

## Effect of Intraperitoneal Administration of L-Thyroxine on Growth and Food Conversion Efficiency in *Oreochromis niloticus*

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**Abstract.** L-Thyroxine ( $T_4$ ) was administered intraperitoneally on a weekly basis to juvenile tilapia (*Oreochromis niloticus*) at doses of 0.25, 0.5, 1.0 and 2.0  $\mu\text{g/g}$  body weight for eight weeks. Fish injected with 0.5 and 1.0  $\mu\text{g/g}$  of  $T_4$  grew significantly faster than the controls. However, the highest dose of  $T_4$  resulted in arrested growth. The effect of  $T_4$  on hepato-, reno-, crania-, and viscerosomatic indices was variable. The results are discussed in the light of existing reports in the literature.

### Introduction

In higher vertebrates, thyroid hormones have been reported to play a significant role for their normal growth and development. In birds and mammals thyroidectomy or hypothyroidism results in retarded growth [1–3] and thyroid hormones increase the protein synthesis in muscle tissue [4, pp. 469-479; 5, pp. 119-157; 6; 7]. The role of these hormones in fish has attracted a great deal of attention for many years and the thyroid may play an important role either directly or indirectly in important biological processes such as oxygen consumption, osmoregulation, migration, skin silvering and sexual maturation [8, pp. 330-356; 9; 10, pp. 1-5; 11, pp. 453-497; 12, pp. 341-346; 13; 14].

There is considerable controversy on the role of the thyroid in normal growth in fish. Barrington *et al.* [15] reported a significant increase in growth of *Salmo gairdneri* after dietary supplementation with thyroxine or when thyroxine was added to the ambient water. Similarly, *Mugil auratus* showed enhanced growth rate after thyroxine treatment [16]. Higgs *et al.* [17–18] administered different doses of thyroxine either intramuscularly, intraperitoneally or by cholesterol implants in coho

salmon and the experimental groups were found to be significantly heavier than the control. Dietary addition of  $T_4$  to juvenile coho salmon and immersion of tilapia larvae in a 0.1 ppm solution of  $T_4$  resulted in a significantly higher growth and survival rate [19–20]. On the other hand, some workers have reported that the administration of thyroid hormones has no effect on *Salmo salar* [21], *Salvelinus fontinalis* [22] and *Oncorhynchus kisutch* [23]. Thyroid hormones retarded the growth of *Oncorhynchus keta* [24]. There are also reports indicating that only triiodothyronine ( $T_3$ ) is effective in stimulating growth in various fish species; thyroxine ( $T_4$ ) has no such effect [14, 19].

Thus the effect of thyroid hormones on fish growth is equivocal. In a recent report, age, dose and temperature dependent effects of both  $T_4$  and  $T_3$  on cellular growth in *Sarotherodon mossambicus* are described [25]. The present study describes the effect of injecting different doses of thyroxine for a period of eight weeks on growth and its related parameters in *Oreochromis niloticus*, a tropical teleost.

### Materials and Method

The fish *Oreochromis niloticus* used in these experiments were collected from Deerab fish station, 80 km south of Riyadh. The animals were transferred in a large tank and after 15 days of acclimatization, they were transferred to five identical tanks of 80 litre capacity with 20 fish (mean weight  $8.31 \pm 0.213$  g) in each. The fish were maintained at  $24.0 \pm 1^\circ\text{C}$  with a photoperiod of 12L : 12D. Fluorescent tubes were used. The water in the tanks was changed every other day and replaced with water of the same temperature. Airstone aeration was supplied. The animals were acclimated for 8 days in these five tanks and after that allocation of tanks to a control and four experimentals was made on a random basis. During the acclimatization as well as the experimental period the fish were fed with fish pellets (proximate analysis = protein, 40%; fats, 8.7%; minerals, 10.9%; and moisture, 6.5%), three times a day. Dechlorinated tapwater was used throughout the experimental period.

### Administration of hormone

Thyroxine ( $T_4$ ) as its sodium salt (Sigma, London) was dissolved (mg/ml) in 50% alkaline propylene glycol. The concentrations of this stock solution were so adjusted that the required dose of  $T_4$  was contained in  $50 \mu\text{l}$  of the solvent. The hormone was injected intraperitoneally (ip) into the anaesthetised fish at 0.25, 0.5, 1.0 and  $2.0 \mu\text{g/g}$  body weight. Controls were injected with the solvent only. The above experimental protocol was followed on a weekly basis for a period of eight weeks. The fish were fed *ad libitum* three times a day during the investigation period. However, no food was given to the fish on the morning of the injection day.

### Weighing and measuring procedure

After every seven days, the fish were anaesthetised with benzocaine (ethyl-4-aminobenzoate, BDH, 40 mg/l) and weighed individually to the nearest 0.01 g on an electric digital balance (Metler, H-80), after removal of the excess water by an absorbent paper towel. Total length was also measured biweekly to the nearest 0.1 cm.

### Analysis of experimental data

The index of fatness or leanness, the condition factor 'K' was computed from the formula:

$$K = W \text{ (g)} \times 100 / L^3 \text{ (cm)}$$

Growth rate (GR =  $dw/dt$ ) and specific growth rate (SGR =  $d \log_e W$  or  $L/dt$ ) were computed from the weekly increments in weight and length data, as described by Stebbing [26]. Food conversion efficiency was derived from the weight gain (g) to food given (g), and the different tissue-body indices were computed as a percentage of the total body weight [27]. For calculating different tissue body indices six fish were sampled from each group at the end of the experiment and in selecting the fish care was taken to choose the fish from each group which did not deviate in weight and length by more than 5% from the mean of the sample. The experimental data were subjected to single factor analysis of variance and Duncan's multiple range test was applied to find out the difference between groups [28, p. 276].

