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EFFECTIVENESS OF USING PAPAYA SEED EXTRACT (*Carica papaya* L.) AS A GONAD MATURITY INHIBITOR FOR NILE TILAPIA (*Oreochromis niloticus*)

Efektivitas Penggunaan Ekstrak Biji Pepaya (*Carica papaya* L.) Sebagai Penghambat Kematangan Gonad Bagi Ikan Nila (*Oreochromis niloticus*)

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ABSTRACT

One of the rapidly growing cultivation businesses in the Sidenreng Rappang Regency is tilapia cultivation, but in the cultivation process of tilapia commodities, problems are often found, including stunted growth, relatively long harvest times, and large amounts of feed consumption, which increase the number of losses during cultivation. In other words, tilapia is a type of commodity that has a fast fertilization process, so an alternative material is needed to determine good gonads in the growth and survival of tilapia, so the alternative material used is papaya seed extract. This study aims to determine and study the dosage of papaya seed extract used to see the level of gonad maturity, growth, and survival of female tilapia. This study will be conducted from February to May 2025, located at Jl. Ahmad Taufik, Panca Rijang District, Sidenreng Rappang Regency, South Sulawesi, Indonesia. The study used 4 treatments, namely treatment A. Commercial feed 100 gr + Papaya seed extract 0 ml / kg feed (100% feed), in treatment B. Commercial feed 100 gr + Papaya seed extract 0.1 ml / kg feed, while treatment C. Commercial feed 100 gr + Papaya seed extract 0.2 ml / kg feed, and treatment D. Commercial feed 100 gr + Papaya seed extract 0.3 ml / kg feed, with 3 repetitions. Data analysis used Analysis of Variance (ANOVA) and a simple analysis was carried out in looking at primary metabolites in the liquid.
Keywords: Gonad Maturity, Growth, Papaya Seed Extract, Survival, Tilapia

ABSTRAK

Salah satu usaha budidaya yang berkembang pesat di wilayah Kabupaten Sidenreng Rappang ialah budidaya ikan nila namun dalam proses budidaya pada komuditas ikan nila sering kali didapati permasalahan diantaranya pertumbuhan yang terhambat, usia panen nya relatif lama, dan jumlah komsumsi pakan yang banyak sehingga menambah angka kerugian pada saat budidaya berlangsung, dengan kata lain ikan nila adalah jenis komoditas yang memiliki proses pembuahan yang cepat, sehingga dibutuhkan satu bahan alternatif yang akan digunakan dalam menentukan gonad yang baik dalam pertumbuhan dan kelangsungan hidup ikan nila, maka bahan alternatif yang digunakan ialah Ekstak biji pepaya, Penelitian ini bertujuan untuk mengetahui dan mempelajari dosis ekstrak biji pepaya yang digunakan untuk melihat tingkat kematangan gonad, pertumbuhan, serta kelangsungan hidup ikan nila betina. Penelitian ini akan dilaksanakan pada bulan Februari sampai dengan Mei 2025, bertempat di Jl. Ahmad taufik, Kec, Panca Rijang, Kab, Sidenreng Rappang, Sulawesi Selatan, Indonesia. Penelitian menggunakan 4 perlakuan yaitu perlakuan A. Pakan komersial 100 gr + Ekstrak biji pepaya 0 ml/ kg pakan (100% pakan), pada perlakuan B. Pakan komersial 100 gr + Ekstrak biji pepaya 0.1 ml/ kg pakan, sedangkan perlakuan C. Pakan komersial 100 gr + Ekstrak biji pepaya 0,2 ml/ kg pakan, dan perlakuan D. Pakan komersial 100 gr + Ekstrak biji pepaya 0,3 ml/ kg pakan, dengan 3 kali pengulangan. Analisis data menggunakan *Analysis of Variance* (ANOVA) serta dilakukan analisis sederhana dalam melihat metabolit primer pada cairan.

