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## **The effect of *Yucca schidigera* extract dietary supplementation on growth performance, feed and protein utilization of European seabass, *Dicentrarchus labrax*, fingerlings**

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### **Abstract**

A total of 180 apparent healthy European seabass, *Dicentrarchus labrax*, fingerlings ( $5.0 \pm 0.5$  g fish<sup>-1</sup>) were used to investigate the effect of different levels of *Yucca*, *Yucca schidigera*, extract (YE) on growth performance, feed utilization and survival rate. *Yucca* extract were used at level of 0, 0.25, 0.50 and 1 g YE kg<sup>-1</sup> diet for 45 days. The fish were reared in twelve glass aquaria (three replicates per treatment), measuring (70x40x30 cm; approximately 70 liters each) at an initial stocking density of 15 fish aquarium<sup>-1</sup>. The aquaria were supported with artificial aeration and the water exchange rate was 15% with saline ground water (32‰). The results indicated that YE dietary supplementation significantly improved final weight, weight gain and specific growth rate in groups fed on 0.50 and 1 g YE kg<sup>-1</sup> diet compared to the control. Feed intake tended to increase with all groups fed on YE supplemented diet. The feed conversion ratio and protein efficiency ratio improved significantly with the highest two level of YE compared to the control. From the above mentioned, it can be concluded that YE can be used at level of 1 g YE kg<sup>-1</sup> diet as feed additive in sea bass fingerlings diet for improving growth performance and FCR and protein metabolism and maintaining survival rate.

## Introduction

European sea bass, *Dicentrarchus labrax*, is a very important commercial marine fish species in Atlantic coastal lines from Norway to Morocco, the Mediterranean Sea and in the Black Sea (Altan & Korkut, 2010). Also, it is one of the most economically important fish species farmed in temperate areas (Eroldogan *et al.*, 2004). In Egypt, the production of European sea bass is second to gilthead sea bream, with an estimated production of 30720 tonnes in 2015 and its production has an expectation to be duplicate in the nearest time (GAFRD, 2016).

The intensification of fish farming is an utmost way in the aquaculture industry to cover the human need of protein and attenuate the pressure on the nature fisheries resource (Aubin *et al.*, 2019). With more fish intensification the consumed feed and subsequent excreted nitrogen is increased, which could affect fish metabolism and ambient water bodies (Besson *et al.*, 2016 and Aubin *et al.*, 2019). Therefore, the improvement of protein metabolism and reduction of nitrogen excretion is an inevitable consequence for sustainable aquaculture sector development (Kelly & Kohler, 2003).

Feed additives including nature (beneficial microorganisms, prebiotics and phytobiotics) and synthetic substances (antibiotics, growth-promoting and hormones) have been used in fish diet to improve fish growth, feed utilization, physiological and immunological, and disease resistance (Lundebye *et al.*, 2010 and Chakraborty & Hancz, 2011 and Mansour *et al.*, 2017 and Mansour *et al.*, 2018). Meanwhile, due to the public health concerns, the using of synthetic chemicals in animal feed has been minimized or banned because of the residues accumulation risk in the edible tissues (Lundebye *et al.*, 2010) and/or multi-resistant pathogenic bacterial strains (Albuquerque *et al.*, 2007).

The *Yucca schidigera* is a plant native to the arid region of southwestern USA and Mexico deserts (Headon & Dawson, 1990). The extract of *Yucca schidigera* (YE) is a rich source of saponins compounds including, steroidal and glyco-components saponin fractions (Cheeke, 2000 and Ayasan *et al.*, 2005). The YE active components have been used in aquaculture, poultry and livestock industries, mainly to minimize ammonia excretion as an end product of protein metabolism and reduce animal ambient environmental pollution (Headon & Dawson, 1990 and Hristov *et al.*, 1999 and Sarkar, 1999 and El-Saidy & Gaber, 2004). The direct application of YE to aquatic animal rearing systems proved apposite effect on reducing ammonia levels in a dose dependent manner in both

fresh and salt water. Whereas, YE exhibit a potentially safe substance suitable for ammonia reduction and water quality management (Santacruz-Reyes & Chien, 2010 and Khalil *et al.*, 2015 and Fayed *et al.*, 2019).