## Results

### Weight and length

The weight and length data accumulated over a period of sixty days of  $T_4$  administration are presented in Fig. 1 and 2, respectively. The growth curves for the different groups remained almost parallel during the initial period but started to diverge during the second week (weight) and fourth week (length) of the experiment. The experimental groups which received 0.5 and 1.0  $\mu\text{g/g}$  of the hormone grew faster than the controls throughout the experimental period. At the end of the experiment, these two experimental groups were significantly heavier and longer than the controls. The groups receiving 0.25  $\mu\text{g/g}$  of the hormone had an almost parallel growth (both in weight and length) to that of the controls (not significantly different), although the former group was slightly heavier and longer throughout the experimental period. The fourth group receiving the highest dose (2.0  $\mu\text{g/g}$ ) showed significantly poorer growth both in weight and length than the controls and the other experimental groups.

### Growth rate (GR) and specific growth rate (SGR)

Table 1 gives GR and SGR values for the different groups of *O. niloticus*. The GR was not uniform throughout the experiment in any of the groups studied. GR

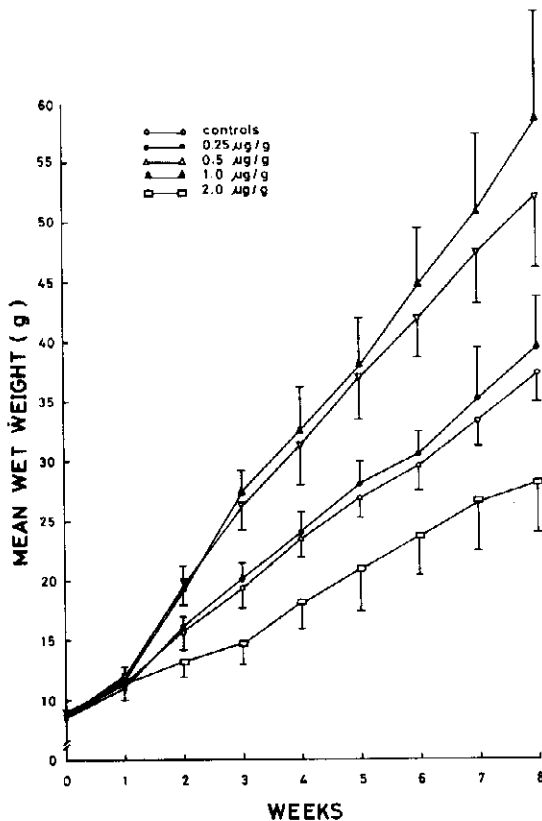


Fig. 1. Mean weight of different groups of *O. niloticus* receiving different doses of  $T_4$  intraperitoneally on a weekly basis. Where standard error bars have been omitted, the values were almost equivalent in the comparable groups. Grouping of level means at the end of experiment by Duncan's multiple-range at 5% level of significance

$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$
2.0	C	0.25	0.5	1.0

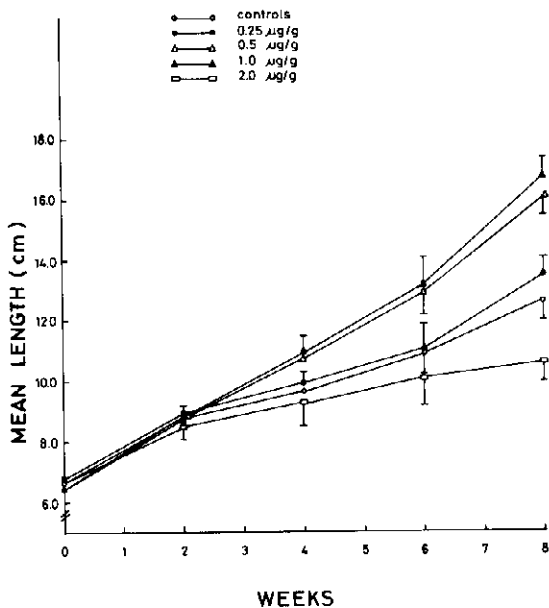


Fig. 2. Changes in total body length of *O. niloticus* injected with different doses of  $T_4$  intraperitoneally on a weekly basis. Where standard error bars have been omitted, the values were almost equivalent in the comparable groups. Grouping of level means at the end of experiment by Duncan's multiple-range test at 5% level of significance

$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$
2.0	C	0.25	0.5	1.0

increases with the size of the organisms because the products of growth also grow. Therefore, change in growth rate can be detected more readily when this factor is considered despite the size of the organism. Thus the rate of change of biomass is better expressed as a function of the biomass or specific growth rate [26]. The SGR values for the fish administered 0.5 and 1.0  $\mu\text{g/g}$  of the hormone were higher than those of the controls and the other two experimental groups. The highest mean GR over the eight week period ( $6.329 \text{ week}^{-1}$ ) and SGR ( $1.283 \text{ g week}^{-1}$ ) is seen in the groups which received 1.0  $\mu\text{g/g}$  of  $T_4$ . The mean GR and SGR values showed a gradual increase with the increase in dose of  $T_4$  from 0.25 to 1.0  $\mu\text{g/g}$  followed by sharp decline in fish injected with the highest dose. The trend in GR and SGR in fish length was similar as described above for the weight.

#### Condition factor

At the start of the experiment the mean value for 'K' ranged from 2.42 to 3.14 among various groups. This was followed by a progressive decline in 'K' value in all the groups. This decline was more pronounced in fish administered 0.5 (from 3.18 to 1.25) and 1.0  $\mu\text{g/g}$  (from 3.05 to 1.24) of thyroxine. A comparison of the mean values of condition factor in various groups over the eight weeks of experimental period indicate that it was maximal in the controls (Table 2).

