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Effects of Partial Replacement of Fish Meal by Dried Baker's Yeast (*Saccharomyces cerevisiae*) on Growth Performance, Feed Utilization and Digestibility in Koi Carp (*Cyprinus Carpio* L., 1758) Fingerlings

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Abstract: This study was conducted to investigate the effects of dietary dried baker's yeast on growth performance, feed utilization and feed ingredients digestibility of koi carp (*Cyprinus carpio* L., 1758) fingerlings. In the experiment, a total of 270 koi carp fingerlings were equally divided into six groups of 45 (three replicates of 15 koi carp fingerlings each). Six levels (0, 10, 20, 30, 40 and 50%) of Dried Baker's Yeast (DBY) were included in isonitrogenous and isocaloric diets. The experimental period lasted 2 months (4 fortnight period). At the end of the experiment, there were no significant differences among the groups of 0, 10, 20, 30% in Body Weight (BW), Feed Intake (FI), Protein Intake (PI), Feed Conversion Ratio (FCR) and apparent digestibility ($p>0.05$). The inclusion of DBY at the level of 40% to the diets reduced Weight Gain (WG), FCR, Specific Growth Rate (SGR), Protein Efficiency Ratio (PER) and apparent digestibility ($p<0.05$). The highest dry matter, protein, fat, fiber, ash, nitrogen free extract and organic matter digestibility coefficients were observed in fish fed diet T (control diet 0% dry yeast replacement) which were significantly higher than those observed for the other diets ($p<0.05$). It is concluded that DBY can be used up to 30% in the diets of koi carp fingerlings without adverse effects on the measured parameters.

Key words: Dried baker's yeast (*Saccharomyces cerevisiae*), fish meal, koi carp, feed conversion ratio, specific growth ratio, protein efficiency ratio, digestibility

INTRODUCTION

Aquaculture continues to be the fastest growing animal food-producing sector. World aquaculture has grown dramatically in the last 50 years. From a production of <1 million ton in the early 1950s, production in 2006 was reported to have risen to 51.7 million ton. Aquaculture sector has maintained an average annual growth rate of 8.7% worldwide since 1970 (Anonymous, 2009). As intensive aquaculture continues to expand so does the requirement for high quality protein sources (Hardy, 1996; Carter and Hauler, 2000). Fish Meal (FM) is considered the most desirable animal protein ingredient in aquaculture diets because of its high protein content, balanced amino acid profiles, high digestibility and palatability and as a source of essential n-3 polyenoic fatty acids (Muzinic *et al*., 2006). The aquaculture sector consumed about 3.06 million ton (56.0%) of world fish meal production and 0.78 million ton (87.0%) of total fish oil production in 2006. The total amount of fishmeal and fish oil used in aquafeeds is estimated to have grown >3 fold between 1992 and 2006 from 0.96-3.06 million ton and from 0.23-0.78 million ton, respectively. Aquafeed manufacturers are increasing their use of fishmeal and fish oil at the expense of all other sectors (e.g., human consumption, industrial and pharmaceutical) (Anonymous, 2009).

FM is one of the most expensive macro-ingredients (used in high percentages) in an aquaculture diet. The high cost of FM and concerns regarding its future availability have made it imperative for the aquaculture industry to reduce or eliminate FM from fish diets. One approach aquaculture nutritionists have embraced is to partially or totally substitute FM with less expensive animal and/or plant protein sources (Muzinic *et al*., 2006). Therefore, the evaluation of alternative protein sources to fishmeal is a research priority. Among these, plant feedstuffs have received most attention in recent years; however due to amino acid unbalances, presence of anti-nutritional factors and low palatability, a high level of

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replacement of fish meal with plant feedstuffs is generally not well accepted. (Oliva-Teles and Goncalves, 2001). Single Cell Proteins (SCP) include micro algae, bacteria and yeast and are alternative non-conventional protein sources that are frequently used as feed ingredients for fish due to the nutritional value of their nutrients such as proteins, B-vitamins, pigments and complex carbohydrates, such as glucans (Sanderson and Jolly, 1994; Tacon, 1994; Oliva-Teles and Goncalves, 2001). Among SCP, yeasts have been the most used within aquafeeds (Tacon, 1994; Oliva-Teles and Goncalves, 2001). The nutritive value of yeast products differs according to its type.

