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# Growth Performance and Feed Utilization of Koi Carp (*Cyprinus carpio* L., 1758) Fed Partial or Total Replacement of Fish Meal with Hazelnut Meal and Soybean Meal

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**Abstract:** The effects of soybean and hazelnut meals as partial and total replacements of fish meal were studied on the growth performance and feed utilization of Koi carp juvenile (*Cyprinus carpio* L., 1758). Juvenile fish (mean initial weights 0.12 g) were fed five isonitrogenously (350 g $kg^{-1}$) and isocalorically (4.20 kcal $g^{-1}$) diets with 50 or 100% of the fish meal protein replaced by soybean and hazelnuts protein. The control diet contained fish meal as major protein source. A total of 225 juvenile koi were randomly assigned to each treatment which was replicated three times with 15 fish each. The fish were fed with these diets for 65 days. The results showed that Final Body Weight (FBW), Relative Growth Rate (RGR) and Protein Efficiency Rate (PER) were not significantly different among groups. Fish fed control, 50% SM, 50% HM and 100% SM diets had similar Specific Growth Rate (SGW) values while fish fed the 100% HM diet had lower SGR values than those of other diets ($p<0.05$). The FCR values of fish fed 50% SM and 50% HM diets were significantly different than that of 100% HM ($p<0.05$). The highest FCR was obtained from 50% HM diet. The results indicated that koi carp juvenile can be cultivated with feeding diets containing 50% SM, 100% SM and 50% HM without any adverse effect on growth performance of fish.

**Key words:** Koi, hazelnut meal, soybean meal, fish meal, growth performance

## INTRODUCTION

Production of cost-effective nutritionally balanced diets for fish are main factor affecting intensive aquaculture because of its influence on growth, health and production cost. Fish Meal (FM) is an important ingredient in aquaculture diets due to its high protein quality. However, as FM is the most expensive of all diet ingredients, the use of less expensive animal or plant protein sources are needed as partial or total replacements for FM. This became an important research interest in aquaculture nutrition (Yigit *et al*., 2006).

FM is the major protein source in aqua feeds globally averaged about 6.5 million metric tonnes (mmt), over the past 20 years. However, the production may fluctuate year by year. Variability in production is associated with variability in landings of fish used to make FM (Hardy, 2010). The percentage of annual global production of FM being utilized in aquafeeds has increased steadily over the last two decades from approximately 15-65% (Tacon and Metian, 2008). Over 11% of the FM used in the aquafeeds sector went into feeds for Carp in 2006. Surprisingly, in feeds for fry and fingerling of omnivorous species was used about 21% (Hardy, 2010). Nevertheless, continued growth of aquaculture production is fundamentally unsustainable if FM remains the primary protein and oil sources used in aquafeed. Sooner or later, supplies will be insufficient. However, alternatives to FM are available from other sources, mainly plant (grains/oil seeds) and animal proteins. The price of fish meal increased significantly from 400 US dollars to over US dollars 1500 per metric ton in 2006. This increased pressure to replace FM with plant protein ingredients. Therefore, replacement of FM with alternative proteins with sustainable supplier or less expensive protein sources would be beneficial in reducing feed costs. Proteins from soybean are widely available economical protein source with relatively high digestible protein and energy contents (Hertrampf and Piedad-Pascual, 2000). Use of soybean proteins as a dietary protein has been examined for many commercial important fish species (Refstie *et al*., 2000).

Hazelnut is commercially cultivated in Turkey, the USA, Italy, Spain, China and Australia. Turkey, the World's largest hazelnut producer, produces about 70-80% of the world supply. Hazelnut production in Turkey was about 825.000 tons (in shells) in 2008.

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Hazelnuts are extensively used in confections, together with chocolate in truffle and other products and in biscuits and cakes. Excess hazelnuts are used to produce oil for human consumption. The production of hazelnut oil and meal reduces the value to one-fifth of its value for domestic sales as hazelnuts. Hazelnut meal produced after oil extraction has high nutritive value due to its high protein and low fibre contents (Yalcin *et al*., 2005; Ergun *et al*., 2008).