Kata Kunci: Ekstrak Biji Pepaya, Ikan Nila, Kematangan Gonad, Kelangsungan Hidup, Pertumbuhan

INTRODUCTION

Tilapia is one of the freshwater fish that is widely cultivated because it easily adapts to unfavorable environments and is easy to spawn, so its distribution in nature is very wide, both in tropical and temperate regions (Eka, 2020). Increasing tilapia production through intensive cultivation requires the provision of quality feed, as well as appropriate cultivation techniques (Putra *et al*., 2011). Tilapia has the ability to grow faster and has a large environmental tolerance limit so it has great potential for development and optimal potential for tilapia growth is found in highlands 500 meters above sea level (masl) (Ardita *et al*., 2015). In terms of potential, Indonesian fisheries are the largest in the world, the achievement of aquaculture production has increased every year. From 2015 to 2019, aquaculture production increased by an average of 1.12 percent annually, from 15.63 million tons in 2019 to 16.33 million tons in 2019 (KKP, 2020).

Sidenreng Rappang Regency has significant potential for developing tilapia aquaculture. The number of farmers in Sidenreng Rappang Regency has continued to increase annually from 2019 to 2024 (BPS, 2024). However, the sustainability of tilapia aquaculture often faces challenges, such as rapid fertilization, which disrupts and slows development and growth. Due to these issues, fish farmers in the Lamongan region have taken preventative measures by adding birth control pills to tilapia feed (Manshuri, 2013). This is because birth control pills are believed to inhibit fish from laying eggs. Birth control pills suppress ovulation and prevent implantation (Suratun, 2008). The health risks of using birth control pills in fish feed, according to Jobling & Tyler (2003), and in agreement with Walayani (2015), include negative side effects, particularly for the fish themselves and for human consumers. Long-term effects of synthetic hormones can increase the risk of infertility, metabolic disorders such as insulin resistance and obesity, and cancer. The risks of using birth control pills in fish feed also impact the environment, as the hormones in the pills can pollute the environment and disrupt aquatic ecosystems (Nugroho *et al*., 2024). Birth control pills are a form of contraception in pill or tablet form that contains the hormones estrogen and progesterone, or contains only progesterone (Yanti & Wirastri, 2023).

One solution to reduce birth control pill use and alleviate problems for fish farmers is to add papaya seed extract to the feed before feeding it to the cultivated organisms. Papaya seeds (*Carica papaya* L.) are one of the traditional medicinal ingredients that are often used as a medicine for roundworms, digestive disorders, diarrhea, male contraception, and raw materials for cold medicine (Awaliah, 2020), WHO formed a working group to find and develop male fertility regulation one way by exploring ingredients or substances from plants as antifertility agents that are expected to be safe, effective and acceptable (Lolok *et al*., 2017). Papaya seeds also contain antimicrobials that are effective in producing male tilapia mixed through feed (Ristianti *et al*., 2015). Research on reducing antifertility has also been conducted by (Khalil *et al*., 2019) regarding the effectiveness of using papaya seeds in reducing the reproductive function of gift tilapia where papaya seed flour mixed in feed affects the morphology and abnormalities in tilapia sperm, Papaya seeds contain alkaloids, terpenoids and saponins that can inhibit gonadal maturity in fish. According to research (Fajriyah *et al*., 2021), papaya seed extract is highly effective as an antifertility agent, acting by suppressing spermatogenesis, disrupting the pituitary gland, affecting reproduction, and reducing egg quality. Therefore, it is highly effective for application in tilapia cultivation. Based on the above background, research on the effectiveness of papaya seed extract as a gonad maturation inhibitor in tilapia is warranted.

METHODS

Time and Location

This research was conducted from February to May 2025, at Jl. Ahmad Taufiq, Panca Rijang District, Sidenreng Rappang Regency, South Sulawesi, Indonesia.

Tools and Materials

The tools and materials used in this research are presented in Tables 1 and 2.

Table 1. Tools Used in the Research

No	Tool	Function
1	Measuring Cup	Determining the position of the observation station
2	Basin	Recording the data obtained
3	Digital Water Thermometer	Measuring the water temperature
4	Fish Filter	Catching fish
5	Water Hose	Removing water from the container
6	Aerator	Adding oxygen to the container
7	Electric Blender	Crushing the papaya seeds
8	Digital Scale	Measuring the papaya seeds
9	pH Meter	Measuring the pH of the water in the container
10	DO Meter	Measuring the DO in the container
11	Green Screen	Closing the container
12	70 cm Plastic Clip	Storing the papaya seed extract

Table 2. Materials Used in the Research

No	Material	Function
1	Papaya Seeds	Test material
2	Commercial Feed	Fish feed
3	Aluminum Foil	As a covering agent
4	Ethanol	Mixture in papaya seed extract
5	Tilapia	Test animals
6	N-Thiosulfate	Addition to papaya seed extract
7	Chlorine	Ingredients for water sterilization

Test Animals and Test Feed

The test animals used were female tilapia fry measuring approximately 8 cm and weighing approximately 7 grams. The female tilapia fry were obtained from hatcheries at the

Passeno Fish Seed Center. The stocking density of the test animals was 20 per container, with a daily feed intake of 5% of the total fish weight.