Candida sp., *Hansenula* sp., *Pichia* sp. and *Saccharomyces* sp. have special importance as components in fish feeds (Ebrahim and Abou-Seif, 2008). Compared to fish meal, the majority of the SCP are either deficient in one or more amino acids or they suffer from an amino acid imbalance (Kiessling and Askbrandt, 1993; Oliva-Teles and Goncalves, 2001). According to Bergstrom (1979), Spinelli *et al*. (1979), Mahnken *et al*. (1980) and Murray and Marchant (1986), the supplementation of yeast-based diets with the deficient amino acids was shown to have beneficial effects on fish growth (Oliva-Teles and Goncalves, 2001). The objective of this study was to determine the extent to which Dried Baker's Yeast (DBY) (*Saccharomyces cerevisae*) could replace fish meal in koi carp fingerlings (*Cyprinus carpio*) diet while maintaining nutritional quality almost identical to the diet based on fish meal and thus be an ideal cost effective renewable alternative.

MATERIALS AND METHODS

Experimental fish and planning: The experiment was conducted in eighteen glass aquariums in the Fish Nutrition Laboratory, Department of Aquaculture and Fisheries, Faculty of Agriculture, University of Ankara, Ankara, Turkey. Each aquarium was filled with water up to level of 30 cm and this level was maintained throughout the experimental period of 2 months. About 270 healthy koi carp fingerlings used in the experiment were purchased from Akdeniz Fisheries Research Institute, Ministry of Agriculture and Rural Affairs, Kepez-Antalya, Turkey. The fingerlings were acclimatized in glass aquaria for 2 weeks. During this period, the fingerlings were fed with control diet. After acclimatization, 15 koi carp fingerlings were randomly transferred to each of the eighteen aquaria. The average initial body weight and length of fingerlings were 1.09±0.01 g and 3.95±0.02 cm, respectively. Three replicates were followed for each treatment.

Preparation of experimental diets and feeding regime: In this study, the experimental diets reported by Sarigun (2002), ingredient composition and chemical content in this experiment were used. The experimental diets are shown in Table 1. Fish were fed by hand ad libitum throughout the 2 months experimental period (El Sayed, 1990). The feed was administered twice daily (morning and evening). The morning feeding was done at 8:00 am and the evening feed was applied at 20:00 pm. Water quality parameters including water temperature,

Table 1: Inclusion levels of ingredients in experimental diets and its proximate composition

Feed ingredients (%)	T-control	T_1	T_2	T_3	T_4	T_5
Fish meal	25.000	22.500	20.000	17.500	15.000	12.547
Corn meal	27.865	27.865	27.865	27.865	27.865	27.865
Liquid vegetable oil	5.000	5.000	5.000	5.000	5.000	5.000
Wheat bran	2.139	0.000	0.000	0.000	0.000	0.000
Wheat meal	3.297	3.983	3.185	2.405	1.607	1.091
Soybean meal	30.961	30.961	30.961	30.961	30.961	30.961
DCP	3.638	4.000	3.747	3.476	3.212	2.640
Vitamin mix*	1.000	1.000	1.000	1.000	1.000	1.000
Mineral mix**	0.100	0.100	0.100	0.100	0.100	0.100
Metionin	0.000	0.300	0.560	0.820	1.090	1.340
Salt	0.500	0.500	0.500	0.500	0.500	0.500
Cr_2O_3	0.500	0.500	0.500	0.500	0.500	0.500
Dried baker's yeast	0.000	3.291	6.582	9.873	13.165	16.456
Dried baker's yeast (%)	0.000	10.000	20.000	30.000	40.000	50.000
Proximate composition of experimental diets (%)						
Dry matter	93.560	92.790	93.340	94.050	94.110	93.890
Moisture	6.440	7.210	6.660	5.950	5.890	6.110
Crude protein	34.990	34.920	34.850	34.840	34.760	34.650
Crude lipid	8.890	8.670	8.600	8.440	8.260	8.100
Crude fibre	2.890	2.650	2.720	2.690	2.500	2.460
Crude ash	12.600	12.390	11.310	11.120	10.150	9.790
Nitrogen-free extract	34.190	34.160	35.860	36.960	38.440	38.890
Digestible energy (kcal kg^{-1})	3000.000	3000.000	3000.000	3000.000	3000.000	3000.000

