

**FISH MEAL REPLACEMENT BY YEAST PROTEIN
(SACCHAROMYCES CEREVISIAE) SUPPLEMENTED WITH
BIOGENIC L-CARINTINE AS A SOURCE OF METHIONINE
PLUS LYSINE MIXTURE IN FEED FOR NILE TILAPIA
(OREOCHROMIS NILOTICUS) FINGERLINGES**

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Abstract

This experiment was carried out to study the effect of partial to total replacement of animal protein (fish meal) in the diet with yeast protein *Saccharomyces cerevisiae* supplemented with biogenic L-carintine (methionine plus lysine mixture) on growth performance, carcass composition and feed utilization of fingerlings Nile tilapia with average body weight 1.5 g/fish. Five experimental diets were prepared, control diet (T₁) contained 20% (fish meal) as animal protein and 80% (soybean meal) plant protein. Tested diets (T₂-T₅) contained 3.57, 7.14, 10.71 and 14.29% yeast in order to substituting about 25, 50, 75 and 100% of fish meal diet respectively. These diets supplemented with a mixture of methionine plus lysine (biogenic L-carintine) at a rate of 100mg/100g diet. The experiment was conducted in 15 aquaria. Each aquarium supplied with well-aerated and dechlorinated tap water for 112 days. The results revealed that, tilapia fed on T₃ and T₄ diets which containing 7.14 and 10.71% yeast (50 and 75% replacement) supplemented with 100mg/100g diet biogenic L-carintine recorded the best growth performance, feed and protein utilization than other experimental and control diet. Tilapia fed on diet T₅ which containing 14.29% yeast (fish meal free diet) had poor growth and did not differ significantly ($p < 0.05$) from the T₂ or T₁ (control). Diet composition did not significantly affect on carcass composition. These data suggested that, yeast *Saccharomyces cerevisiae* supplemented with biogenic L-carintine (methionine plus lysine mixture) can totally replace of fish meal (animal protein) in fingerlings tilapia diet without any adverse effect on growth performance. Moreover, fish meal partially replacement by yeast, gave superior growth performance than other diets under the present experimental condition.

INTRODUCTION

Yeasts are a rich source of protein and B-complex vitamins. They have been used successfully as a complementary protein source in fish diet (Liao, 1977). Also, they have been used as a supplement in animals feed to compensate for the amino acid and vitamin deficiencies of cereals, and are recommended as a substitute for soybean oil in diets for fowl (Gohl, 1991). In addition they are considered a cheaper dietary supplement as they are easily produced on an industrial level from a number of carbon-rich substrate by-products (Lee and Kim 2001). Crude protein content in the yeast compared favorably with other conventional protein source (Ballerini and

Thonon,1980).The percentage of crude protein varies among yeast products and ranges from 38.8% in *Candida boidinii* to 67.8% in *Candida lipolytica* (Shulz and Oslage,1976). Therefor the nutritive value of yeast products differs according to its type. *Candida sp,Hansenula sp, Pichia sp.and Saccharomyces sp.* are special importance as components in fish feeds. However, the presence of high percentage of non protein nitrogen sets some limitation against yeast consumption. Yeast and soybean have almost the same essential amino acid composition (Yeh, 1963). However, crude protein of yeasts comprises a high content of non-protein nitrogen (De Groot, 1977). Lysine content in yeast varies according to yeast strain and ranges between 4.1 in *Torulopsis candida* (Ballerini and Thonon, 1977) to 8.4% of dietary protein in *Saccharomyces sp.* (Schulz and Oslage, 1976). Methionine content as percentage of dietary protein ranges from 0.9 in *Candida tropicalis* (Ballerini and Thonon, 1980) to 1.9 in *Pichia aganobii* (Ohkouchi *et al.*, 1980).Yeast based starters fed to carp larvae resulted in higher survival rate and better growth performance (Kamler *et al.*, 1987).When fodder yeast was fed to young carp, fish gained more weigh than control diet based on live food. Matty and Smith (1978) reported that, yeast *Candida lipolytica* was the most efficient feed for growth of rainbow trout. Omar *et al.* (1989) found that, active yeast (*Saccharomyces cerevisiae*) significantly growth of tilapia and carp over inactive yeast.(Abd EL-Halim *et al.*,1992) reported that, tilapia fed on diet containing 100% yeast protein instead of fish meal protein gave the lowest growth performance and feed utilization than those control diet which contained 100% fish meal protein. A similar result were obtained by Murai *et al.* (1989) on carp fingerlings fed on diet containing 10.5% fish meal and 13.6% torula yeast. Kohler and Pagan-Font (1978) found that, tilapia fed on diet containing distilleries yeast give the lowest growth performance than those tilapia fed on diets containing fish meal. Similarly rainbow trout fed the yeast *Hansenula anomala* as the only protein source showed significant reduction in food intake, weight gain and length increment (Sanchez- Muniz *et al.* 1983). Mahnken *et al.* (1980) when used *Candida sp.* As a substitute for fish meal in salmonid diets found that, these fishes accepted substitution levels from 25 to50%. A slight growth reduction was noted at the highest inclusion level, though this was improved with addition of methionine.