Furthermore, dietary supplementation of YE significantly increased growth performance of Nile tilapia at level of 0.75 mg kg<sup>-1</sup> diet and improved the growth of fish fed plant protein based diets to equalized fish fed fish meal based diets (Gaber, 2006). YE was used as a fish feed additive to enhance the protein metabolism and reduce ammonia excretion (Kelly & Kohler, 2003 and El-Saidy & Gaber, 2004 and Gaber, 2006). In addition, *Y. schidigera* and *Y. saponaria* induce progressive effects on growth performance and haematological parameters, and decrease total ammonia-nitrogen excretion in striped catfish, *Pangasianodon hypophthalmus* (Güroy *et al.*, 2014).

To the authors' knowledge, there is no literature investigating the use of YE as feed additives in the feeding of European sea bass. Therefore, the aim of this study was to evaluate the effects of YE on growth performance, feed utilization, hematological and immunological status, and stress resistance of European sea bass, *Dicentrarchus labrax*.

## MATERIALS AND METHODS

### Fish and experimental facilities

A total of 180 apparent healthy European seabass, *Dicentrarchus labrax*, fingerlings with an average body weight of 5.0±0.5 g fish<sup>-1</sup> were used. The feeding experiment periods were performed at indoor wet lab. at El- Shreif Fish Farm and Hatchery (Mariout valley, Alexandria, Egypt) during April to May, 2017. Prior to the start of the experiment, the fish were acclimated to the experimental conditions for two weeks in four indoors circular fiberglass tanks (1 m<sup>3</sup>) and fed the control basil diet (Table 1).

Fish were randomly divided into twelve glass aquaria measuring (70x40x30 cm; approximately 70 liters each) at an initial stocking density of 15 fish aquarium<sup>-1</sup>, representing four experimental treatments (three replicates per treatment). Each aquarium was supported with artificial aeration through air blower and 5 cm sand stone. The water source was a ground water of saline water well with salinity of 32‰, a constant temperature (18± 1°C) and pH (7.0 ± 0.50). Throughout the experimental period, the lightening regime was 12: 12 hour light: dark cycle and the dissolved oxygen was 6.01± 0.2. Fish excreta were removed by manual siphoning and water exchange rate was 15% per day and 60% biweekly during the fish weight sampling.

### Experimental design and diets

The four experimental treatments were fed one of the following four isonitrogenous (~45% crude protein) and isocaloric (~4840 kJ g<sup>-1</sup> DM) experimental diets for 45 days. The first diet was the control without any *Yucca schidigera* extract (YE) supplementation (YE<sub>0</sub>), the second diet was supplemented with 0.25 g *Y. schidigera* extract kg<sup>-1</sup> diet (YE<sub>0.25</sub>), the third diet was supplemented with 0.5 g *Y. schidigera* extract kg<sup>-1</sup> diet (YE<sub>0.5</sub>) and the fourth diet was supplemented with 1 g *Y. schidigera* extract kg<sup>-1</sup> diet (YE<sub>1</sub>) (Table 1). The selected doses of *Y. schidigera* extract were selected according to the previous work of Kelly and Kohler (2003) and Gaber (2006).

The fish were fed the test diets until apparent satiation two times daily (9:00 a.m. and 2:00 p.m.), six days a week. The apparent satiation was considered after half hour of introducing the diet, then the remaining pellets were collected, dried and weight for accurate determination of feed intake.

The diets were prepared by finely ground the solid ingredients to powder and manually mixed in a plastic container for about 15 minutes to assure its homogeneity. Oil and additives were slowly added to the mixture. Warm distilled water was added gradually until the diet began to clump forming a dough-shaped paste then passed through a commercial meat grinder to form a spaghetti-like extruded diet (0.3 ml in diameter). The resultant pellets were dried using drying air force oven at 40-45°C to a moisture level <10% and stored at -20°C until used. The proximate chemical composition (%) of the formulated diets was analyzed according to (AOAC, 2000) and presented in Table 1.