*Rovimix 107 (Vit A: 4000000 IU kg^{-1}, Vit D3: 600000 mg kg^{-1}, Vit E: 40000 mg kg^{-1}, Vit K3: 2400 mg kg^{-1}, Vit B1: 4000 mg kg^{-1}, Vit B2: 6000 mg kg^{-1}, Niacin: 40000 mg kg^{-1}, Cal-D-Pantothenate: 10000 mg kg^{-1}, Vit B6: 4000 mg kg^{-1}, Vit B12: 10 mg kg^{-1}, Folic acid: 1600 mg kg^{-1}, D-Biotin: 100 mg kg^{-1}, Vit C: 40000 mg kg^{-1}, Inositol: 60000 mg kg^{-1}); **Remineral B (Manganesium: 90000 mg kg^{-1}, Iron: 65000 mg kg^{-1}, Zinc: 80000 mg kg^{-1}, Copper: 12500 mg kg^{-1}, Cobalt: 400 mg kg^{-1}, Iodine 1800 mg kg^{-1}, Selenium 150 mg kg^{-1})

dissolved oxygen and pH in each aquarium were monitored through digital meters. The level of dissolved oxygen was maintained using air pump. The range of water quality parameters were: water temperature 22-26°C; pH 7.84±0.03; dissolved oxygen 6.78±0.03 mg L^{-1}; total ammonium nitrogen NH_3-N 0.003±0.001 mg L^{-1} throughout the experimental period.

About 2 h after each feeding, water from the aquaria was removed and the unconsumed feed from each aquarium was collected in separate petri dishes. Each aquarium was filled with water again immediately after the removal of unconsumed feed. The feed so collected was dried in an oven and weighed.

The fingerlings were taken from each replicate on fortnight basis after removing water from the aquarium. The morphometric characteristics, i.e., body weight and total length were recorded to observe their growth performance. The fingerlings were released in water immediately after body measurements.

The feed was stopped a day before the weight was recorded. The mean weight of fingerlings in each aquarium was calculated to determine the Feed Conversion Ratio (FCR) for the next fortnight. The FCR was calculated as follows (Halver, 1972):

$$FCR = FC/(W_2-W_1)$$

Where:

FC = The weight of food consumed by fish during the study period

W_1 = The live weight of fish at the beginning of the study period

W_2 = The live weight of fish at the end of the study period

Specific Growth Rate (SGR) was calculated as % change in body weight per day according to Ricker (1979):

$$SGR(\%) = 100 \times (\ln W_2 - \ln W_1) \times (t_2-t_1)^{-1}$$

Where, W_1 and W_2 are individual fish weights (g) at days t_1 and t_2, respectively. Protein Efficiency Ratio (PER) was calculated from equation (Halver, 1972):

$$PER = \frac{\text{Mean weight gain (g)}}{\text{Crude protein fed (g)}}$$

Feces were collected from each aquarium daily (8 h after feeding) by siphoning with rubber tube and were oven dried at 48°C (DeSilva and Anderson, 1995). Chromic oxide content of diets and feces were analysed by acid digestion according to Furukawa and Tsukahara (1966). The Apparent Digestibility Coefficient (ADC) of the experimental diets was calculated as follows (Halver, 1972):

$$ADC(\%) = 100-\left[\frac{\%\ Cr_2O_3\ \text{in feed}}{\%\ Cr_2O_3\ \text{in faeces}} \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in feed}}\right]$$

Analytical procedures: Proximate analyses of the feed ingredients, experimental diets and fecal samples were performed following the AOAC procedures (AOAC, 1992) as follows: moisture was determined by oven drying at 105°C for 24 h; crude protein (nitrogen ×6.25) was determined by Kjeldahl method, after acid hydrolysis; lipid was extracted by petroleum ether (boiling point 40-60°C) for 7-8 h in a Soxhlet apparatus followed by determination of lipid gravimetrically; ash was determined by combustion at 550°C in a muffle furnace, till a constant weight and crude fiber was determined due to the loss on ignition of dried residue remaining after digestion of sample with H_2SO_4 and NaOH solution under specific conditions. Water quality parameters were determined during the feeding trial by the procedures of APHA (1995).