The present study aims to evaluate the effect of partial to total replacement of fish meal (animal protein) in the diet with yeast (*Saccharomyces cerevisiae*) supplemented with biogenic L-carintine (methionine plus lysine mixture) on growth performance, carcass composition and feed utilization by Nile tilapia (*Oreochromis niloticus*) fingerlings.

MATERIALS AND METHODS

Experimental procedure

A total number of 300 Nile tilapia fingerlings (*Oreochromis niloticus*) were obtained from the Department of Hatchery and Reproductive fish in Central Lab. for Aquaculture Research, Agriculture Research Center. The fish obtained were apparently healthy, and free from any infection. Fish with an average body weight of (1.5g/fish), were placed in a fiberglass tank, and fed on control diet (T₁) which contained fish meal protein. After two week acclimation period, the fingerlings were divided into five equal groups. Each group with three replicates and each aquarium contain 20 fish and transferred into glass aquaria, each aquarium measured (50 X 50 X 60cm), and it was supplied with dechlorinated aerated tap water. The aquaria were cleaned and water was changed biweekly. Dissolved oxygen was maintained at an acceptable range (5.5 – 6.5 mg/L), water temperature was thermostatically controlled at the range of 27 ± 2°C, with thermostatic heater and water Ph adjusted at 7.4. Nile tilapia fingerlings were fed twice a day for 6 days a week. Daily feed allowance was determined as percentage of fish live body weight and readjusted biweekly. Feeding rate were 4% of the fish fresh body weight on five experimental diets to evaluate the effect of replacing dietary fish meal T₁ (control) by 25, 50, 75 and 100% yeast (*Saccharomyces cerevisiae*) supplemented with 100mg/100g diet biogenic L-carintine on growth performance, carcass composition and feed and nutrient utilization.

The formulation and chemical composition of the experimental diets used were illustrated in Table (1). At the end of the experiment, fish in each aquarium were counted, weighted, growth and feed parameters were calculated as described previously by Ebrahim (2007). The chemical analysis of both diets and fish were determined by Stander Methods According to (AOAC, 1984).

Tested yeast

Tested yeast obtained from the Egyptian Company for Starch and yeast products are commercial products of the yeast (*Saccharomyces cerevisiae*) grown on molasses. Yeast was dried at lower temperature (37 °c). After that, cooled and saved in plastic bags and stored in refrigerator at 5 °c until add to the experimental diets to avoid the nutrients deterioration. The dried yeast is approximately composed of 42.03% protein, 6.52%fat, 6.15%ash, 39.78% carbohydrate and 8.52% moisture.

Statistical methods

The data obtained in the present study were statically analyzed according to F-test : Analysis of variance (ANOVA) was carried out following the method described for one way classification (Snedecor and Cochran,1982), and for comparing the different mean values with Duncan's multiple range tests (Duncan,1955).

RESULTS AND DISCUSSION

Growth performance

Results summarized in Table (2) showed the effect of partial to total replacement of animal protein (fish meal) with yeast protein supplemented with 100mg/100g diet biogenic L-carintine on growth performance and survival rate of Nile tilapia (*Oreochromis niloticus*) fingerlings. It could be noticed that, the initial body weight of tilapia in all treatments were approximately similar and there were no significant differences among them.

Concerning with final body weigh, the obtained results revealed that, no significant differences were observed between diets T₂, T₅ and control (T₁). While the diets T₃ and T₄ which contained 50 and 75% replacement were significantly superior than control (T₁) and other diets (T₂ and T₅) respectively.