Various protein sources have evaluated for fish feeds but the results were inconsistent. Unfortunately, attempts by feed manufacturers and nutritionists to replace the FM component of practical fish feeds with alternative protein sources have generally led to reduced feed efficiency and growth (Tacon and Jackson, 1985; Xie *et al*., 2001). Poor palatability, low digestibility, poor utilization of proteins and amino acids, anti-nutritional factors and other unknown factors are important to consider in utilization of plant proteins as replacement of FM (Becker and Makkar, 1999; Xie *et al*., 2001).

Ornamental fish production is an important component of the aquaculture industry. The ornamental Carp, *Cyprinus carpio* var. Koi L. is very popular to fish hobbyists throughout the World. Commonly known as koi, this fish has a market for individuals over 4 g and requires only about 3-5 months of grow out to attain saleable size (Jha *et al*., 2007). Protein requirement of koi is reported as 250-450 g $kg^{-1}$ of the diet. It is therefore of interest to replace some of the FM with protein-rich ingredients. Previous studies with koi have focused on some plant protein sources such as corn gluten meal, rapeseed meal, peanut cake, potato protein concentrate, cotton seed meal and soybean meal etc (Xie *et al*., 2001). The aim of this study was to investigate the effects of partial or total replacement of fish meal with hazelnut and soybean meals on growth performance and nutrient utilization of koi carp juvenile (*C. carpio*).

## MATERIALS AND METHODS

The experiment was conducted at Sinop University, Sinop Faculty of Fisheries, Fresh Water Fish Farming Unit (Sinop, Turkey).

**Preparation of experimental diets:** Diet ingredients were provided by a local fish feed manufacturer (SIBAL Inc., Sinop-Turkey). Hazelnut Meal (HM) was provided by FISKOBIRLIK Inc. (Giresun-Turkey). Five practical diets were formulated with commercially available ingredients and were produced in the Fish Nutrition Laboratory of Sinop University in Sinop, Turkey. The diets were isonitrogenous and isocaloric on a crude protein (350 g $kg^{-1}$) and gross energy (4.20 kcal $g^{-1}$) basis.

The experimental diets were formulated by substituting FM protein for Soybean Meal (SM) and HM protein at levels of 50 and 100% replacement on a crude protein basis. Ingredients and chemical compositions of the diets are shown in Table 1. The proximate analyses and amino acid contents of protein sources are shown in Table 2. The amino acid profiles of diets were estimated according to Kaushik (1995) and shown in Table 3. Total n-3 Highly Unsaturated Fatty Acid (HUFA) contents were calculated according to the equation given below and ranged from 1.45 g $kg^{-1}$ for diet to 2.15 g $kg^{-1}$ for the diet containing (Table 1).

Total n-3 HUFA in diet, g $kg^{-1}$ = (total fish oil in diet, g $kg^{-1}$) × (% n-3 HUFA in fish oil used). The dry ingredients and oil were mixed in a food mixer for 15 min. Tap water was then blended into the mixture to attain a consistency appropriate for passing the mixture through a meat grinder. After pelleting, the diets were dried to a moisture content of 7-8% and stored in a deep freeze (-20°C) until use.

**Fish and experimental rearing conditions:** The feeding experiment was performed on koi carp juveniles. The juveniles were obtained from the experimental fish farm at Faculty of Fisheries, Fresh Water Fish Farming Unit in

Table 1: Formulation and nutrient composition of the diets used in the experiment