**Table (1):** Ingredients and chemical composition (g kg<sup>-1</sup>) of the experimental diets

<b>Ingredients</b>	Yucca <sub>0</sub>	Yucca <sub>0.25</sub>	Yucca <sub>0.5</sub>	Yucca <sub>1</sub>
Fish meal, 68 %	300.00	300.00	300.00	300.00
Soy bean meal, 47%	375.00	375.00	375.00	375.00
Corn gluten, 60%	90.00	90.00	90.00	90.00
Rice bran, 12%	65.00	65.00	65.00	65.00
Wheat medling, 13%	70.00	69.75	69.50	69.00
Soy bean oil	40.00	40.00	40.00	40.00
Fish oil	48.80	48.80	48.80	48.80
Dicalcium Phosphate	8.00	8.00	8.00	8.00
Vitamins and Minerals mixture*	2.00	2.00	2.00	2.00
Vitamin C	0.20	0.20	0.20	0.20
Yucaa, <i>Yucaa shadegree</i> , extract <sup>‡</sup>	-	0.25	0.50	1.00
<b>Chemical composition (g kg<sup>-1</sup> DM)</b>				
Dry matter (DM)	938.00	937.50	931.80	937.70
Crude protein (CP)	448.60	448.80	449.70	446.00
Ether extract (EE)	125.60	131.00	129.80	128.90
Nitrogen free extract (NFE) <sup>†</sup>	283.40	257.00	266.50	278.60
Crude fiber (CF)	32.40	43.20	34.00	32.00
Ash	110.00	120.00	120.00	114.50
Gross energy (GE; kj 100 g <sup>-1</sup> DM) <sup>‡</sup>	2041.01	2017.35	2031.08	2039.62

\*Composition of vitamin and mineral mixture of 1 kg: vitamin A – 50 00 000 IU; vitamin D3 – 10 00 000 IU; vitamin B2 – 2.0 g; vitamin E – 750 units; vitamin K – 1.0 g; calcium pantothenate 2.5 g; nicotinamide – 10.0 g; vitamin B12 – 6.0 g; choline chloride – 150.0 g; calcium – 750.0 g; manganese – 27.5 g; iodine – 1.0 g; iron – 7.5 g; zinc – 15.0 g; copper – 2.0 g; cobalt-0.45 g, calcium carbonate up to (1000 g). †NFE: nitrogen-free extract calculated using the following equation: NFE = 100-(crude protein + ether extract + crude fibre + ash).

‡Yucaa, *Yucaa shadegree*, extract: Vime Yucaa (P)<sup>®</sup> contain 33% yucca extract with 12% saponin, produced by Vemedim Corporation, Vietnam.

†NFE: nitrogen-free extract calculated using the following equation: NFE = 100-(crude protein + ether extract + crude fiber + ash).

‡GE: gross energy calculated on the basis of 23.6, 39.4 and 17.2 k joule gross energy g<sup>-1</sup> protein, ether extract and NFE respectively (National research council, 2011).

## Measured parameters

### Growth performance and survival

The mean final body weight (FBW) in experimental treatment was determined by dividing the total fish weight in each aquarium by the number of fish. Weight gain (WG), specific growth rate (SGR) and survival (%) were calculated using the following equations according to (Castell & Tiewes, 1980):

$$\text{WG (g fish}^{-1}\text{)} = W_2 - W_1$$

where  $W_1$ : initial weight of the fish (g), and  $W_2$ : FBW of the fish (g).

$$\text{ADG (g fish}^{-1} \text{day}^{-1}\text{)} = (W_2 - W_1) / n$$

where  $W_1$ : initial weight of the fish (g),  $W_2$ : FBW of the fish (g), and  $n$ =days.

