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► To cite this version:

Cf Chang, Sj Lin. Immersion in bovine insulin stimulates growth of tilapia. *Reproduction Nutrition Development*, 1995, 35 (1), pp.95-103. hal-00899736

HAL Id: hal-00899736

<https://hal.science/hal-00899736>

Submitted on 11 May 2020

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Immersion in bovine insulin stimulates growth of tilapia

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(Received 10 January 1994; accepted 21 October 1994)

Summary — The objective of this study was to investigate the effects of bovine insulin on growth responses in tilapia. Juvenile hybrid male tilapia (*Oreochromis niloticus* x *O. aurea*; $n = 135$) were subjected to 1 of 3 treatments. Each treatment was subdivided into 3 replicates of 15 fish each. The fish were immersed into 1 of 2 doses (10 and 100 $\mu\text{g}/100$ ml water) of insulin or no hormone for 15 min per week for 8 weeks. Body weight, growth and feed conversion efficiency were significantly higher as a result of the first 4 weeks of insulin treatment as compared to control fish. The lower dose of insulin had a better stimulation effect than that of the higher doses. Insulin also stimulated feed consumption. Liver protein and protein/DNA ratio were higher in both insulin-treated groups than in the control group. Muscle proximate composition and hepatosomatic index were similar in the insulin-treated and control groups. The experimental findings suggest that insulin administered by immersion can enhance growth, feed consumption, food utilization and liver cell size in tilapia.

insulin / growth stimulation / feed conversion efficiency / tilapia

Résumé — L'immersion en présence d'insuline bovine stimule la croissance du tilapia. L'objet de ce travail est de tester les effets d'insuline bovine sur la croissance du tilapia. Des hybrides mâles juvéniles de tilapia (*Oreochromis niloticus* x *O. aurea*; $n = 135$) ont été soumis à l'un des 3 traitements suivants. Chaque traitement a été appliqué à 3 lots de 15 poissons. Les animaux ont été immergés dans l'eau (0, 10 ou 100 μg d'insuline par ml d'eau) pendant 15 min par semaine durant 8 sem. Le poids corporel, la croissance et l'indice de consommation étaient significativement supérieurs au témoin après 4 sem de traitement. La dose inférieure était plus stimulante que la dose supérieure. L'insuline induisait aussi la consommation d'aliments. Les protéines du foie et le rapport protéines/ADN dépassaient les niveaux des témoins dans les 2 groupes de poissons stimulés. La composition des muscles et l'index hépatosomatique étaient semblables dans tous les groupes. Les résultats suggèrent que l'insuline administrée par immersion peut modifier la croissance, la consommation de nourriture, l'utilisation des aliments et la taille des cellules hépatiques chez le tilapia.

insuline / stimulation de la croissance / indice de consommation / tilapia

INTRODUCTION

Several anabolic hormones have been studied with respect to their involvement in fish growth (reviewed by Donaldson *et al*, 1979). These hormones have included growth hormone (Higgs *et al*, 1975, 1977; Degani and Gallagher, 1985), several androgens (Degani and Gallagher, 1985; Lone, 1989), estradiol (Lone and Matty, 1983), insulin (Ablett *et al*, 1981a; Plisetskaya *et al*, 1984; Degani and Abraham, 1992), thyroxine (Matty, 1986), triiodothyronine (Higgs *et al*, 1982) and insulin-like growth factor (Bern *et al*, 1991; Mc Cormick *et al*, 1992).

Insulin appears to have an important role in protein, carbohydrate and lipid metabolism. Protein and amino-acid conservation and tissue deposition may be just as important as regulation of carbohydrate metabolism in the physiological function of insulin in fish (reviewed by Matty and Lone, 1985; Hilton *et al*, 1987). Insulin treatment decreased plasma-free fatty-acid and cholesterol levels (Minick and Chavin, 1972; Perez *et al*, 1989), while it enhanced tissue lipid content (Ablett *et al*, 1981a; Perez *et al*, 1989). Insulin has been suggested to involve in growth physiology of fish because it enhances protein anabolic processes (Plisetskaya *et al*, 1991; Sundby *et al*, 1991; Degani and Abraham, 1992). The increased uptake of amino acid by muscle and liver cells was found in fish (Tashima and Cahill, 1968; Ablett *et al*, 1981b). However, Ludwig *et al* (1977) showed that injection of bovine insulin did not significantly increase the growth of coho salmon. Ablett *et al* (1981a) indicated that higher doses of insulin increased growth when administered for 56 d in rainbow trout. Oral administration of insulin significantly stimulated growth in eels (Degani and Abraham, 1992). Still, very little is known about the role of insulin in the general growth of fish. It is also unknown whether insulin influences food intake or cellular growth. Therefore, the objectives of