#### Tissue body indices

Changes in tissue weight relative to body weight i.e. Cranio-somatic (CSI), Hepato-somatic (HSI), Reno-somatic (RSI) and Viscero-somatic (VSI) indices are presented in Fig.3. CSI values are comparable in various groups except the one in which highest dose (2.0  $\mu\text{g/g}$ ) was administered. In the latter group, CSI was significantly higher than in all other groups including the controls. The highest VSI was found in controls and the mean VSI decreased with an increase in hormonal dose in different experimental groups. A similar trend could be seen in HSI. However, RSI showed no significant differences.

#### Food conversion efficiency

Food conversion efficiencies (FCE) in the different groups are given in Table 3. FCE values remained high throughout the experimental period in fish injected with 0.5 and 1.0  $\mu\text{g/g}$  of the hormone as compared to controls and the two other experimental groups. FCE in the group administered the highest hormonal dose (2.0  $\mu\text{g/g}$ ) was almost comparable with the control group during the first four weeks but declined during the last four weeks of the experiment. A comparison of the mean FCE values for groups calculated over the 8 weeks period reveals that it was significantly higher in the fish which received 0.5 and 1.0  $\mu\text{g/g}$   $T_4$  than in the controls. The mean FCE value in controls was significantly higher than the fishes injected with the highest dose. FCE was almost comparable in control and in the group administered with minimum dose of  $T_4$ .

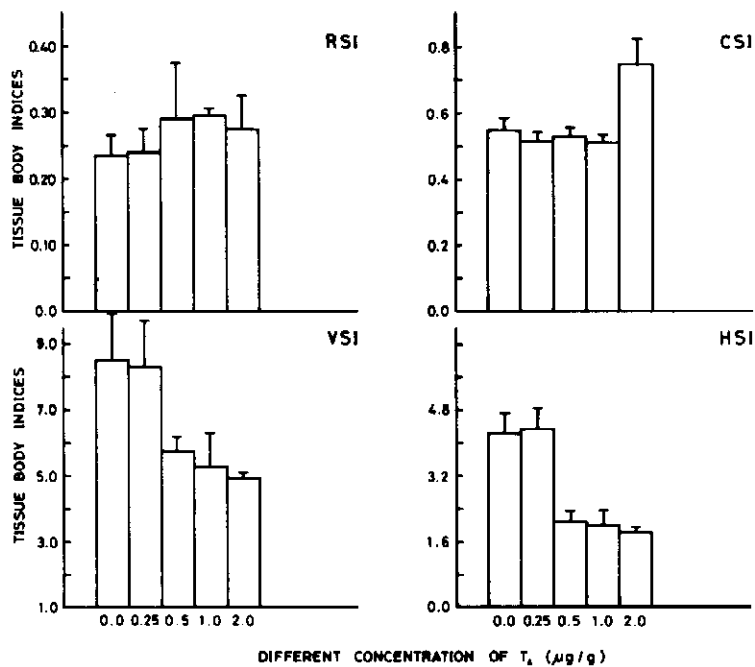


Fig. 3. Effect of intraperitoneal administration of  $T_4$  on a weekly basis for a period of eight weeks on Reno-somatic (RSI), Viscero-somatic (VSI), Hepato-somatic (HSI) and Cranio-somatic (CSI) index in *O. niloticus*.

**Table 1.** Effect of weekly injections of  $T_4$  for eight weeks on the growth rate,  $GR = \frac{dw}{dt}$  and specific growth rate,  $SGR = \frac{d \log_e w}{dt}$  (in parenthesis) of *Oreochromis niloticus* (over the period indicated at the column heads).

Dose of $T_4$ ( $\mu\text{g/g}$ )	Weight				Length			
	Weeks				Weeks			
	0-2	2-4	4-6	6-8	0-8	0-4	4-8	Mean
Control	3.07 (0.983)	3.90 (1.027)	3.18 (0.925)	3.54 (0.979)	3.42 (0.978)	0.76 (0.277)	0.72 (0.267)	0.79 (0.272)
0.25	3.98 (1.037)	4.16 (1.059)	3.10 (0.912)	4.58 (1.107)	3.95 (1.028)	0.74 (0.272)	0.68 (0.251)	0.71 (0.261)
0.50	5.89 (1.233)	5.75 (1.221)	5.16 (1.167)	5.19 (1.169)	5.49 (1.197)	1.08 (0.366)	1.34 (0.420)	1.21 (0.393)
1.00	5.72 (1.218)	6.43 (1.277)	6.21 (1.259)	6.95 (1.316)	6.32 (1.283)	1.12 (0.376)	1.45 (0.939)	1.28 (0.407)
2.00	2.48 (0.798)	2.26 (0.754)	2.98 (0.892)	2.13 (0.725)	2.46 (0.792)	0.69 (0.255)	0.30 (0.051)	0.49 (0.153)

**Table 2.** Effect of weekly injections of  $T_4$  for eight weeks on the condition factor of *Oreochromis niloticus*.

Dose of $T_4$ ( $\mu\text{g/g}$ )	Weeks					
	0	2	4	6	8	Mean
Control	3.16	2.81	2.59	2.34	1.85	2.15 $\pm$ 0.35
0.25	2.42	2.26	2.50	2.30	1.97	2.30 $\pm$ 0.08
0.50	3.18	2.94	2.55	2.33	1.25	2.45 $\pm$ 0.29
1.0	3.02	3.26	2.47	1.91	1.24	2.38 $\pm$ 0.32
2.0	2.91	2.16	2.16	2.28	2.34	2.38 $\pm$ 0.12

**Table 3.** Effect of weekly injections of  $T_4$  for eight weeks on the food conversion efficiency of *Oreochromis niloticus*.