| Ingredients (g $kg^{-1}$) | Control (FM) | 50% SM | 50% HM | 100% SM | 100% HM |
|---|---|---|---|---|---|
| Fish meal | 32.00 | 16.00 | 16.00 | - | - |
| Soybean meal | - | 22.50 | - | 45.00 | - |
| Hazelnut meal | - | - | 23.00 | - | 47.50 |
| Sunflower meal | 32.00 | 34.50 | 35.80 | 36.00 | 36.50 |
| Corn meal | 27.10 | 14.30 | 13.10 | 5.30 | 3.00 |
| Wheat starch | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| Fish oil | 2.40 | 6.20 | 5.60 | 7.20 | 6.50 |
| Vitamin premix[1] | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Mineral premix[2] | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Colin 60 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| **Proximate compositon (% on air dry basis)** | | | | | |
| Moisture | 7.15 | 8.44 | 7.59 | 7.75 | 8.24 |
| Dry matter | 92.85 | 91.56 | 92.41 | 92.25 | 91.76 |
| Crude lipid | 9.36 | 9.21 | 9.25 | 9.17 | 9.16 |
| Crude ash | 6.81 | 6.33 | 6.82 | 6.71 | 6.90 |
| Crude protein | 35.26 | 35.55 | 35.75 | 35.11 | 35.32 |
| Crude cellulose | 6.38 | 7.41 | 6.65 | 7.99 | 8.08 |
| Nitrogen-free extract[3] | 35.04 | 33.06 | 33.94 | 33.27 | 32.30 |
| GE (kcal $g^{-1}$)[4] | 4.31 | 4.23 | 4.29 | 4.21 | 4.19 |
| P:E (mg $kcal^{-1}$) | 81.73 | 83.95 | 83.41 | 83.29 | 84.37 |
| Crude fat (%) | 7.70 | 7.70 | 7.70 | 7.70 | 7.70 |
| Fat from fish meal (%) | 2.46 | 1.23 | 1.23 | 0.00 | 0.00 |
| Total fish oil in diet (%) | 4.86 | 7.23 | 6.83 | 7.20 | 6.50 |
| n-3 HUFA in fish oil (%)[5] | 29.76 | 29.76 | 29.76 | 29.76 | 29.76 |
| Total n-3 HUFA in diet (%) | 1.45 | 2.15 | 2.03 | 2.14 | 1.93 |

[1]Vit. Mix.: Rovimix 107 (g $kg^{-1}$ mix); Vit. A 8000 IU, Vit D3 800 IU, Vit. E 80 mg, Vit. K3 4.8 mg, Vit. B1 8 mg, Vit. B2 12 mg, Vit. B6 8 mg, Vit. B12 0.02 mg, Vit. C 80 mg, Niasin 80 mg, Folik asit 2.4 mg, Kalsyum D-Pantothenate 20 mg, Biotin 0.2 mg, Inositol 120 mg. [2]Min. Mix.: Remineral B Balik 97 (g $kg^{-1}$ mix); Fe 97.5 mg, Cu 18.75 mg, Mn 135 mg, Cb 0.6 mg, Zn 120 mg, I 2.7 mg, Se 0.225 mg. [3]Nitrogen Free Extract (NFE) = Dry matter - (Percentage of crude protein+Percentage of crude lipid + Percentage of crude ash+Percentage of crude cellulose). [4]Calculated according to 5.65 kcal $g^{-1}$ protein, 9.45 kcal $g^{-1}$ lipid, 4.1 kcal $g^{-1}$ nitrogen free extract; [5]According to Guner *et al*. (1998)

Table 2: Proximate analyses and amino acid profiles of fish meal, soybean meal, hazelnut meal, Sunflower meal and corn meal

| Parameters | Fish Meal | Soybean meal | Hazelnut meal | Sunflower meal | Corn meal |
|---|---|---|---|---|---|
| **Proximate analysis (%)** | | | | | |
| Moisture | 8.00 | 11.00 | 10.00 | 7.00 | 14.70 |
| Protein | 65.90 | 47.70 | 43.60 | 34.30 | 10.50 |
| Lipid | 7.70 | 1.60 | 3.70 | 6.90 | 7.70 |
| Ash | 18.40 | 6.40 | 6.00 | 7.30 | 2.00 |
| **Amino acids (DM%)** | | | | | |
| Arg | 4.11 | 3.41 | 2.15 | 3.45 | 0.43 |
| Cys | 0.66 | 0.63 | NA | 0.69 | 0.22 |
| His | 1.76 | 1.26 | 0.38 | 0.90 | 0.26 |
| Ile | 3.38 | 2.92 | 0.52 | 2.25 | 0.35 |
| Leu | 5.43 | 4.02 | 1.03 | 2.47 | 1.21 |
| Lys | 5.49 | 3.10 | 0.47 | 1.61 | 0.25 |
| Met | 2.16 | 0.72 | 0.15 | 0.94 | 0.17 |
| Mey+cys | 2.82 | 1.35 | NA | 1.63 | 0.39 |
| Phe | 3.03 | 2.45 | 0.68 | 1.80 | 0.48 |
| Phe+tyr | 5.47 | 4.17 | 1.11 | 2.80 | 0.86 |
| Thr | 3.00 | 1.92 | 0.50 | 1.37 | 0.35 |
| Trp | 0.82 | 0.68 | NA | 0.50 | 0.08 |
| Tyr | 2.44 | 1.72 | 0.44 | 1.00 | 0.38 |
| Val | 3.81 | 2.53 | 0.63 | 2.01 | 0.44 |