Statistical analysis: The effects of partial replacement of fish meal by dried baker's yeast (*S. cerevisae*) on growth performance and feed utilization and digestibility of feed ingredients in koi carp fingerlings were determined by the one-way Analysis of Variance (ANOVA) using the statistical programme SPSS for windows, version 15.0 (SPSS Inc., Chicago, IL). The differences between means were compared by Duncan's multiple range test. The statistical software MSTATS was used for the analysis of data.

RESULTS

Growth performance and feed utilization parameters: Results of growth parameters, feed conversion and protein efficiency ratios are shown in Table 2. Experimental fish utilized the six feeds at varying levels brought about significant variations ($p<0.05$) in some of the growth parameters considered. Initial Body Weight (IBW) did not differ among treatments ($F = 0.143$, $p = 0.979$). The highest mean Final Body Weight (FBW) (2.95 g $fish^{-1}$) and the lowest FBW (2.37 g $fish^{-1}$) were recorded in fish fed diet T (control diet) and diet T_5 containing 0 and 50% DBY, respectively. Differences observed among the experimental groups in FBW were significant ($F = 92.694$, $p = 0.001$).

The Average Weight Gain (AWG) was least in fish fed diet T_5 50% DBY replacement (1.29 g) and highest in fish fed diet T (1.87g) ($F = 132.549$, $p = 0.001$). SGR was

Table 2: Growth performance and nutrient utilization in koi carp fingerling

Parameters	Experimental diets T (control)	T_1	T_2	T_3	T_4	T_5
IBW (g/fish)	$1.08\pm0.02^{a*}$	1.09 ± 0.00^{a}	1.09 ± 0.01^{a}	1.08 ± 0.02^{a}	1.09 ± 0.01^{a}	1.08 ± 0.02^{a}
FBW (g/fish)	2.95 ± 0.06^{a}	2.94 ± 0.01^{a}	2.95 ± 0.02^{a}	2.91 ± 0.02^{a}	2.41 ± 0.02^{b}	2.37 ± 0.03^{b}
AWG (g/fish)**	1.87 ± 0.04^{a}	1.85 ± 0.01^{a}	1.86 ± 0.01^{a}	1.82 ± 0.03^{a}	1.32 ± 0.02^{b}	1.29 ± 0.01^{b}
ADWG (g/fish/day)***	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}
FC (g/fish)	4.12 ± 0.02^{b}	4.12 ± 0.04^{b}	4.20 ± 0.04^{a}	4.22 ± 0.04^{a}	4.06 ± 0.04^{c}	4.04 ± 0.01^{c}
PI (g/fish)	1.44 ± 0.01^{a}	1.44 ± 0.01^{a}	1.46 ± 0.02^{a}	1.47 ± 0.01^{a}	1.41 ± 0.01^{b}	1.40 ± 0.01^{b}
RGR (%)****	172.39 ± 1.05^{a}	168.58 ± 0.95^{a}	169.81 ± 1.19^{a}	168.24 ± 5.48^{a}	121.73 ± 0.85^{b}	119.89 ± 0.72^{b}
SGR (% day^{-1})	1.67 ± 0.01^{a}	1.65 ± 0.01^{a}	1.66 ± 0.01^{a}	1.64 ± 0.03^{a}	1.33 ± 0.01^{b}	1.31 ± 0.00^{b}
FCR	2.21 ± 0.06^{b}	2.23 ± 0.02^{b}	2.26 ± 0.03^{b}	2.32 ± 0.05^{b}	3.07 ± 0.05^{a}	3.13 ± 0.05^{a}
PER	1.30 ± 0.03^{a}	1.28 ± 0.01^{a}	1.27 ± 0.02^{a}	1.24 ± 0.03^{a}	0.94 ± 0.02^{b}	0.92 ± 0.01^{b}