The papaya seeds obtained were processed into flour. The papaya seeds were washed thoroughly and then dried in the sun for 2-3 days. Once dry, the papaya seeds were ground into a flour, diluted with water. Once homogeneous, the mixture was sprayed onto the test feed and allowed to stand for 20 minutes before being fed to the test animals. The test feed used in this study was a commercial feed with the nutritional content shown in (Table 3):

Table 3. Feed Ingredients

Feed Ingredients	
Crude Protein	38-40 %
Fat	10-15 %
Fiber	8-10 %
Ash	Max 14 %
Moisture Content	8-10 %

Source: FAO (2016)

Research Containers and Media

The containers used in this study were 12 50-L basins filled with 20 liters of fresh water per container. All containers were thoroughly washed before use. The water was first filtered using a 10-µm filter bag, then disinfected with 100 ppm chlorine, and allowed to stand for 24 hours. The chlorine was then neutralized using thiosulfate and fully aerated for 24 hours. The treated water was covered and allowed to stand until the fish were stocked.

Preparation of Papaya Seed Extract

The method used refers to (Purwaningdyah *et al*., 2015). The extract was prepared by maceration. Eighty-five grams of papaya seed powder was placed in a beaker, soaked in 500 ml of 95% ethanol, then covered with aluminum foil and left for four days, stirring occasionally. The remaining residue was then re-macerated in 250 ml of 95% ethanol. The container was then covered with aluminum foil and left for two days, stirring occasionally. After two days, the sample was filtered to produce the filtrate and residue. Filtrate 1 and filtrate 2 were mixed and evaporated using a rotary evaporator, followed by a water bath to obtain a thick extract. The resulting thick extract was weighed and stored in a sealed glass container before being used for testing.

Maintenance

The maintenance of the test fish began with sorting the test fish by weighing and measuring their length as baseline data. The fish were then stocked into a maintenance container/basin previously filled with water and the water quality was measured. Weighing was performed using an electronic scale with an accuracy of 1 gram, and initial length measurements were made using a slide rule with an accuracy of 0.01 cm as baseline data. During rearing, the test fish were fed commercial feed at a rate of 5% of their biomass per day, fed twice daily (7:00-08:00 AM and 5:00-6:00 PM WITA). Feeding was done manually or by scattering the feed directly into each experimental unit.

Visual observations were made daily to monitor fish development. Any remaining feed in the container was removed before the next feeding. A 50% water change was performed weekly. Fish gonads were collected on days 15, 30, 45, and 60. Gonad collection was performed by dissecting the fish's abdomen vertically from the anus to the vertebrae, then horizontally to the ventral fin. Once the abdomen was opened, the gonads could be observed to determine their maturity. The gonads were removed by gently separating them from the digestive tract to avoid damage. The gonads were then weighed using an electric scale on pre-weighed filter paper.

Tilapia Sex Identification

Tilapia sex can be identified morphologically by observing their characteristics. Male tilapia have a slightly rounder body shape and are shorter than female tilapia. Male tilapia are generally brighter and more attractive in color than female tilapia. The anus of male tilapia has elongated and brightly visible genitalia. This study will use female tilapia fry, so sex identification is necessary to prevent contamination of the test animals with male tilapia.