In respect of total weight gain (g/fish), daily weight gain (g/fish), specific growth rate (%/d) and relative growth rate (%) recorded the same trend of the final body weight. Our data are in agreement with those obtained by Abou –seif (2006) who found that, using biogenic L-carintine in monosex Nile tilapia diet during a single stage nursery-rearing system improve growth performance and survival rate, (Mahnken *et al.* 1980) they found that, no reduction in the growth of trout fed diets without fish meal. Also, they found that, salmonids accepted substitution fish meal by yeast (*Candida sp.*) at a rate of 25 to 50% replacement. A slight growth reduction was noted at the highest inclusion level and Olvera-Novoa *et al.* (2002) they revealed that, it is possible to replace up to 65% of animal protein with a mixture of plant protein, including 30% from torula yeast, in tilapia fry diets without adverse effects on fish performance and culture profit. Moreover, (Abd EL-Halim *et al* 1995) in tilapia,(Murai *et al.* 1989) in carp and (Sancheze Muniz *et al.*1983) in rainbow trout ,they showed that, total replacement fish meal protein by yeast protein gave the slight growth reduction than those control diet which contained 100% fish meal protein, though this was slightly improved with addition of methionine. These results evidence that the biogenic L-carintine (methionine plus lysine) supplementation is useful only when yeast is partialy replaced by animal protein. Wu and Jam(1977) attributed the poor body weight gain of tilapia when fed on 100% yeast diet to poor digestibility of yeast and amino acid composition or

imbalance, Windell *et al.* (1974) suggested that, the poor digestibility of yeast was attributed to an inferior processing method having severely reduced carbohydrate digestibility and consequently the total nitrogen digestibility of yeast for fish and Tacon and Cooke (1980) suggested that, the poor digestibility of yeast is due to high nucleic acid content.

Concerning with survival rate, it could be noticed that, survival rate was not affected by replacing fish meal by yeast protein (*Saccharomyces cerevisiae*) where, survival rate values were not significantly fluctuated between all tested and control diets. These values are in the same line with those reported by Abd EL-Halim *et al.* (1995) they found that, yeast (*Saccharomyces cerevisiae*) can replace about 50% of fish meal in fingerlings tilapia diet without any adverse effect of survival rate or compromising growth

Carcass composition

Chemical analysis of fish carcass at the end of the experiment is shown in Table(3), the obtained results showed that, dry matter, crude protein, body fat and body ash content were slightly fluctuated among all the experimental diets without significantly differences. These results took the same trend of those results obtained by Olvera-Novoa *et al.* (2002) they observed no differences in carcass composition when substituting animal protein with a mixture of plant feedstuffs including 25,30,35,40 and 45% of the protein with torula yeast (*Candida utilis*) in diet for tilapia (*Oreochromis mossambicus*) fry, Omar *et al.* (1989) they showing no significant differences in carcass composition of tilapia fed active or inactive yeast and Ebrahim *et al.* (2008) they reported that, carcass composition was not affected by dietary protein sources.

Feed and nutrient utilization

Results of feed and nutrient utilization are presented in Table (4), the obtained results revealed that, diets T₃ and T₄ which contained 50 and 75% replacement were significantly higher in dry feed intake and feed conversion ratio (FCR) than control diet (T₁) and other tested diets. Increasing dry feed intake and FCR in diets T₃ and T₄ reflect on the protein retention efficiency (PRE) and energy retention efficiency (ERE) which were significantly increased in the same diets (T₃ and T₄) than among all the experimental diets. These results agreement with results were reported by Abd EL-Halim *et al.* (1995) they found that, protein and energy retention efficiency were lower in the diet containing 30% yeast and diet free from animal protein (100% yeast). But protein and energy retention efficiency were higher in the diets containing above 30% yeast and diet containing animal protein (control). Concerning with protein efficiency ratio (PER), it could be noticed that, no

significant differences observed between all the experimental diets (T₁-T₅). These results evidence that, tilapia was able to utilize yeast protein efficiency comparing with fish meal diet (T₁).

In conclusion, the present study clearly confirm the possibility of replacing the expensive protein source (fish meal) with less expensive source (*Saccharomyces cerevisiae*) in tilapia fingerlings diets, whereas tilapia grew better when fed on diets contained yeast plus fish meal at a rate of 50/50 and 75/25 yeast/fish meal % than diets contained yeast or fish meal alone as a source of animal protein.