*Data on amino acids contents of fishmeal and soybean meal from Halver (1991) of hazelnut meal from Koksal *et al.* (2006) of Sunflower meal and corn meal from Bilguven; NA = Not Available

Table 3: Essential amino acid contents of the experimental diets

| Amino acids (DM%)[1] | Control (FM) | 50% SM | 50% HM | 100% SM | 100% HM |
|---|---|---|---|---|---|
| Arg | 2.54 | 2.68 | 2.44 | 2.80 | 2.29 |
| Cys | 0.49 | 0.52 | 0.38 | 0.54 | 0.26 |
| His | 0.92 | 0.91 | 0.73 | 0.90 | 0.52 |
| Ile | 1.90 | 2.02 | 1.51 | 2.14 | 1.08 |
| Leu | 2.86 | 2.80 | 2.15 | 2.76 | 1.43 |
| Lys | 2.34 | 2.17 | 1.60 | 1.99 | 0.82 |
| Met | 1.04 | 0.86 | 0.74 | 0.67 | 0.42 |
| Mey+cys[2] | 1.53 | 1.37 | 1.09 | 1.21 | 0.61 |
| Phe | 1.68 | 1.73 | 1.35 | 1.78 | 0.99 |
| Phe+tyr | 2.88 | 2.90 | 2.25 | 2.93 | 1.58 |
| Thr | 1.49 | 1.43 | 1.13 | 1.38 | 0.75 |
| Trp | 0.44 | 0.47 | 0.32 | 0.49 | 0.18 |
| Tyr | 1.20 | 1.18 | 0.90 | 1.15 | 0.59 |
| Val | 1.98 | 1.94 | 1.53 | 1.89 | 1.05 |

[1]Calculated from data in Table 2. [2]There is no cystine in hazelnut meal

Sinop University. In the experiment, five homogeneous groups consisting of 15 koi (initial mean body weight: 0.116 g) were randomly distributed in 15 rectangular plastic tanks (12 l capacity). Fish were fed with diets of control (FM), 50% SM, 50% HM, 100% SM and 100% HM for 65 days. Each experimental group had three replicates.

Continuous aeration was provided by air pumps. Dissolved oxygen ranged from 8-10 mg $L^{-1}$ during experiments period. Fish were fed by hand *ad libitum* (visual observation of the first uneaten feed) twice a day (09.00 and 18.00 h) and daily feed intake was recorded. About 30% of tank water was replaced by fresh water everyday. The experimental tanks were cleaned daily to remove uneaten feed and fecal material. Fish were exposed to natural light regime (14 h light: 10 h dark) for a 65 days period.

Fish were weighed at the start of experiment and beginning of each month and end of the experiment. Before weighing, fish were deprived of feed for a day. The feed consumption was recorded every day. Water temperature was maintained at mean 24°C with water heaters and natural photoperiod was used during the experiment.

**Chemical analyses:** The chemical composition of the diets was determined according to AOAC (1984) guidelines as follows: dry matter after drying at 105°C for 24 h, ash by combustion at 550°C for 12 h, crude protein (N×6.25) by the Kjeldahl method after acid digestion and crude lipid by petroleum ether extraction in a Soxhlet System; nitrogen free extracts was calculated by difference (NFE = Dry matter - (Crude protein + Crude lipid + Crude ash + Crude cellulose).

**Calculations:** Relative Growth Rate (RGR), Specific Growth Rate (SGW), Feed Conversion Rate (FCR) and Protein Efficiency Rate (PER) were calculated as described by Watanabe *et al.* (1987) and Yigit *et al.* (2002).