*Mean values with the different superscript along the same row are significantly different (p<0.05); **AWG = $\sum(W_2-W_1)$/fish numbers, ***ADWG = $AWG/(t_2-t_1)$, **** RGR = $[100*(W_2-W_1)/W_1*(t_2-t_1)^{-1})]$

Table 3: The apparent digestibility coefficients of feed ingredients in koi carp fingerlings

Digestibility (%)	Experimental groups T (control)	T_1	T_2	T_3	T_4	T_5
Dry matter	78.41 ± 0.54^{a}	78.56 ± 0.35^{a}	77.87 ± 0.25^{a}	76.18 ± 0.21^{b}	$75.87\pm0.24^{b,c}$	75.02 ± 0.24^{c}
Crude protein	78.48 ± 0.10^{a}	78.42 ± 0.10^{a}	78.23 ± 0.06^{ab}	78.01 ± 0.05^{b}	76.29 ± 0.11^{c}	75.17 ± 0.09^{d}
Crude fat	39.22 ± 0.55^{a}	$38.72\pm0.38^{a,b}$	37.62 ± 0.33^{b}	36.12 ± 0.16^{c}	34.67 ± 0.72^{d}	33.81 ± 0.30^{d}
Crude fiber	18.25 ± 0.07^{a}	18.19 ± 0.04^{a}	$17.96\pm0.04^{a,b}$	$17.84\pm0.15^{a,b}$	$17.74\pm0.13^{b,c}$	17.40 ± 0.23^{c}
Crude ash	50.22 ± 0.08^{a}	49.99 ± 0.08^{a}	48.95 ± 0.06^{b}	48.52 ± 0.22^{b}	48.41 ± 0.13^{b}	47.18 ± 0.41^{c}
Nitrogen free extract	$47.83\pm0.28^{a,b}$	47.95 ± 0.28^{a}	$47.41\pm0.10^{a,b,c}$	$47.24\pm0.16^{b,c,d}$	$46.95\pm0.09^{c,d}$	46.64 ± 0.16^{d}
Organic matter	73.26 ± 0.03^{a}	$73.05\pm0.05^{a,b}$	72.81 ± 0.21^{b}	72.11 ± 0.01^{c}	71.45 ± 0.21^{d}	70.27 ± 0.06^{e}

*Mean values with the different superscript along the same row are significantly different (p<0.05)

highest in fish fed diet T (1.67%), while it was least in fish fed diet T_5 50% DBY replacement (1.31%) (F = 133.808, p = 0.001). The highest Relative Growth Rate (RGR) and lowest RGR were determined in fish fed diet T and diet T_5, respectively. The best FCR value was recorded as 2.21 in fish fed diet T and the poorest FCR value was determined as 3.13 in fish fed diet T_5. FCR values of the fish fed diet T and diets T_1-T_3 did not differ significantly from each other (p>0.05). But FCR values of the fish fed diets T_4 and T_5 were significantly different from the others (F = 104.897, p = 0.001). PER was highest in fish fed T (1.30) while it was least in fish fed diet T_5 50% DBY replacement (0.92) (F = 74.673, p = 0.001). WG, Protein Intake (PI), SGR, RGR, FCR and PER values of 0, 10, 20 and 30% yeast replacement groups were significantly different from the 40 and 50% yeast replacement groups (p<0.05). These values of 40 and 50% DBY replacement groups was similar (p>0.05).