this study were to investigate the effects of bovine insulin on growth, feed conversion efficiency, body composition, feed consumption and liver protein/liver DNA ratio in the tilapia.

MATERIALS AND METHODS

Fish

Fingerlings of all male hybrid tilapia (*Oreochromis niloticus* x *O. aurea*) obtained from the tilapia hatchery of YC Tsay, Taiwan, were reared in a fiber-glass tank filled with 2.0 tons of freshwater. Fish were fed with eel feed (Fwu Sow Feed Co, Taiwan) before their transfer to experimental tanks. A total of 135 tilapia, mean weight 1.02 ± 0.04 g were selected for the experiment.

Experimental design

Fish ($n = 135$) were randomly divided into 3 groups (1 control and 2 treatment groups) and then each group was subdivided randomly into 3 replicates of 15 fish, which were maintained in separate experimental tanks (60 x 30 x 35 cm) in a recirculation system. All fish in each tank were netted and immersed once a week for 15 min in a solution which contained either no bovine insulin (control), or 10 µg/100 ml (treatment I) or 100 µg/100 ml (treatment II) bovine insulin (Sigma Chemical Co, Saint Louis, MO). Bovine insulin was dissolved in distilled water as a dipping solution. All fish were fed to satiation twice daily (9–10 am and 4–5 pm) with the same diet. The formulated diet (NRC, 1983) contained the following proximate composition: protein, 21.9%; lipid, 10.4%; moisture, 11.9%; and ash, 13.5%. The diet particle size was adjusted in relation to fish size.

Each replicate of fish was weighed biweekly and feed consumption was measured daily. Photoperiod (12 h light/12 h dark) was kept constant during the experimental period. Water temperatures were no different between aquaria in the recirculation system. Daily changes of water temperatures ranged from 27–31°C throughout the experimental period. At the end of 8 weeks of

treatment, 3 tilapia from each replicate were removed for dissection. Muscle tissue and livers were quickly dissected, frozen and kept in a freezer at -135°C until assay.

Analyses

In relation to the proximate analyses of the diet and muscle samples, protein content was determined by the Kjeldahl method ($\%N \times 6.25$) and lipid was measured by the procedures of Bligh and Dyer (1959). Ash content was obtained after heating at 600°C for 6 h. Moisture content in muscle was obtained by drying in an oven for a minimum of 8 h at 90°C .

Soluble protein in liver was measured by the Biuret method with a kit (E Merck, Germany).

Bovine serum albumin was used as a standard. Total DNA was extracted with a cold, sterile, phosphate-buffered saline with an ultrasonic cell disrupter (Ferguson and Drahushchak, 1989). The homogenates were centrifuged and RNase was added to digest RNA. Ethidium bromide was added to excite the emission of fluorescence according to the procedures of Ferguson and Drahushchak (1989). Herring sperm DNA was used as a standard.

Percentage gain over initial weight was calculated according to the formula $(\text{final wt} - \text{initial wt}) \times 100 / \text{initial wt}$. Specific growth rate for weight was computed according to the formula $(\text{Ln final wt} - \text{Ln initial wt}) \times 100 / \text{feeding duration (d)}$. Food conversion efficiency was calculated as $\text{wet weight gain (g)} \times 100 / \text{wet weight of feed (g)}$ as fed. Data were expressed as mean $\pm$ SD (stan-

Table 1. Effects of insulin on growth, food intake and feed conversion efficiency of tilapia.