Experimental Design

The experimental design in this study, using CRD, consisted of four treatments with three replications, resulting in 12 experimental units. The treatments tested were different doses of papaya seed extract in commercial feed, namely:

A = 100 g Commercial Feed + 0 ml Papaya Seed Extract/kg Feed
B = 100 g Commercial Feed + 0.1 ml Papaya Seed Extract/kg Feed
C = 100 g Commercial Feed + 0.2 ml Papaya Seed Extract/kg Feed
D = 100 g Commercial Feed + 0.3 ml Papaya Seed Extract/kg Feed

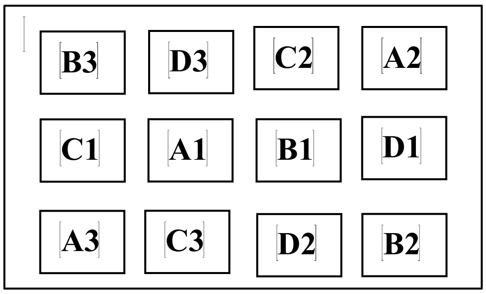

Figure 1. Experimental Design

Where:
A – D = Treatments
1 – 3 = Replications

Gonad Maturity Parameters

To determine the level of gonad maturity, tilapia are divided every two weeks. Gonad maturity is calculated directly. The gonad maturity index is calculated based on the gonad weight (BG) and body weight (BT) multiplied by 100 percent (Effendie 1979) in Zulfahmi (2014):

$$\text{IKG} = \left(\frac{\text{BG}}{\text{BT}}\right) \text{X } 100\%$$

Where:
IKG = Gonad maturity index (%);
BG = Gonad weight (g);
BT = Body weight (g).

Growth

Individual growth rates are calculated according to (Dehaghani *et al*., 2015).

$$\text{SGR } (\%) = \left[\frac{\ln \ln W2 - \ln \ln W1}{T}\right] \text{x}100$$

Where:
W2 = Average individual weight at the end of the study (g)
W1 = Average individual weight at the beginning of the study (g)

T = Duration of the study.

Survival Rate

The survival formula calculation according to Goddaard (1996) in Pindonta (2014) is as follows:

$$SR = Nt/No \times 100\%$$

Where:
Nt = Number of fish surviving at the end of the study (fish);
No = Number of fish at the beginning of the study (fish).

Data Analysis

The data will be statistically analyzed using Analysis of Variance (ANOVA). If the results are significant, a Tukey W-test will be used to determine which treatment yields the best results.

Liquid Analysis

Liquid analysis tests are conducted using test solutions such as Lugol's solution to test for carbohydrate content, tissue or blotting paper to test for fat content, and NaOH and CuSO4/biuret to test for protein content in the samples.

RESULTS

Gonad Maturity Level

Gonad maturity level refers to the period that occurs in fish before and after spawning. Based on observations during the research, the Gonad Maturity Level in tilapia can be seen in the following table:

Table 4. Analysis of Gonad Maturity Level

Treatment	Parameter ± Std
	Gonad Maturity (%)
A	0.28 ± 0.01^a
B	0.50 ± 0.02^a
C	0.27 ± 0.05^a
D	0.22 ± 0.02^b

Note: Different superscript letters in the same column indicate significant differences between treatments at the 95% confidence level (P<0.05).

Growth

The growth of the test animals in this study was measured using four treatments: Treatment A: 100 g Commercial Feed + 0 ml Papaya Seed Extract/kg of feed; Treatment B: 100 g Commercial Feed + 0.1 ml Papaya Seed Extract/kg of feed; Treatment C: 100 g Commercial Feed + 0.2 ml Papaya Seed Extract/kg of feed; and Treatment D: 100 g Commercial Feed + 0.3 ml Papaya Seed Extract/kg of feed. The results of these four treatments are shown in the following table:

Table 5. Analysis of Test Animal Growth

Treatment	Parameter ± Std
	Growth (%/day)
A	2.996 ± 0.03^a
B	3.080 ± 0.18^a
C	3.200 ± 0.13^a

Treatment	Parameter ± Std
	Growth (%/day)
D	3.523 ± 0.06[b]

Note: Different superscript letters in the same column indicate significant differences between treatments at the 95% confidence level (P<0.05).

Survival

The survival of test animals fed commercial feed mixed with papaya seed extract in different treatments (0 ml papaya seed extract), (0.1 ml papaya seed extract), (0.2 ml papaya seed extract), and (0.3 ml papaya seed extract) is shown in the following table:

Table 6. Analysis of Test Animal Survival

Treatment	Parameter ± Std
	Survival (%)
A	66.67 ± 2.88[a]
B	70.00 ± 5.00[a]
C	73.33 ± 2.88[a]
D	86.67 ± 2.88[b]

Note: Different superscript letters in the same column indicate significant differences between treatments at the 95% confidence level (P<0.05).