$$\text{SGR (\% day}^{-1}\text{)} = 100 \times (\ln W_2 - \ln W_1) / \text{days}$$

where  $\ln$  is the natural log.

$$\text{Survival (\%)} = 100 \times (\text{final number of fish} / \text{initial number of fish}).$$

### Feed and protein utilization

The feed intake was calculated by dividing the total feed intake (g) for each group on the number of fish in the same group. The feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated as follow:

$$\text{FCR} = \text{feed intake (g)} / \text{weight gain (g)}.$$

$$\text{PER} = \text{weight gain (g)} / \text{protein intake (g)}.$$

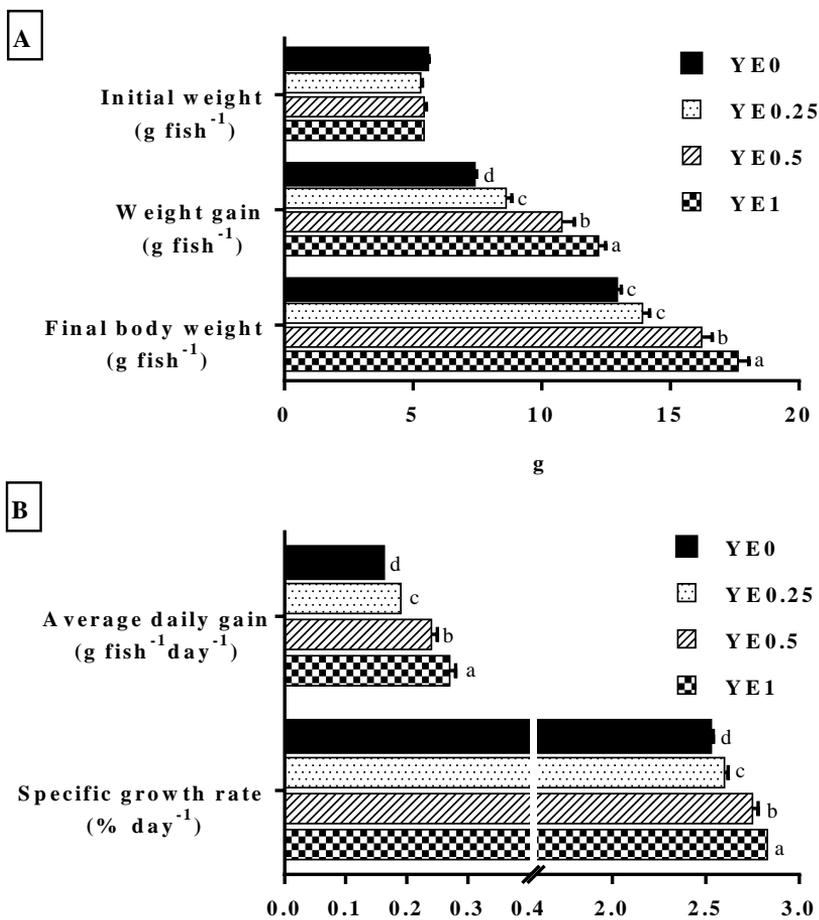
### Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA), when a significant difference was found among treatments. Duncan's multiple range test was performed to rank the groups (Duncan, 1955), using SPSS (Version 17) statistical software.