Dose of $T_4$ ( $\mu\text{g/g}$ )	Weeks				Mean
	0-2	2-4	4-6	6-8	
Control	0.41	0.49	0.57	0.70	0.54
0.25	0.43	0.51	0.59	0.70	0.55
0.50	0.62	0.66	0.72	0.76	0.69
1.0	0.61	0.65	0.80	0.84	0.72
2.0	0.40	0.40	0.37	0.36	0.38

### Discussion

The results of the present study indicate that intraperitoneal injection of thyroxine ( $T_4$ ) increases growth in tilapia. The enhancement in growth was more pronounced in fish administered 0.5 and 1.0  $\mu\text{g/g}$   $T_4$  weekly, whereas the lowest dose (0.25  $\mu\text{g/g}$ ) was without significant effect and the highest dose (2.0  $\mu\text{g/g}$ ) resulted in growth retardation.

These results substantiate the earlier findings that although  $T_4$  increases growth at lower doses it becomes deleterious and detrimental to growth at higher doses [25, 29]. This decrease in growth rate at higher doses is probably due to catabolic action and a decrease in appetite of the organism. High doses of  $T_4$  have been reported to be catabolic in terms of total proteins and RNA contents of liver and muscle in fish [25, 30] and mammals [4]. The mechanism(s) for these opposite effects in the same animals are not known. Although, the biochemical parameters of growth were not investigated in the present studies, it has been established that growth in fish is largely protein accretion [31, p. 395; 32, p. 943; 33, p. 258] and thyroid hormones increase the turnover rate of cellular RNA and total protein contents [25, 30, 34]. It is possible that at higher hormonal doses turnover rate becomes much higher and the degradation rate surpasses the synthesis rate [35]. An increased level of thyroid hormone increases the lysosomal enzyme cathepsin D, which degrades the proteins in different tissues [36].

The growth-promoting effect of  $T_4$  in tilapia injected with 0.5 and 1.0  $\mu\text{g/g}$  agrees with previous reports on other fish species [15, 20, 37, 38, 39]. However, there was no positive effect of  $T_4$  on the growth of *Oncorhynchus kisutch* [23, 24] and *S. mossambicus* and *C. carpio* [40]. These discrepancies could be due to differences in the method of hormonal administration. The workers who failed to obtain any growth promoting response, administered  $T_4$  orally along with the diet, and in such circumstances the amount of hormone taken by the fish remains unknown. In the present studies,  $T_4$  was injected intra-peritoneally and thus the exact dose given to the fish was certain. Moreover, there are a number of possible explanations for this lack of growth promotion by  $T_4$  administered by oral route. Firstly, when  $T_4$  is given orally it probably becomes more tightly bound to the contents in the alimentary canal and is metabolised (deiodinated) at a higher rate. The above hypothesis has been confirmed in brook trout *Salvelinus fontinalis* by Law and Eales [41]. High level of  $T_4$  probably inhibit conversion of  $T_4$  to  $T_3$  [12].  $T_3$  is more potent than  $T_4$  in accelerating fish growth [11, 25].

Food conversion efficiency (FCE) in the various groups corresponds to their growth responses. In comparison to controls, FCE values were higher in the groups (0.5 and 1.0  $\mu\text{g/g}$ ) which exhibited maximum growth but lower in fish receiving 2.0

$\mu\text{g/g}$   $T_4$  per week. These results differ from earlier reports in which the FCE of  $T_4$  treated fish always remained high irrespective of its effect on growth [23, 40, 42]. This difference is difficult to explain, but increased food conversion is perhaps the product of increased digestion and assimilation of food and thus should account for increased growth.

Administration of  $T_4$  also affected the relative weight of different tissues (tissue body indices) except the kidney. There was significant increase ( $p < 0.01$ ) in the weight of brain relative to body weight in tilapia injected with  $2.0 \mu\text{g/g}$  of  $T_4$  weekly. This increase in relative weight of the brain may not be due to an increase in the total weight of brain *per se*, but due to a decrease in the total weight of fish. In contrast to an earlier report [40], hepato-somatic index (HSI) and viscero-somatic index (VSI) decreased in all experimental groups in the present studies. Furthermore, the effect of  $T_4$  on HSI and VSI appears to be dose dependent (Fig. 3). The decrease in VSI is probably due to the mobilization of the abdominal fat contents. A decrease in visceral fat reserve under the influence of thyroid hormones has been reported in *Salmo gairdneri* and *Salvelinus fontinalis* [15, 43, 44]. Similarly, a decrease in HSI in the treated groups may be due to the depletion of hepatic glycogen [12, 45]. It seems that under the influence of  $T_4$  visceral fat depots and liver glycogen are being mobilised for energy requirements and proteins are saved to induce body growth in this fish species.

It may however be pointed out that the findings reported above are of preliminary nature and should be confirmed in future experimentation based on improved experimental design and analysis.

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تأثير حقن هرمون الثيروكسين في أسماك البلطي (*Oreochromis niloticus*)  
على النمو والتحولات الغذائية