Digestibility of feed ingredients: The Apparent Digestibility Coefficients (ADCs) of the experimental diets are shown in Table 3. Replacement of fishmeal by the DBY affected digestibility of dry matter, protein, lipid, fiber, ash, nitrogen free extract and organic matter. The highest apparent digestibility coefficients for dry matter, protein, fat, fiber, ash, nitrogen free extract and organic matter were observed in fish fed diet T (control diet 0% dried baker's yeast replacement) which were significantly higher than those observed for the other diets (p<0.05). There was a significant effect of DBY content on the ADCs of all nutrient ingredients. ADCs decreased together with increasing of the content of DBY in diet. The dry matter digestibility values are in the range of 78.56 and 75.02%. The highest dry matter digestibility was determined in fish fed diet-T (F = 20.754, p = 0.001). Dry matter digestibility values for T, T_1 and T_2 were found similar (p>0.05). The highest protein digestibility (78.73%) was observed in fish fed the control diet-T which was significantly higher than those observed for the other groups (F = 257.657, p = 0.001). Fat digestibility ranged from 39.22-33.81% (F = 24.154, p = 0.001). The highest digestibility coefficient for fat was determined in the group fed with the control diet-T. Fiber and ash digestibility values were also affected by diet treatments (p<0.05) and the values were found as in the range of 17.40-18.25% (F = 5.795, p = 0.006) and 47.18-50.22% (F = 29.929, p = 0.001) for fiber and ash, respectively.

DISCUSSION

There is no information in the scientific literature concerning the use of DBY in koi carp fingerling diets. Scientific literature is usually related to Nile tilapia and rainbow trout. In this study, when fish meal was replaced by DBY>30%, average FBW, WG, PI, SGR, RGR and PER were significantly decreased compared to all other diets, while FCR was significantly increased ($p<0.05$). Contrary to this, fish fed diets in which 10, 20 or 30% of the fish meal was replaced by DBY were not significantly different from fish fed control diet (T) in terms of growth performance and feed utilization ($p>0.05$). During the experiment no mortality was recorded. Single Cell Proteins (SCP), including yeast and bacteria have been viewed as promising substitutes for fishmeal in fish diets.

Some researchers have studied the nutritional value of yeast *S. cerevisiae* in lake trout (Rumsey *et al*., 2007), rainbow trout (Rumsey *et al*., 1991a), sea bass (Oliva-Teles and Goncalves, 2001), Israeli carp (Noh *et al*., 1994), Nile tilapia (Medri *et al*., 2000, 2005; Abdel-Tawwab *et al*., 2008) by comparing growth performance and feed utilization. According to the results of these studies, DBY could replace up to 30% of fish meal protein without adversely affecting growth of species mentioned above.

However, Mahnken *et al*. (1980) found that salmonids accepted substitution fish meal by yeast (*Candida* sp.) at a rate of 25-50% replacement. Abdel-Halim *et al*. (1992) reported 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. Contrary to this, Zerai *et al*. (2008) found that inclusion of brewer's waste above 50% fish meal protein replacement resulted in a significant decline in the growth of tilapia.

Medri *et al*. (2000) determined that, the substitution of 10, 20 and 30% of the ration by alcohol yeast (*S. cerevisiae*) in balanced experimental diets on the development of alevins of Nile tilapia did not show a harmful effect up to the maximum tested level of 30%. In the present study, apparent digestibility of dry matter, protein, lipids and the other feed ingredients showed a trend similar to that found for the growth performance and feed utilization. In this study, inclusion of DBY above 30% fish meal protein replacement resulted in a significant decline in the digestibility of feed ingredients.

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.

According to Oliva-Teles and Goncalves (2001), the lower digestibility performance of fish diets having high levels of brewer's yeast may be because of intact yeast cells. Rumsey *et al*. (1991b) determined an improvement in the apparent digestibility of brewer's dried yeast after disruption of intact cells. Tacon and Cooke (1980) suggested that the poor digestibility of yeast is due to high nucleic acid content. Similarly, Schneider *et al*. (2004) also roperted that SCP sources have low digestibility as compared to other protein sources and this could be because of the presence of cell wall and high nucleic acid. These results evidence that, inclusion of *S. cerevisae* in fish diets may be at certain level due to high nucleid acid content and presence of cell wall.

CONCLUSION

The inclusion of 10, 20 and 30% of DBY in the diets of koi carp fingerlings gave similar the results to that for the control diet in terms of growth performance and feed utilization. But fish fed diets containing DBY demonstrated lower digestibility performance than fish fed control diet in terms of protein digestibility. In conclusion, findings in the present study indicated that DBY (*S. cerevisiae*) can replace up to 20-30% of FM in koi carp fingerling diets without adverse effects on survival rate, growth performance, feed utilization efficiency and apparent digestibility.

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