Duration (weeks)		Concentration of insulin ($\mu\text{g}/100 \text{ ml water}$)		
		0	10	100
Weight (g)	0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.1
	2	2.2 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
	4	4.4 ± 0.5	$5.4 \pm 0.3^*$	$5.3 \pm 0.3^*$
	6	6.9 ± 1.1	8.7 ± 0.8	8.3 ± 0.3
	8	12.0 ± 2.4	14.9 ± 1.3	14.3 ± 0.3
Gain in weight (%)	0-2	119.0 ± 9.4	$141 \pm 10.2^*$	129 ± 14.4
	0-4	337.8 ± 57.0	$439.6 \pm 29.1^*$	409.8 ± 40.7
	0-6	587.6 ± 117.6	776.3 ± 86.7	693.7 ± 58.0
	0-8	$1\ 095.4 \pm 250.3$	$1\ 389 \pm 115.2$	$1\ 268.7 \pm 62.4$
Specific growth rate (%)	0-2	5.6 ± 0.3	6.3 ± 0.3	5.9 ± 0.5
	0-4	5.5 ± 0.5	6.0 ± 0.2	5.8 ± 0.3
	0-6	4.6 ± 0.4	5.2 ± 0.2	4.9 ± 0.2
	0-8	4.4 ± 0.4	4.8 ± 0.1	4.7 ± 0.1
Feed consumed (g)	0-8	288.0 ± 28.2	$324.6 \pm 7.4^{**}$	$334.6 \pm 1.7^{**}$
Feed conversion efficiency (%)	0-2	54.0 ± 2.7	60.0 ± 4.0	57.3 ± 0.6
	0-4	51.7 ± 5.1	$62.7 \pm 3.8^*$	58.0 ± 2.7
	0-6	51.3 ± 4.7	61.3 ± 5.9	55.0 ± 2.0
	0-8	56.7 ± 5.9	64 ± 4.6	59.3 ± 0.6

The fish were dipped into solutions of the hormone once weekly for 8 weeks. The survival of all groups of fish were 100% regardless of treatment. Data are shown as mean $\pm$ SD ($n = 3$); *, ** significant different ($P < 0.05$ and $P < 0.01$, respectively) from control group.

dard deviation) and analysed by the analysis of variance (ANOVA).

The total of water, lipid, protein and ash of the proximate composition is higher than 100%. The method of sampling and the analysis of crude protein (nitrogen titration) could contribute to this discrepancy.

RESULTS

Data related to body weight, percent gain over initial weight, specific growth rate, feed consumption and feed conversion efficiency are presented in table I. Body weights significantly increased by week 4, in the groups treated with low and high doses of insulin as compared to the control group. Feed conversion efficiency was not significantly increased except during the first 4 weeks of treatment for the group on the low doses of insulin. Percent gain over initial weight significantly increased by weeks 2 and 4 in the groups that received the low dose of insulin. No significant increases of specific growth rate were noted in tilapia treated with low and high doses of insulin. The food intakes of tilapia in the groups of low and high doses of insulin were higher than observed for the control group.

No differences were found in the terminal muscle proximate composition (*eg*, moisture, lipid and protein) of tilapia regardless of

insulin dose (table II). Higher ash content was observed in the insulin-treated tilapia ($p < 0.1$, table II). The liver DNA content was unchanged (table III). Soluble liver protein was significantly higher in the insulin treatment groups.

Further, the low dose of insulin had more pronounced effects than the high dose of insulin (table III). A significant elevation of protein/DNA ratio in the livers of the insulin-treated groups was found (table III).

DISCUSSION

The results of the present study show that bovine insulin can significantly increase the growth of tilapia. The growth effect was found to be more pronounced between weeks 0 and 4 when the fish were administered 10–100 µg insulin/100 ml.

Although growth-promoting responses were observed in the insulin-treated tilapia, the effects were only statistically significant in the first half of the treatment period. The increasing density of fish in the tanks during the final period of the experiment may have resulted in the non-significant differences in growth of the fish between 6 to 8 weeks. Moreover, failure to observe the effects of insulin immersion in larger tilapia may have arisen from the high endogenous production of growth-stimulating hormones or fac-

Table II. Effects of insulin on the terminal proximate composition of muscle of tilapia.

