone administration on RNA and DNA concentration, RNA/DNA ratio, body protein level, protein retention, and daily growth rate depend on fish size, in which the difference of potential growth rate was produced by fish size.

Table 4. Protein content of the body, protein retention and daily growth rate (DGR) of fish from groups I, II, III.

Feed/ T_3 Level (mg/kg of feed)	Protein Content of Body (% of wet weight)	Protein retention (%)	DGR (%/day)
1. Group I (0.39 – 0.42 g/fish)			
A (0.0)	15.12	31.07 ± 0.53	4.20 ± 0.07
B (2.5)	15.83	33.46 ± 0.83 (7.7%)*	4.23 ± 0.04 (0.7%)*
C (5.0)	15.86	35.69 ± 0.53 (14.9%)*	4.33 ± 0.06 (3%)*
D (7.5)	16.11	36.88 ± 0.68 (18.7%)*	4.49 ± 0.07 (6.9%)*
E (10.0)	17.03	40.38 ± 0.21 (30%)*	4.59 ± 0.10 (9.3%)*
2. Group II (19.11 – 21.99 g/fish)			
A (0.0)	17.12	46.91 ± 3.00	2.29 ± 0.11
B (2.5)	17.44	50.09 ± 2.75 (6.8%)*	2.33 ± 0.06 (1.8%)*
C (5.0)	17.66	50.55 ± 1.02 (7.8%)*	2.44 ± 0.07 (6.6%)*
D (7.5)	18.19	53.26 ± 0.48 (13.5%)*	2.50 ± 0.05 (9.2%)*
E (10.0)	18.20	54.67 ± 0.32 (16.5%)*	2.62 ± 0.07 (14.4%)*
3. Group III (37.52 – 40.79 g/.fish)			
A (0.0)	19.55	48.02 ± 1.12	1.71 ± 0.08
B (2.5)	20.14	52.86 ± 0.08 (10%)*	2.01 ± 0.04 (17.5%)*
C (5.0)	17.66	51.18 ± 0.98 (6.6%)*	1.95 ± 0.07 (14%)*
D (7.5)	19.51	46.31 ± 1.17 (-3.6%)	1.68 ± 0.09 (-1.8%)*
E (10.0)	19.10	44.86 ± 0.78 (-6.6%)*	1.65 ± 0.06 (-3.5)*

Protein retention (Y) – T3 level (X) correlation :
Group I : $Y = 30.87 + 0.90X$; $r^2 = 0.94$
Group II : $Y = 46.36 + 0.75X$; $r^2 = 0.96$
Group III : $Y = 49.74 + 1.33X - 0.7X^{2;}$ $r^2 = 0.86$

Daily growth rate (Y) – T3 level (X) correlation :
Group I : $Y = 4.17 + 0.04X$; $r^2 = 0.97$
Group II : $Y = 2.27 + 0.03X$; $r^2 = 0.98$
Group III : $Y = 1.83 + 0.07X - 0.009X^{2;}$ $r^2 = 0.73$

* percentage of increase/decrease compared to control (treatment A).

REFERENCES

Alien, R.E., R.A. Mcrkel and R.B. Young. 1979. Cellular aspect of muscle growth : Myogenic cell proliferation. J. Anim. Sci. 49 : 115 - 127.

Brafield A. E. 1985. Laboratory studies of energetics budgets. *In* Tytler, P. and P. Calow (Eds), Fish Energetics, New Perspectives. Croom Helm, London, p. 257-281.

Djojosocbagio S. 1990. Fisiologi Kclenjar Endokrin I. Pusat Antar Universitas Ilmu Hayat. Institut Pertanian Bogor. 247 p.

Donaldson E.M., U. H. M. Fagcrlund, D.A. Higgs, J. R. McBridge. 1979. Hormonal enhancement of growth. *In* Fish Physiology. I W S Woar, D. J. Randall, and J.R. Brett (Eds.), Vol. 8. Academic Press, New York. p. 453-597.

Fagerlund U. H. M., I. McCallum, D. A. Higgs and J. R. McBride. 1984. Diet composition as a factor in the anabolic efficacy of 3, 5, 3'- Triiodo-L-Thyronine administrated orally to steelhead trout *(Salmo gairdneri).* Aquaculturc, 36 : 49-59.