Liquid Analysis

Following liquid analysis of papaya seeds, results showed that the seeds contain primary metabolites such as fat, protein, and carbohydrates. This can be seen in the table below:

Table 7. Analysis of Papaya Seed Extract Nutrient Liquid

Nutritional Analysis of Papaya Seed Extract	Primary Metabolite Content (%)
Carbohydrates	22.5%
Protein	28.3%
Fat	29.7%

Note: The results of the primary metabolite content in papaya seed extract were obtained from the Pharmaceutical Laboratory at the Muslim University of Indonesia.

DISCUSSION

Gonad Maturation Level

Based on the results of the various experiments above, papaya seed extract containing alkaloid compounds was able to inhibit gonad maturation in test animals, with a significance level of P<0.05. Further Tukey's W-test results showed that treatment B produced the highest results, followed by C and A with nearly equal scores, and treatment D with the lowest score.

The final results of this study, using 100% feed and a mixture of papaya seed extract, showed the highest results in treatment B (0.50%), treatment A (0.28%), treatment C (0.27%), and treatment D (0.22%).

Gonad maturity in test animals is a biological factor, as tilapia are capable of reproducing 6-7 times per year (Amri, 2008 in Yenni *et al*., 2017). This affected treatment D, which obtained the lowest score, with commercial feed of 100g + 0.3ml/kg feed. This indicates that the spawning rate in tilapia varies regardless of length, weight, and age. Tilapia have a high growth rate (Hayati *et al*., 2018).

Based on the results of this study, the treatment with the highest score was treatment B, with commercial feed of 100g + 0.1ml/kg feed. This is in accordance with the statement (Puspaningdiah *et al*., 2014), which states that this is due to food availability in the waters, different fish adaptation patterns, and different growth rates, resulting in different gonad development.

Growth

Based on the results of the analysis of variance above, the provision of commercial feed mixed with Papaya Seed Extract had a significant impact on tilapia growth, with a significance level of $P<0.05$. Further Tukey W-test results showed that treatment D had a significant effect compared to treatments B and C, while treatment A had the lowest value.

The final results of feeding commercial feed mixed with papaya seed extract to test animals showed the highest value in treatment D (3.523%), followed by treatment C (3.200%), treatment B (3.080%), and the lowest in treatment A (2.996%).

Based on the assessment results, treatment D obtained the highest value of the four different treatments, treatment D with the highest value showed a real effect on the growth of tilapia fed commercial feed with a mixture of papaya seed extract, Fahrizal *et al*. (2017) said that in maintaining fish growth, it is necessary to provide food support that meets fish nutrition, in addition to the metabolic process in fish is also one of the supporters of fish growth, Yuniwati & Purwanti (2008) explained that papaya seeds also contain primary metabolite compounds such as: 9.5% fat, 8.5% protein, 9.44% carbohydrates, 1.47% ash, and 71.8% water.

The lowest growth rate for test animals was demonstrated in treatment A, which was fed 100g of commercial feed plus 0 ml/kg of feed, which could be considered 100% commercial feed usage. However, the very low results were due to the ongoing development of fish gonads throughout the research period. This is supported by Muchlisin & Dewiyanti (2012) in At *et al*. (2018) who stated that gonad development is generally influenced by various factors, including physiological conditions, water quality, feed availability, and gonad development.

The growth of test animals in each treatment showed significant results, with both 100% feed and feed supplemented with papaya seed extract. These positive results are supported by Effendi (1997) in Aliyas *et al*. (2016) who stated that, simply put, growth is the process of increasing certain dimensions over a certain period. However, growth in individuals is tissue growth resulting from cell division that occurs due to excess energy and amino acid (protein) input from food.

Survival

Analysis of variance results showed that supplementing with fermented duck bone meal significantly affected tilapia survival ($P<0.005$). Tukey's W-test showed that supplementing with commercial feed mixed with papaya seed extract yielded absolute results in treatment D, but differed in treatments A, B, and C, which were nearly identical.