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ملخص البحث. لقد درس تأثير حقن عدة جرعات من هرمون الثيروكسين (٢٥، ٥٠، ١٠٠، ٢٠٠، ٤٠٠، ٨٠٠، ١٦٠٠، ٣٢٠٠، ٦٤٠٠، ١٢٨٠٠، ٢٥٦٠٠، ٥١٢٠٠، ١٠٢٤٠٠، ٢٠٤٨٠٠، ٤٠٩٦٠٠، ٨١٩٢٠٠، ١٦٣٨٤٠، ٣٢٧٦٨٠، ٦٥٥٣٦٠، ١٣١٠٧٢٠، ٢٦٢١٤٤٠، ٥٢٤٢٨٨٠، ١٠٤٨٥٧٦٠، ٢٠٩٧١٥٢٠، ٤١٩٤٣٠٤٠، ٨٣٨٨٦٠٨٠، ١٦٧٧٧٣٦٠، ٣٣٥٥٤٧٢٠، ٦٧١٠٩٤٤٠، ١٣٤٢١٨٨٠، ٢٦٨٤٣٧٦٠، ٥٣٦٨٧٥٢٠، ١٠٧٣٧٥٠٤٠، ٢١٤٧٥٠٠٨٠، ٤٢٩٥٠٠١٦٠، ٨٥٩٠٠٠٣٢٠، ١٧١٨٠٠٦٤٠، ٣٤٣٦٠١٢٨، ٦٨٧٢٠٢٥٦، ١٣٧٤٤٠٥١٢، ٢٧٤٨٨٠٩٢٤، ٥٤٩٧٦١٨٤٨، ١٠٩٩٥٢٣٦٩٦، ٢١٩٩٠٤٧٣٩٢، ٤٣٩٨٠٩٤٧٨٤، ٨٧٩٦١٨٩٥٦٨، ١٧٥٩٢٣٧١٢٨، ٣٥١٨٤٧٤٢٥٦، ٧٠٣٦٩٤٨٥١٢، ١٤٠٧٣٨٩٠٢٤، ٢٨١٤٧٧٨٠٤٨، ٥٦٢٩٥٥٦٠٩٦، ١١٢٥٩١١٣١٢، ٢٢٥١٨٢٢٦٢٤، ٤٥٠٣٦٤٥٢٤٨، ٩٠٠٧٢٩٠٤٩٦، ١٨٠١٤٥٨٠٩٦، ٣٦٠٢٩١٦١٩٢، ٧٢٠٥٨٣٢٣٨٤، ١٤٤١١٦٦٤٦٨، ٢٨٨٢٣٣٢٩٦، ٥٧٦٤٦٦٥٩٢، ١١٥٢٩٣٣١٨٤، ٢٣٠٥٨٦٦٣٦٨، ٤٦١١٧٣٢٧٣٦، ٩٢٢٣٤٦٥٤٧٢، ١٨٤٤٦٩٣٠٨٤٨، ٣٦٨٩٣٨٦١٧٦، ٧٣٧٨٧٧٢٣٥٢، ١٤٧٥٧٥٤٦٧٠٤، ٢٩٥١٥٠٩٣٤٠٨، ٥٩٠٣٠١٨٦٨١٦، ١١٨٠٦٠٣٧٣٦٣٢، ٢٣٦١٢٠٧٤٦٦٦٤، ٤٧٢٢٤١٤٩٣٣٢٨، ٩٤٤٤٨٢٩٨٦٦٥٦، ١٨٨٨٩٥٧٧٣٣٢٨، ٣٧٧٧٩١٥٤٦٦٥٦، ٧٥٥٥٨٣٠٩٣٣٣٢، ١٥١١١٦٦٠٦٦٦٦٤، ٣٠٢٢٣٣٢١٣٣٢٨، ٦٠٤٤٦٦٤٢٦٦٦٥٦، ١٢٠٨٩٣٢٥٣٣٣٢٨، ٢٤١٧٨٦٥٠٦٦٦٦٥٦، ٤٨٣٥٧٣٠١٣٣٣٢٨، ٩٦٧١٤٦٠٢٦٦٦٥٦، ١٩٣٤٢٩٠٥٣٣٣٢٨، ٣٨٦٨٥٨٠٦٦٦٦٥٦، ٧٧٣٧١٦١٣٣٣٢٨، ١٥٤٧٤٣٢٦٦٦٦٥٦، ٣٠٩٤٨٦٥٣٣٣٢٨، ٦١٨٩٧٣٠٦٦٦٦٥٦، ١٢٣٧٩٤٦١٣٣٣٢٨، ٢٤٧٥٨٩٢٦٦٦٦٥٦، ٤٩٥١٧٨٥٣٣٣٢٨، ٩٩٠٣٥٧٠٦٦٦٦٥٦، ١٩٨٠٧١٤١٣٣٣٢٨، ٣٩٦١٤٢٨٢٦٦٦٥٦، ٧٩٢٢٨٥٦٥٣٣٣٢٨، ١٥٨٤٥٧٣٠٦٦٦٦٥٦، ٣١٦٩١٤٦١٣٣٣٢٨، ٦٣٣٨٢٩٢٦٦٦٥٦، ١٢٦٧٦٥٦٥٣٣٣٢٨، ٢٥٣٥٣١٢٦٦٦٥٦، ٥٠٧٠٦٢٥٣٣٣٢٨، ١٠١٤١٢٥٦٦٦٥٦، ٢٠٢٨٢٥١٣٣٣٢٨، ٤٠٥٦٥٠٢٦٦٦٥٦، ٨١١٣٠٠٥٣٣٣٢٨، ١٦٢٢٦٠١٠٦٦٦٥٦، ٣٢٤٥٢