Concentration of insulin (µg/100 ml)	Moisture (%)	Ash (%)	Crude fat (%)	Crude protein (%)
0	78.4 ± 1.3	6.3 ± 0.1	1.9 ± 0.4	18.7 ± 0.4
10	78.2 ± 0.9	7.5 ± 0.2	2.0 ± 0.4	19.5 ± 0.5
100	78.3 ± 0.3	7.2 ± 0.1	2.1 ± 0.3	19.0 ± 0.5

The fish were immersed in solutions of the hormone once weekly for 8 weeks. Data are shown as mean ± SD ($n =$

Table III. The effects of insulin on the hepatosomatic index (HSI), soluble protein, DNA and protein/DNA ratio in liver of tilapia.

Concentration of insulin ($\mu\text{g}/100\text{ ml}$)	HSI (%)	Protein (mg/g)	DNA ($\mu\text{g}/\text{g liver}$)	Protein/DNA $\times 10^3$
0	3.3 ± 0.7	46.5 ± 9.8	11.8 ± 2.0	3.9 ± 0.3
10	3.2 ± 0.7	$72.0 \pm 1.7^*$	12.0 ± 0.6	$6.0 \pm 0.3^{**}$
100	3.1 ± 0.6	$67.2 \pm 5.3^*$	13.2 ± 0.6	$5.1 \pm 0.5^*$

Data are shown as mean $\pm$ SD ($n = 9$); *, ** significantly different ($P < 0.05$ and $P < 0.01$, respectively) from the control group. Refer to table I for additional information.

tors. The decreasing capacity of insulin uptake by large fish and development of the resistance to insulin should also not be excluded.

Insulin administration by the immersion procedure rather than giving the hormone via the oral route or injection was used in this experiment to avoid degradation of the peptide by digestive enzymes or handling stress due to injection. Injection is one of the popular routes for insulin administration in fish studies (Ludwig *et al*, 1977; Ablett *et al*, 1981a). In this study, dipping the fish in a solution of insulin for 15 min seemed to be enough for the uptake of insulin. Bovine serum albumin was detectable in the plasma of chum salmon when it was administered by immersion for only 3 min (Ototake *et al*, 1992). The uptake of the insulin may have been rapid because the molecular weight (6 000 Da) of the hormone is not large. The amount of insulin uptake in circulation was not measured in this study because the fish size was too small to bleed.

The uptake of exogenous compounds by bath immersion was first reported in fish by Amend and Fender (1976). Administration of an enkephalin analog to tilapia by immersion for 15 min every week was also found to enhance growth (Chang and Lin, 1991). Tatner and Horne (1983) showed

that immersion of rainbow trout in a solution of *Vibrio anguillarum* vaccine for just 10 s was long enough for vaccine uptake. The gills (Zapata *et al*, 1987, Kawahara and Kusuda, 1988), oral-digestive tract (Tatner, 1987) and lateral line (Fender and Amend, 1978) were the likely sites of absorption of the exogenous insulin. The oral absorption of biologically active bovine insulin was shown in common carp and European eel (Degani and Abraham, 1992; Hertz *et al*, 1992). The exact mechanism of insulin entry in this experiment remains unknown and needs to be studied further.

The administration of bovine insulin to coho salmon tended to increase their specific growth rate and food utilization (Ludwig *et al*, 1977). Insulin was first found to stimulate feed consumption in fish. In human, insulin has different effects on the stimulation of food consumption depending on dose (Morley, 1987). Insulin was also found to enhance feed conversion efficiency in this study and a low dose was more efficient than a high dose in this regard. Both of the foregoing mechanisms are therefore likely to account for the effect of insulin on the stimulation of growth in tilapia.

Liver soluble protein and protein/DNA ratio also significantly increased in the treatment groups. However, no change in liver

DNA content was observed. Protein/DNA can be used as an indicator of cell size whereas the absolute amount of DNA provides information about the cell numbers in an organ. Therefore, bovine insulin treatment induced hypertrophy of the liver. This induction further supports the growth responses of tilapia to insulin. An enkephalin analog and anabolic steroids such as methyltestosterone and ethylestrenol have also been found to stimulate higher levels of liver protein, protein/DNA ratio, and body growth in tilapia and carp (Lone and Matty, 1980, 1983; Korsgaard, 1990; Chang and Lin, 1991). However, hepatosomatic index was not higher in the insulin-treated tilapia as compared to that in the control group. Hepatosomatic index may not be able to reflect the hypertrophy of the liver because other liver components such as liver glycogen often decrease in fish after insulin treatment (Ottolenghi *et al*, 1982; Ince, 1983). The insulin-treated fish also showed a lower hepatosomatic index although the content of liver protein was higher (Lewander *et al*, 1976; Carneiro and Amaral, 1983).