Based on research with test animals fed with different papaya seed extract mixtures in each treatment, treatment D demonstrated the highest value (86.67%). This high value was attributed to the papaya seed extract's non-toxic content, which is detrimental to fish survival. This finding is supported by the opinion Sukadana *et al*. (2008) that seeds contain triterpenoids, which are the main components of papaya seeds and have physiological antibacterial activity. This opinion is supported by scientific evidence from various studies showing that papaya seeds are not toxic to fish when administered in appropriate doses as a feed additive. On the other hand, papaya seeds have positive benefits, such as increased growth, increased feed efficiency, and even antibacterial properties that support fish health. It is important to refer to existing research to determine a safe and effective dosage (Putra *et al*., 2015).

The osmoregulation process also produces waste products such as feces and ammonia, resulting in cloudy culture media due to the large amount of feces excreted by the fish (Marshall *et al*., 2006 in Aliyas *et al*., 2016). In their research, Aliyas *et al*. (2016) also stated that the impact of nitrogen excretion will affect the fish's life, specifically the ambient conditions, which ultimately affect their immune systems. Once the tolerance limit is exceeded, the fish will die. Since not all fish die, it is certain that the tolerance levels of fish populations in tanks vary. This is likely due to differences in body condition prior to introduction into the media, including parasite intensity, stress levels, and other factors. In freshwater, organs involved in osmoregulation include the gills, intestines, and kidneys.

The lowest values in this study were shown by treatments A and B, with values for A (66.67%) and B (70.00%), respectively, each of which was below 73%. According to Andriyan *et al*. (2018), a good average fish survival rate ranges from 73.5 to 86.0%. Research conducted by Murjani (2011) states that fish survival is highly dependent on the fish's adaptability to food and the environment, fish health status, stocking density, and adequate water quality to support growth. In this study, the mortality of the test animals, namely tilapia, was largely suspected to be a stress response to handling during data collection and water changes after siphoning.

Liquid Analysis

Based on the results of the analysis of the liquid content of papaya seed extract, it was found that the primary metabolites contained in it provide standard nutrition for the growth and survival of the test animals. Carbohydrates in the form of simple sugars and starch in papaya seeds ranged from 20–25% of the dry weight. The carbohydrates in papaya seeds can be used as a low-cost energy source for tilapia. According to Abdel-Tawwab *et al*. (2021), providing plant-based carbohydrate sources in tilapia feed can increase feed efficiency by reducing the use of protein as the primary energy source. The protein content of papaya seeds ranges from 27–30% and contains sufficient amounts of essential amino acids. This protein supports tissue growth, immunity, and feed conversion efficiency. A study by Mohammed *et al*. (2023) reported that papaya seed flour has a protein profile that has the potential to be used in fish feed formulations. The fat content in papaya seeds ranges from 28–32%, particularly polyunsaturated fatty acids (oleic and linoleic). These fats are essential for energy reserves and maintaining healthy cell membranes. According to Khalifa *et al*. (2022), essential fatty acids play a crucial role in the optimal growth and endurance of tilapia. So the source of protein, fat and carbohydrate content really supports the nutritional needs of tilapia fish, plus the bioactive compounds in papaya seeds (flavonoids, alkaloids, saponins) act as natural immunostimulants and improve fish health.

CONCLUSION

The content of bioactive compounds in papaya seed extract mixed with commercial feed produces a significant effect on inhibiting gonad maturity, growth, and survival, in seeing gonad maturity, real results are seen in treatment B with commercial feed treatment of 100gr + 0.1ml papaya seed extract then the results obtained are at a value of 0.50%, this is produced from a mixture of appropriate papaya seed extract so that the bioactive compounds in papaya seeds are superior to primary metabolites, then in growth, the highest value is found in treatment D with commercial feed administration of 100gr + 0.3ml papaya seed extract then the results obtained are 3.523%, this highest value is obtained from the influence of primary metabolites contained in appropriate papaya seed extract can increase the nutritional content of the feed so that the needs of test animals in terms of nutrition are met, then we can see in survival where the highest value is in treatment D with commercial feed treatment of 100gr + 0.3ml papaya seed extract, the results obtained from this treatment are 86.67%, the highest result This is obtained from the content of papaya seed extract which can be used as an antibacterial. In other words, papaya seed extract which is added appropriately to feed does not contain toxic substances which can affect the health of fish and most likely kill fish.

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