٠٢١٣٣٣٢٨، ٦٤٩٠٤٠٤٢٦٦٥٦، ١٢٩٨٠٨٠٥٣٣٣٢٨، ٢٥٩٦١٦٠٦٦٦٥٦، ٥١٩٢٣٢١٣٣٣٢٨، ١٠٣٨٤٦٢٦٦٥٦، ٢٠٧٦٩٢٥٣٣٣٢٨، ٤١٥٣٨٥٠٦٦٥٦، ٨٣٠٧٧٠١٣٣٣٢٨، ١٦٦١٥٤٠٦٦٥٦، ٣٣٢٣٠٨٠٥٣٣٣٢٨، ٦٦٤٦١٦٠٦٥٦، ١٣٢٩٢٤٠٥٣٣٣٢٨، ٢٦٥٨٤٨٠٦٥٦، ٥٣١٦٩٦٠٥٣٣٣٢٨، ١٠٦٣٣٦٠٦٥٦، ٢١٢٦٧٢٠٥٣٣٣٢٨، ٤٢٥٣٤٤٠٦٥٦، ٨٥٠٦٨٨٠٥٣٣٣٢٨، ١٧٠١٣٧٠٦٥٦، ٣٤٠٢٧٤٠٥٣٣٣٢٨، ٦٨٠٥٤٨٠٥٣٣٣٢٨، ١٣٦١٠٩٦٠٥٣٣٣٢٨، ٢٧٢٢١٩٢٠٥٣٣٣٢٨، ٥٤٤٤٣٨٤٠٥٣٣٣٢٨، ١٠٨٨٨٧٦٠٥٣٣٣٢٨، ٢١٧٧٧٥٢٠٥٣٣٣٢٨، ٤٣٥٥٥٠٤٠٥٣٣٣٢٨، ٨٧١١٠٠٨٠٥٣٣٣٢٨، ١٧٤٢٢٠١٦٠٥٣٣٣٢٨، ٣٤٨٤٤٠٣٢٠٥٣٣٣٢٨، ٦٩٦٨٨٠٦٤٠٥٣٣٣٢٨، ١٣٩٣٧٦٠٩٦٠٥٣٣٣٢٨، ٢٧٨٧٥٢١٩٢٠٥٣٣٣٢٨، ٥٥٧٥٠٤٣٨٤٠٥٣٣٣٢٨، ١١١٥٠٠٨٧٦٠٥٣٣٣٢٨، ٢٢٣٠٠١٧٥٢٠٥٣٣٣٢٨، ٤٤٦٠٠٣٥٠٤٠٥٣٣٣٢٨، ٨٩٢٠٠٧٠٠٨٠٥٣٣٣٢٨، ١٧٨٤٠١٤٠١٦٠٥٣٣٣٢٨، ٣٥٦٨٠٢٨٠٣٢٠٥٣٣٣٢٨، ٧١٣٦٠٥٦٠٦٤٠٥٣٣٣٢٨، ١٤٢٧٢١٢٠١٢٨٠٥٣٣٣٢٨، ٢٨٥٤٤٢٤٠٢٥٦٠٥٣٣٣٢٨، ٥٧٠٨٨٤٨٠٥١٢٠٥٣٣٣٢٨، ١١٤١٧٦٩٦٠٢١٢٠٥٣٣٣٢٨، ٢٢٨٣٥٣٩٢٠٤٢٤٠٥٣٣٣٢٨، ٤٥٦٧٠٧٨٤٠٨٤٨٠٥٣٣٣٢٨، ٩١٣٤١٥٦٨٠١٦٩٦٠٥٣٣٣٢٨، ١٨٢٦٨٣١٢٠٣٣٩٢٠٥٣٣٣٢٨، ٣٦٥٣٦٦٢٤٠٦٧٩٢٠٥٣٣٣٢٨، ٧٣٠٧٣٢٤٨٠١٣٥٨٤٠٥٣٣٣٢٨، ١٤٦١٤٦٤٩٦٠٢٧١٦٨٠٥٣٣٣٢٨، ٢٩٢٢٩٢٩٩٢٠٥٤٣٣٦٠٥٣٣٣٢٨، ٥٨٤٥٨٥٩٨٤٠١٠٨٦٧٢٠٥٣٣٣٢٨، ١١٦٩١٧١٩٦٠٢١٧٣٤٤٠٥٣٣٣٢٨، ٢٣٣٨٣٤٣٩٢٠٤٣٤٦٨٨٠٥٣٣٣٢٨، ٤٦٧٦٦٨٧٨٤٠٨٦٩٣٧٦٠٥٣٣٣٢٨، ٩٣٥٣٣٧٥٦٨٠١٧٣٨٧٥٢٠٥٣٣٣٢٨، ١٨٧٠٦٧٥١٣٦٠٣٤٧٧٥٠٥٣٣٣٢٨، ٣٧٤١٣٥٠٢٧٢٠٦٩٥٥٠٥٣٣٣٢٨، ٧٤٨٢٧٠٠٥٤٤٠١٣٩١٠٥٣٣٣٢٨، ١٤٩٦٥٤٠١٠٨٨٠٢٧٨٢٠٥٣٣٣٢٨، ٢٩٩٣٠٨٠٢١٦٦٤٠٥٣٣٣٢٨، ٥٩٨٦١٦٠٤٣٣٢٠٥٣٣٣٢٨، ١١٩٧٢٣٠٨٦٦٤٠٥٣٣٣٢٨، ٢٣٩٤٤٦٠١٧٣٢٠٥٣٣٣٢٨، ٤٧٨٨٩٢٠٣٤٦٤٠٥٣٣٣٢٨، ٩٥٧٧٨٤٠٦٩٢٨٠٥٣٣٣٢٨، ١٩١٥٥٦٠١٣٨٥٦٠٥٣٣٣٢٨، ٣٨٣١١٢٠٢٧٧١٢٠٥٣٣٣٢٨، ٧٦٦٢٢٤٠٥٥٤٢٤٠٥٣٣٣٢٨، ١٥٣٢٤٤٠١١٠٨٤٨٠٥٣٣٣٢٨، ٣٠٦٤٨٨٠٢٢١٦٩٦٠٥٣٣٣٢٨، ٦١٢٩٧٦٠٤٤٣٣٦٠٥٣٣٣٢٨، ١٢٢٥٩٥٢٠٨٨٦٧٢٠٥