Huisman, E.A. 1976. Food convcrtion cfficicnces at maintenance and production levels for carp, *Cyprinus carpio* L and rainbow trout, *Salmo gairdneri.* Aquaculturc, 9 : 259 - 273.

Jackim and La Roche. 1973. Protein synthesis in *Fundulus leteroctilus* muscle. Comp. Biochem. Physiol. A., 44:851-866.

Matty A. J. 1985. Fish Endocrinology. Croom Helm, London. 267 p.

Matty A. J. and K. P. Lone. 1985. The hormonal control of metabolism and feeding. *In:* Fish Energetics. Tytler P. and P Calow (Eds.). New Perspectives, Croom Helm, London, p. 185-209.

Matty A. J., M. A. Chaudhry, and K. P. Lone. 1982. The effect of thyroid hormone and temperature on protein and nucleic acid content of liver and muscle of *Sarotherodon mossambica.* Gen. Comp. Endocrinol., 47 : 497 - 507.

Medda, A. K. and A. K.. Ray. 1979. Thyroxine and muscle of lata fish, Gen. Comp. Endocrinol., 9 : 473-480.

Mokoginta I., M. A. Suprayudi, and M. Setiawati. 1994. Kcbutuhan nutrisi ikan gurame *(Oshpronemus gouramy* Lac.) untuk pertumbuhan dan rcproduksi. Lap. Pen. HB. II/2, Dirbinlitabnas, Dikti, Dcpdikbud. IPB.

Mokoginta I., M.A. Suprayudi and M. Setiawati. 1995a. Kebutuhan optimum protein dan energi makanan bcnih ikan gurame *(Oshpronemus gouramy Lac.).* Jur. Perikanan Indonesia, 1 (3) : 82 - 94.

Mokoginta I., M. A. Suprayudi, and M. Setiawati. 1995b. Kebutuhan nutrisi ikan gurame *(Oshpronemus gouramy* Lac.) untuk pertumbuhan dan reproduksi. Lap. Pen. HB. *11/3,* Dirbinlitabnas, Dikti, Depdikbud. IPB.

Mokoginta I., M. A. Suprayudi, and M. Setiawati. 1996. Kebutuhan nutrisi ikan gurame *(Oshpronemus gouramy* Lac.) untuk pertumbuhan dan reproduksi. Lap. Pen. HB. II/4, Dirbinlitabnas, Dikti, Dcpdikbud. IPB.

Narayansingh T. and J. G. Eales. 1975. Effect of thyroid hormones on *in vivo* [1 - ^{14}C] L - Icucine incorporation in plasma and tissue protein of brook trout *(Salvelinus fontinalis)* and rainbow trout *(Salmo gairdneri).* Comp. Biochem. Physiol. B., 52: 399 - 405.

Plummer, D.T., 1979. An introduction to practical biochemistry. McGraw-Hill. New Delhi. 362 p.

Steel R.; G. D., and J. H. Torrie. 1993. Prinsip dan Proscdur Statistika. PT Gramcdia Pustaka Utama, Jakarta. 748 p.

Takcuchi, T. 1988. Laboratory work chemical evaluation of dietary nutrients. *In:* Fish Nutrition and Mariculturc. Watanabc (Ed.). JICA Text Book. The General Aquaculture Course, Japan, p. 179-190.

Widlcr I. B. and J.G. Stanley. 1983. RNA - DNA ratio as an index to growth in Salmonid fishes in the laboratory and in stream contaminated by Carbaryl. J. Fish Biol., 22 : 165-172.

Woo, N. Y. S., A. S. B. Chung, and T. B. Ng. 1991. Influence of oral administration of 3, 5, 3'-Triiodothyroninc on growth, digestion, food conversion and metabolism in the undcrycarling Red sea Bream *(Crysophrys major).* J. Fish Biol., 39 : 459-468.

Yang X. and T. A. Dick. 1993. Effect of dietary fatty acids on growth, feed efficiency and liver RNA and DNA content of Arctic charr, *Salve/inns alpinus* (L). Aquaculture, 116 : 57 - 70.