**Statistical analysis:** The data on growth, feed conversion ratio and protein efficiency rate of fish were expressed as means and ±standard error. Data from each treatment diet for each sampling period were analysed by one-way ANOVA and significant differences (if present) were ranked with Tukey's multiple comparison test at the 5% level of significance using the MINITAB Release 13.1 Statistical Analysis Software Program for Windows, Version 10.0.1 (Minitab Inc., Chicago, Illinois, USA).

## RESULTS

At the end of the 65 days of growth trial, there were no significant differences among the treatments in survival rates. Fish meal protein by different protein sources had no effect on survival rates. All the experimental diets were well accepted by fish. The growth performance and feed utilization values were shown in Table 4. Mean Final Body Weights (FBW) ranged from 0, 28 to 0, 46 g in experiment groups. There were no significantly differences in FBW, Relative Growth Rate (RGR) and Protein Efficiency Ratio (PER) between experiment diet groups. Even though difference was not significant and FBW, RGR and PER in fish fed 50% HM diet better concluded than the other groups. Diet 100% HM gave lowest values of FBW and RGR. Fish fed control, 50% SM, 50% HM and 100% SM diets had similar SGR values while fish fed the 100% HM diet had lower SGR values than those of other diets ($p<0.05$). The FCR values of fish fed 50% SM and 50% HM diets were significantly different than that of 100% HM ($p<0.05$). The best FCR values were obtained with 50% HM, 50% SM, 100% SM and control diets, respectively.

Table 4: Growth performance and feed utilization in fish fed with the experimental diets

| Parameters | Control | 50% SM | 50% HM | 100% SM | 100% HM |
|---|---|---|---|---|---|
| IBW (g) | $0.1116\pm0.03^{a}$ | $0.116\pm0.020^{a}$ | $0.116\pm0.030^{a}$ | $0.116\pm0.0300^{a}$ | $0.116\pm0.030^{a}$ |
| FBW (g) | $0.374\pm0.230^{a}$ | $0.392\pm0.130^{a}$ | $0.399\pm0.160^{a}$ | $0.384\pm0.2300^{a}$ | $0.319\pm0.110^{a}$ |
| RGR (%) | $222.70\pm6.3500^{a}$ | $240.81\pm36.520^{a}$ | $243.68\pm12.270^{a}$ | $234.77\pm24.5100^{a}$ | $170.69\pm47.190^{a}$ |
| SGR (% day$^{-1}$) | $1.953\pm0.030^{a}$ | $2.065\pm0.230^{a}$ | $2.057\pm0.060^{a}$ | $2.010\pm0.1180^{a}$ | $1.48\pm0.3500^{b}$ |
| FCR | $2.811\pm0.212^{ab}$ | $2.344\pm0.111^{a}$ | $2.262\pm0.206^{a}$ | $3.040\pm0.2260^{ab}$ | $3.887\pm1.730^{b}$ |
| PER | $1.013\pm0.008^{a}$ | $1.202\pm0.060^{a}$ | $1.243\pm0.108^{a}$ | $0.94\pm0.07000^{a}$ | $1.05\pm0.0060^{a}$ |

*Values (mean±standard deviation of data for triplicate groups) with the different superscript along the same row are significantly different ($p<0.05$). IBW, initial Body Weight; FBW, Final Body Weight; RGR, Relative Growth Rate = [(FBW- IBW)/IBW]×100; SGR, Specific Growth Rate (percent increase in body weight per day) = [(ln FBW-ln IBW)/days]×100; FCR, Feed Conversion Ratio = feed/weight gain; PER, Protein Efficiency Ratio = weight gain/protein intake