٣٣٣٢٨، ٢٤٥١٩٠٤١٧٣٤٤٠٥٣٣٣٢٨، ٤٩٠٣٨٠٨٣٤٦٨٠٥٣٣٣٢٨، ٩٨٠٧٦١٦٧٣٣٦٠٥٣٣٣٢٨، ١٩٦١٥٢٣٤٦٦٧٢٠٥٣٣٣٢٨، ٣٩٢٣٠٤٦٩٣٣٤٠٥٣٣٣٢٨، ٧٨٤٦٠٩٣٨٦٦٧٢٠٥٣٣٣٢٨، ١٥٦٩٢١٧٧٣٣٤٠٥٣٣٣٢٨، ٣١٣٨٤٣٤٦٩٣٣٦٠٥٣٣٣٢٨، ٦٢٧٦٨٦٩٣٨٦٦٧٢٠٥٣٣٣٢٨، ١٢٥٥٣٧٧٧٧٣٣٦٠٥٣٣٣٢٨، ٢٥١٠٧٥٥٤٦٩٣٣٦٠٥٣٣٣٢٨، ٥٠٢١٥١٠٩٣٨٦٦٧٢٠٥٣٣٣٢٨، ١٠٠٤٣٠٢١٧٧٣٣٦٠٥٣٣٣٢٨، ٢٠٠٨٦٠٤٣٥٤٦٩٣٣٦٠٥٣٣٣٢٨، ٤٠١٧٢٠٨٧٠٩٣٨٦٦٧٢٠٥٣٣٣٢٨، ٨٠٣٤٤١٧٤١٨٧٦٦٧٢٠٥٣٣٣٢٨، ١٦٠٦٨٣٤٨٣٥٤٦٩٣٣٦٠٥٣٣٣٢٨، ٣٢١٣٦٦٩٦٧١١٦٩٣٣٦٠٥٣٣٣٢٨، ٦٤٢٧٣٣٩٣٤٢٣٣٦٠٥٣٣٣٢٨، ١٢٨٥٤٦٧٦٦٨٤٦٩٣٣٦٠٥٣٣٣٢٨، ٢٥٧٠٩٣٥٣٣٦٠٥٣٣٣٢٨، ٥١٤١٨٧٠٦٦٧٢٠٥٣٣٣٢٨، ١٠٢٨٣٤١٣٣٦٠٥٣٣٣٢٨، ٢٠٥٦٦٨٢٦٦٧٢٠٥٣٣٣٢٨، ٤١١٣٣٦٥٣٣٦٠٥٣٣٣٢٨، ٨٢٢٦٧٣٠٦٦٧٢٠٥٣٣٣٢٨، ١٦٤٥٣٤٦٦٧٢٠٥٣٣٣٢٨، ٣٢٩٠٦٩٣٣٦٠٥٣٣٣٢٨، ٦٥٨١٣٨٦٦٧٢٠٥٣٣٣٢٨، ١٣١٦٢٧٣٣٦٠٥٣٣٣٢٨، ٢٦٣٢٥٤٦٦٧٢٠٥٣٣٣٢٨، ٥٢٦٥٠٩٣٣٦٠٥٣٣٣٢٨، ١٠٥٣٠١٦٦٧٢٠٥٣٣٣٢٨، ٢١٠٦٠٣٣٦٠٥٣٣٣٢٨، ٤٢١٢٠٦٦٧٢٠٥٣٣٣٢٨، ٨٤٢٤١٣٣٦٠٥٣٣٣٢٨، ١٦٨٤٨٢٦٧٢٠٥٣٣٣٢٨، ٣٣٦٩٦٦٧٢٠٥٣٣٣٢٨، ٦٧٣٩٣٣٦٠٥٣٣٣٢٨، ١٣٤٧٨٦٧٢٠٥٣٣٣٢٨، ٢٦٩٥٧٣٣٦٠٥٣٣٣٢٨، ٥٣٩١٤٦٧٢٠٥٣٣٣٢٨، ١٠٧٨٢٧٣٣٦٠٥٣٣٣٢٨، ٢١٥٦٥٤٦٧٢٠٥٣٣٣٢٨، ٤٣١٣٠٩٣٣٦٠٥٣٣٣٢٨، ٨٦٢٦١٦٧٢٠٥٣٣٣٢٨، ١٧٢٥٢٣٣٦٠٥٣٣٣٢٨، ٣٤٥٠٤٦٧٢٠٥٣٣٣٢٨، ٦٩٠٠٩٣٣٦٠٥٣٣٣٢٨، ١٣٨٠١٦٧٢٠٥٣٣٣٢٨، ٢٧٦٠٣٣٦٠٥٣٣٣٢٨، ٥٥٢٠٦٧٢٠٥٣٣٣٢٨، ١١٠٤١٣٣٦٠٥٣٣٣٢٨، ٢٢٠٨٢٦٧٢٠٥٣٣٣٢٨، ٤٤١٦٣٣٦٠٥٣٣٣٢٨، ٨٨٣٢٦٧٢٠٥٣٣٣٢٨، ١٧٦٦٣٣٦٠٥٣٣٣٢٨، ٣٥٣٢٦٧٢٠٥٣٣٣٢٨، ٧٠٦٥٣٣٦٠٥٣٣٣٢٨، ١٤١٣٦٧٢٠٥٣٣٣٢٨، ٢٨٢٧٣٣٦٠٥٣٣٣٢٨، ٥٦٥٤٦٧٢٠٥٣٣٣٢٨، ١١٣٠٩٣٣٦٠٥٣٣٣٢٨، ٢٢٦١٨٦٧٢٠٥٣٣٣٢٨، ٤٥٢٣٦٧٢٠٥٣٣٣٢٨، ٩٠٤٧٣٣٦٠٥٣٣٣٢٨، ١٨٠٩٤٦٧٢٠٥٣٣٣٢٨، ٣٦١٨٩٣٣٦٠٥٣٣٣٢٨، ٧٢٣٧٨٦٧٢٠٥٣٣٣٢٨، ١٤٤٧٥٣٣٦٠٥٣٣٣٢٨، ٢٨٩٥٠٦٧٢٠٥٣٣٣٢٨، ٥٧٩٠١٣٣٦٠٥٣٣٣٢٨، ١١٥٨٠٢٦٧٢٠٥٣٣٣٢٨، ٢٣١٦٠٦٧٢٠٥٣٣٣٢٨، ٤٦٣٢