Table 1. The formulation and chemical composition of the tested diets

Diets	Control	Tested diets			
		Fish meal/yeast %	75/25	50/50	25/75
Diet No.	T ₁	T ₂	T ₃	T ₄	T ₅
Fish meal (59.1%CP)	10.15	7.61	5.07	2.54	-
Soybean meal (49.80%CP)	48.22	48.22	48.22	48.22	48.22
Yeast (42.03%CP)	-	3.57	7.14	10.71	14.29
Starch	34.24	33.20	32.27	31.33	30.39
Fish oil	1.46	1.60	1.73	1.87	2.00
Vegetable oil	2.93	2.70	2.47	2.23	2.00
Vitamin premix ¹	1.00	1.00	1.00	1.00	1.00
Mineral premix ²	1.00	1.00	1.00	1.00	1.00
Biogenic L-carintine ³	0.10	0.10	0.10	0.10	0.10
Carboxymethyl cellulose (CMC)	0.50	0.50	0.50	0.50	0.50
Vit C	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100
Chemical composition (on as fed basis)					
Dry matter (DM%)	90.16	91.17	91.20	90.51	91.67
Crude protein (CP%)	29.66	29.60	29.50	29.60	29.70
Ether extract (E.E%)	7.80	7.85	8.00	8.18	8.30
Crude fiber (CF%)	3.22	3.30	3.42	3.44	3.45
Ash (%)	6.55	6.55	6.00	5.89	5.70
N.F.E (%) ⁴	42.93	43.87	44.28	43.40	44.52
Gross energy (GE) (Kcal/100g) ⁵	427.76	431.97	434.95	433.79	440.00
Digestible energy (DE) (Kcal/100g) ⁶	310.80	312.99	314.90	315.30	319.14
P:E ratio ⁷	69.34	68.52	69.58	68.24	67.50
Protein energy ⁸	39.52	39.06	38.66	38.90	38.48

¹Vitamin premix = each 2.5Kg contain Vit A 10MIU, D₃ 1MIU, E 10gm, K gm, B₁ gm, B₂ 4gm, B₆ 1.5gm, B₁₂ 10gm, Pantothenic acid 10gm, Nicotinic acid 20gm, Folic acid 1000gm, Biotin 50gm And Coline chloride 500gm.

²Mineral premix. No # 5 (tilapia) sources: Jauncey and Ross, 1982.

³Biogenic L-carintine = methionine plus lysine mixture

⁴N.F.E = Nitrogen free extract = 100 — (moisture + protein + lipid + fiber + ash)

⁵Gross energy (kcal/100g) ,based on 5.7 kcal/g protein,9.5 kcal/g lipid,4.0 kcal/g carbohydrate.

⁶Digestible energy (kcal/100g) , based on 5.0 kcal/g protein,9.0 kcal/g lipid,2.0 kcal/g carbohydrat

⁷P/E ratio = Protein to energy ratio in mg protein/kcal of gross energy

⁸Protein energy = (energy in protein/gross energy) X 100.

Table 2. Effect (mean±S.E) of replacing fish meal with yeast protein supplemented with biogenic L-carintine on growth performance of Nile tilapia fingerlings

Diets	Control	Tested diets			
Fish meal/yeast %	100/0.0	75/25	50/50	25/75	0.0/100
Diet No.	T ₁	T ₂	T ₃	T ₄	T ₅
Initial body weight (g/fish)	1.57±0.18 ^a	1.57±0.24 ^a	1.50±0.17 ^a	1.53±0.25 ^a	1.53±0.20 ^a
Final body weight (g/fish)	10.17±0.53 ^b	10.00±0.52 ^b	12.33±0.40 ^a	12.33±0.65 ^a	9.87±0.53 ^b
Total body weight gain (g/fish)	8.60±0.53 ^b	8.43±0.52 ^b	10.83±0.40 ^a	10.80±0.65 ^a	8.34±0.53 ^b
Daily body weight gain (g/fish) ¹	0.077±0.04 ^b	0.077±0.06 ^b	0.097±0.06 ^b	0.100±0.05 ^a	0.077±0.06 ^b
Specific growth rate (%) ²	1.67±0.16 ^b	1.65±0.20 ^b	1.82±0.15 ^a	1.82±0.19 ^a	1.64±0.16 ^b
Relative growth rate (%) ³	547.5±4.3 ^b	536.9±3.9 ^b	722.2±3.0 ^a	706.0±5.8 ^a	544.9±4.3 ^b
Survival rate (%) ⁴	90.00±0.52 ^a	88.33±0.40 ^a	91.65±0.56 ^a	93.33±0.40 ^a	86.65±0.40 ^a