## DISCUSSION

The present investigation showed the potential of HM and SM meals for inclusion in granule diets of koi carp juveniles. Several researches reported that FM in fish diets can be replaced by plant protein sources. Use of plant feedstuffs as protein sources in fish diet limited for HM in koi juveniles diets. There has been number of different studies especially on the evaluation of HM and SM in diets for different fish species. Buyukcarpar and Kamalak (2007) found that HM can replace up to 35% of protein in FM and 40% of the protein in SM in fingerling mirror carp diets without adverse effects on growth performance, feed utilization and body composition. Bilgin *et al*. (2007) determined the growth performance and feed utilization in Rainbow trout and reported that hazelnut meal had the potential to substitute 20-30% of soybean meal in extruded feeds. Ergun *et al*. (2008) found that turbot diets of SM can be replaced up to 20% of the FM without reducing the growth performance and nutrient utilization and hazelnut meal can be incorporated into diet with 20% rate. Sevgili determined that the inclusion of >10% HM in mirror carp diets resulted in lowering the growth and feed utilization. The findings in present study agreed with those reported in other studies. FBW, RGR and SGR values decreased as hazelnut and soybean meal inclusion rates were increased but acordingly PER among groups and the fact that fish were fed adlibitum determine that problem of palatability of diets is not showed. Rodehutscord *et al*. (1995) reported that fish meal could totally be replaced by a mixture of wheat glutein and crystalline amino acids without negative effects on growth in rainbow trout.

The experimental diets supplied the essential fatty acid requirements of koi carp juveniles. However, control diets contained low n-3 HUFA due to the lower content of anchovy oil (Table 1). The inadequate fatty acid content of control diets probably resulted in low growth performance of fish. Yigit *et al*. (2006) also stated that low performance of the poultry by-product meals was related to the insufficient essential amino acid contents as well as fatty acid levels.

Deficiencies in essential amino acids and negative effects of anti-nutritional factors can be determined by inclusion of higher levels of some plant protein sources. In the present study, the low growth performance of koi juvenile fed with 100% HM diets was largely caused by most of the amino acids. Lysine and methionine were mostly reported to be growth limiting amino acids (Tacon and Jackson, 1985). Therefore, lysine and methionine containing of 100% HM diets were lower growth performance than other diets (Table 3).

Reduced growth performance of juveniles fed with 100% HM, 100% SM and control diets may be due to their fat contents. Optimal dietary fat levels should be lower than 12% in the practical diets of cyprinids (Kaushik, 1995). Dietary fat levels of experiment groups were equated. But, control diets contained low n-3 HUFA than the other diet groups. Although, n-3 HUFA contents in control diet is <100% HM diet treatment. Control group showed higher growth performance than 100% HM. Therefore, fatty acids contents may have not an effect on growth performance. However, low growth performance in the experiments was related to the amino acid contents of experimental diets. Some of the amino acid levels exceeded the stated requirements of common carp (Xie *et al*., 2001). Even though, the diets were not fortified with essential amino acids, the imbalances of amino acid composition between experimental groups were definite (Table 3). Lysine and methione+cysteines amounts in 100% HM group were 200 and 150% less than control group, respectively. Lysine and methione + cysteines which are generally considered the important limiting amino acids for most fish species. Thus, mean final body weights of fish fed with 100% HM diet were at least 15% lower compared with the other diet groups (Table 4).

Protein contents of soybean products ranged from 36% to over 70%. Soybeans like other plant-derived protein sources have anti-nutritional factors that can reduce the palatability, protein utilization or growth (Hardy, 2000). Trypsin inhibitors decrease the activity of trypsin, a digestive enzyme that breaks down proteins in the intestine. Trypsine inhibitors were also reported lowering the protein digestibility in diets of salmon and trout (Arndt *et al.*, 1999). In this study, results of the growth performance were not showed that trypsin inhibitors were not affected protein digestibility in the diets. The result showed increased SM and HM percentages in koi juvenile diets decreased the growth performances in koi juveniles.

SGR value of koi juveniles fed with 100% HM diet was significantly different than that of other diet groups ($p<0.05$). Buyukcarpar and Kamalak (2007) reported that the PER decreased and FCR increased with increasing inclusion levels of HM and SM diets. The results may reflect a deficiency of one or more amino acids in the diets. Similar result, in this study was determined that FCR was significantly different in experimental diet groups and the best FCR's were obtained from 50% HM and 50% SM diet groups, respectively (Table 4).

## CONCLUSION

This research indicates that growth performances of koi carp juveniles were negatively affected with 100% HM diet. The results clearly indicated that growth performances were decreased with increasing inclusion level of plant protein sources.

In the present study, koi carp juveniles were tested and the plant proteins were incorporated at relatively high levels. Future experiments should be carried out on koi of different sizes to determine the suitable inclusion levels of various plant proteins that yield the best economic gains.

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