٠٦٧٢٠٥٣٣٣٢٨، ٩٢٦٤٠٦٧٢٠٥٣٣٣٢٨، ١٨٥٢٨٦٧٢٠٥٣٣٣٢٨، ٣٧٢٥٧٣٣٦٠٥٣٣٣٢٨، ٧٤٥١٤٦٧٢٠٥٣٣٣٢٨، ١٤٩٠٣٣٦٠٥٣٣٣٢٨، ٢٩٨٠٦٧٢٠٥٣٣٣٢٨، ٥٩٦١٣٣٦٠٥٣٣٣٢٨، ١١٩٢٢٦٧٢٠٥٣٣٣٢٨، ٢٣٨٤٤٦٧٢٠٥٣٣٣٢٨، ٤٧٦٨٩٣٣٦٠٥٣٣٣٢٨، ٩٥٣٧٨٦٧٢٠٥٣٣٣٢٨، ١٩٢٧٥٣٣٦٠٥٣٣٣٢٨، ٣٨٥٥٠٦٧٢٠٥٣٣٣٢٨، ٧٧١٠١٣٣٦٠٥٣٣٣٢٨، ١٥٤٠٢٦٧٢٠٥٣٣٣٢٨، ٣٠٨٠٤٦٧٢٠٥٣٣٣٢٨، ٦١٦٠٩٣٣٦٠٥٣٣٣٢٨، ١٢٣٢١٦٧٢٠٥٣٣٣٢٨، ٢٤٦٤٣٣٦٠٥٣٣٣٢٨، ٤٩٢٨٦٧٢٠٥٣٣٣٢٨، ٩٨٥٧٣٣٦٠٥٣٣٣٢٨، ١٩٧١٤٦٧٢٠٥٣٣٣٢٨، ٣٩٤٢٩٣٣٦٠٥٣٣٣٢٨، ٧٨٨٥٨٦٧٢٠٥٣٣٣٢٨، ١٥٧٦٧٣٣٦٠٥٣٣٣٢٨، ٣١٥٣٤٦٧٢٠٥٣٣٣٢٨، ٦٣٠٦٩٣٣٦٠٥٣٣٣٢٨، ١٢٦١٣٦٧٢٠٥٣٣٣٢٨، ٢٥٢٢٧٣٣٦٠٥٣٣٣٢٨، ٥٠٤٥٤٦٧٢٠٥٣٣٣٢٨، ١٠٠٩٠٦٧٢٠٥٣٣٣٢٨، ٢٠١٨٠٦٧٢٠٥٣٣٣٢٨، ٤٠٣٦٠٦٧٢٠٥٣٣٣٢٨، ٨٠٧٢٠٦٧٢٠٥٣٣٣٢٨، ١٦١٤٠٦٧٢٠٥٣٣٣٢٨، ٣٢٢٨٠٦٧٢٠٥٣٣٣٢٨، ٦٤٥٦٠٦٧٢٠٥٣٣٣٢٨، ١٢٩١٢٦٧٢٠٥٣٣٣٢٨، ٢٥٨٢٤٦٧٢٠٥٣٣٣٢٨، ٥١٦٤٨٦٧٢٠٥٣٣٣٢٨، ١٠٣٢٩٦٧٢٠٥٣٣٣٢٨، ٢٠٦٥٨٦٧٢٠٥٣٣٣٢٨، ٤١٣١٦٧٢٠٥٣٣٣٢٨، ٨٢٦٣٢٦٧٢٠٥٣٣٣٢٨، ١٦٥٢٦٧٢٠٥٣٣٣٢٨، ٣٣٢٥٢٦٧٢٠٥٣٣٣٢٨، ٦٦٥٠٤٦٧٢٠٥٣٣٣٢٨، ١٣٢٩٦٧٢٠٥٣٣٣٢٨، ٢٦٥٩٢٦٧٢٠٥٣٣٣٢٨، 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٢٩٦٩٢٦٧٢٠٥٣٣٣٢٨، ٥٩٣٨٤٦٧٢٠٥٣٣٣٢٨، ١١٨٧٦٧٢٠٥٣٣٣٢٨، ٢٣٧٥٢٦٧٢٠٥٣٣٣٢٨، ٤٧٥٠٤٦٧٢٠٥٣٣٣٢٨، ٩٤٠٠٨٦٧٢٠٥٣٣٣٢٨، ١٨٨٠١٦٧٢٠٥٣٣٣٢٨، ٣٧٦٠٣٦٧٢٠٥٣٣٣٢٨، ٧٥٢٠٦٧٢٠٥٣٣٣٢٨، ١٤٨٠٦٧٢٠٥٣٣٣٢٨، ٢٩٦٠٣٦٧٢٠٥٣٣٣٢٨، ٥٩٢٠٦٧٢٠٥٣٣٣٢٨، ١١٨٠١٦٧٢٠٥٣٣٣٢٨، ٢٣٦٠٣٦٧٢٠٥٣٣٣٢٨، ٤٧٤٠٦٧٢٠٥٣٣٣٢٨، ٩٤٠٠٦٧٢٠٥٣٣٣٢٨، ١٨٨٠١٦٧٢٠٥٣٣٣٢٨، ٣٧٦٠٣٦٧٢٠٥٣٣٣٢٨، ٧٥٢٠٦٧٢٠٥٣٣٣٢٨، ١٤٨٠٦٧٢٠٥٣٣٣٢٨، ٢٩٦٠٣٦٧٢٠٥٣٣٣٢٨، ٥٩٢٠٦٧٢٠٥٣٣٣٢٨، ١١٨٠١٦٧٢٠٥٣٣٣٢٨، ٢٣٦٠٣٦٧٢٠٥٣٣٣٢٨، ٤٧٤٠٦٧٢٠٥٣٣٣٢٨، ٩٤٠٠٦٧٢٠٥٣٣٣٢٨، ١٨٨٠١٦٧٢٠٥٣٣٣٢٨، ٣٧٦٠٣٦٧٢٠٥٣٣٣٢٨، ٧٥٢٠٦٧٢٠٥٣٣٣٢٨، ١٤٨٠٦٧٢٠٥٣٣٣٢٨، ٢٩٦٠٣٦