Means with different superscript letters within a row are significantly different (P<0.05).

$$^1 \text{ Daily weight gain} = (W_1 - W_0) \div T$$

$$^2 \text{ Specific growth rate (\%)} = [(Ln_{w_1} - Ln_{w_0}) \div T] \times 100.$$

$$^3 \text{ Relative growth rate (\%)} = [(W_1 - W_0) \div W_0] \times 100.$$

Where, Ln = natural log, W₀ = Initial body weight (g), W₁ = Final body weight (g) and T = Time (day)

$$^4 \text{ Survival rate (\%)} = \text{Fish No. at the end} \div \text{Fish No. stocked at the beginning}.$$

Table 3. Body composition (on Dry matter basis) of Nile tilapia fingerlings fed diets containing different levels of yeast protein supplemented with biogenic L-carintine

Diets	Control	Tested diets			
Fish meal/yeast %	100/0.0	75/25	50/50	25/75	0.0/100
Diet No.	T ₁	T ₂	T ₃	T ₄	T ₅
Chemical composition	%				
Dry matter (%)	27.60±0.53 ^a	28.40±0.51 ^a	27.97±0.37 ^a	28.40±0.39 ^a	27.80±0.53 ^a
Crude protein (%)	56.70±0.48 ^a	57.00±0.49 ^a	57.75±0.43 ^a	57.40±0.41 ^a	56.60±0.33 ^a
Crude lipid (%)	23.10±0.52 ^a	22.71±0.47 ^a	23.04±0.18 ^a	23.16±0.27 ^a	23.04±0.42 ^a
Ash (%)	20.20±0.60 ^a	20.29±0.41 ^a	19.21±0.38 ^a	19.44±0.45 ^a	20.36±0.57 ^a

Means with different superscript letters within a row are significantly different (P<0.05)

Table 4. Effect (mean \pm S.E) of replacing fish meal with yeast protein supplemented with biogenic L-carintine on feed and nutrient utilization by Nile tilapia fingerlings

Diets	Control	Tested diets			
Fish meal/yeast%	100/0.0	75/25	50/50	25/75	0.0/100
Diet No	T ₁	T ₂	T ₃	T ₄	T ₅
Dry matter feed intake (g/fish)	19.70 \pm 0.68 ^b	19.27 \pm 0.40 ^b	23.20 \pm 0.54 ^a	23.17 \pm 0.76 ^a	19.07 \pm 0.47 ^b
Feed conversion ratio (FCR) ¹	2.30 \pm 0.15 ^a	2.30 \pm 0.18 ^a	2.14 \pm 0.11 ^b	2.15 \pm 0.19 ^b	2.30 \pm 0.14 ^a
Protein utilization					
Protein intake(g)	5.84	5.70	6.84	6.86	5.66
Protein gain(g)	0.90	1.20	1.95	1.60	0.80
Protein retention efficiency (%) ²	15.41 0.61 ^c	21.05 0.65 ^b	28.51 0.39 ^a	23.32 0.66 ^b	14.13 0.68 ^c
Protein efficiency ratio ³	1.46 0.11 ^a	1.55 0.15 ^a	1.55 0.18 ^a	1.57 0.17 ^a	1.47 0.11 ^a
Energy utilization					
Energy consumed (Kcal)	82.21	81.17	98.32	97.89	81.77
Energy retained (Kcal)	12.65	10.66	18.02	17.17	11.52
Energy retention efficiency (%) ⁴	15.39 \pm 0.54 ^b	13.13 \pm 0.19 ^c	18.33 \pm 0.45	17.54 \pm 0.52 ^a	14.09 \pm 0.46 ^b

Means with different superscript letters within a row are significantly different ($P < 0.05$).

¹Feed conversion ratio = Feed consumed (g) / Total weight gain (g).

²Protein retention efficiency (%) = [Final body protein (g) – Initial body protein (g) \div Dietary protein consumption (g)] X 100.

³Protein efficiency ratio = Live weight gain (g) \div Protein intake (g).

⁴Energy retention efficiency (%) = [Final body energy (Kcal) – Initial body energy (Kcal) \div Dietary energy consumption (Kcal)] X 100

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