The general effects of insulin on carbohydrate and protein metabolism are to increase uptake of substrate by cells. The most obvious response of carbohydrate metabolism to exogenous insulin administration in fish is hypoglycemia (Ablett *et al*, 1981a; Ince, 1983). Insulin has a variable effect on liver glycogen and increases muscle glycogen (Tashima and Cahill, 1968; Lewander *et al*, 1976; Ablett *et al*, 1981a; Carneiro and Amaral, 1983). However, the hepatosomatic index was not different between insulin-treated and control groups in this experiment. Liver glycogen showed a decrease whereas in muscle there was an increase after insulin treatment in fish (Ottolenghi *et al*, 1982; Perez *et al*, 1989). Ablett *et al* (1981a) indicated that the role of insulin in carbohydrate metabolism may

be directed to oxidative reaction of glucose but not generally to glycogen deposition.

The absence of any effect of insulin on lipid content in muscle was found in this study. Insulin decreases plasma-free fatty acid concentrations and has antilipolytic effects in most fish species (Minick and Chavin, 1972; Ince and Thorpe, 1975; Lewander *et al*, 1976). Furthermore, no significant effect on lipid muscle content was observed in sea bass by insulin injection (Perez *et al*, 1989). The role of insulin in the regulation of protein anabolic effects has been studied and this may be one of the major functions of insulin in fish (Murat *et al*, 1981). For instance, insulin has been found to significantly lower plasma amino acid nitrogen in the European silver eel (*Anguilla anguilla*), northern pike (*Esox lucius*) and rainbow trout (Ince and Thorpe, 1974; Thorpe and Ince, 1974; Ablett *et al*, 1981b). Furthermore, insulin has been noted to increase the incorporation of amino acids into the muscle and liver protein of fish (Tashima and Cahill, 1968; Lewander *et al*, 1976). The increases in liver protein and protein/DNA ratio noted in the insulin-treated tilapia in this study probably reflect an increased uptake of amino acids and enhanced proteogenesis in the liver.

Insulin treatment did not alter the proximate composition of muscle in tilapia. The crude protein content in muscle estimated from the total percentage of nitrogen might be not an appropriate method, however, to reflect the anabolic effect of insulin on muscle protein. Insulin-treated tilapia had a higher ash content in muscle compared with the control group. The action of insulin on the inorganic ion metabolism in fish is not known. Harper (1973) indicated that insulin could stimulate the uptake of phosphate in muscle protein. Insulin may also act through the receptors of insulin-like growth factor (IGF) to stimulate sulfate incor-

puration in certain tissues (Bern *et al*, 1991; McCormick *et al*, 1992).

It is not known whether insulin acts alone or in combination with one or more of the other anabolic and metabolic hormones to enhance tilapia growth. For instance, the interaction of insulin and growth hormone in the growth of fish is not clear. A balance between insulin and growth hormone seems to exist in fish fed at different levels. In fasted fish, insulin level is low and growth hormone level is high (Plisetzkaya, 1990; Sumpter *et al*, 1991). In mammals, growth hormone is less effective in the absence of insulin (Daughaday *et al*, 1975) and this may also be true in fish. The involvement of IGF and receptor of IGF in growth regulation in fish under the effects of insulin immersion should also not be excluded (Bern *et al*, 1991; Duan and Hirano, 1992; McCormick *et al*, 1992). Insulin could bind IGF receptor and stimulate growth effect (Bern *et al*, 1991).

In conclusion, our experimental finding suggest that insulin administered by immersion can enhance growth, feed consumption, food utilization and liver cell size in tilapia.

ACKNOWLEDGMENT

This work was supported in part by the Council of Agriculture (CA 77-AD-7. 1-F53, CA 78-AD-7. 1-F-06), Taiwan, Republic of China.

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