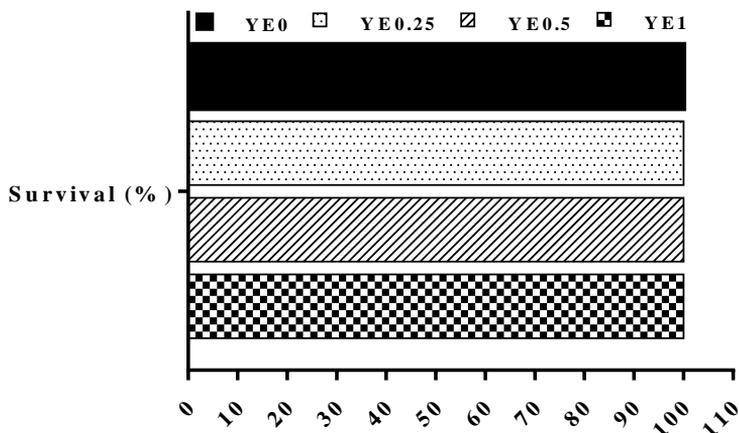
## Results

### Growth performance

The results of growth performance of different dietary levels of *Yucca schidigera* extract on the growth performance of culture European sea bass (*Dicentrarchus labrax*) presented in Table (2). The growth performance of European sea bass, *D. labrax*, fed supplemented diets with different levels of YE (0, 0.25, 0.5 and 1 g kg<sup>-1</sup> diet) showed a significant increase of FBW with 0.5 and 1 g YE kg<sup>-1</sup> compared to the control. Moreover, WG, ADG and SGR improved significantly with all YE supplemented diets. The survival (%) was up to 100% for all treatments (Figure 1).



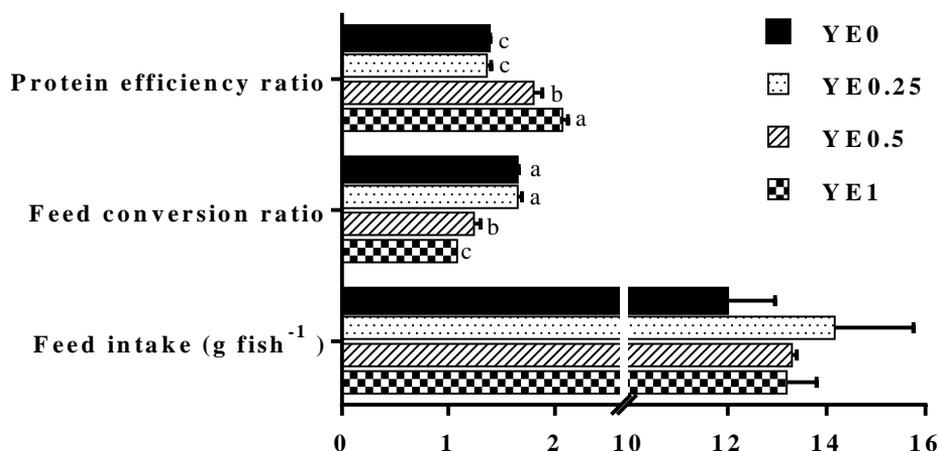
**Figure 1.** Growth performance (A and B) of European sea bass, *Dicentrarchus labrax*, fed supplemented diets with different levels of *Yucca schidigera* extract (g kg<sup>-1</sup>) for 45 days (n=3; means ± SE). Columns bearing with different superscript differ significantly ( $P \leq 0.05$ ).



**Figure 2.** Survival (%) of European sea bass, *Dicentrarchus labrax*, fed supplemented diets with different levels of *Yucca schidigera* extract ( $\text{g kg}^{-1}$ ) for 45 days ( $n=3$ ; means  $\pm$  SE).

### Feed and protein utilization

The effect of YE supplementation on feed intake and protein utilization presented in Figure 3. The results showed that feed intake did not differ significantly among different treatments. Meanwhile, FCR improved significantly with all YE supplemented treatments compared to the control group. The PER increased significantly with the higher two doses of YE ( $0.5$  and  $1 \text{ g YE kg}^{-1}$ ) compared to the control group.



**Figure 3.** Feed intake feed and protein utilization of European sea bass, *Dicentrarchus labrax*, fed supplemented diets with different levels of *Yucca schidigera* extract (g kg<sup>-1</sup>) for 45 days (n=3; means ± SE). Columns bearing with different superscript differ significantly ( $P \leq 0.05$ ).

## Discussion

As marine fish feed cost is one of the detrimental factors affecting mariculture expanding, improving the metabolic efficiency and feed utilization of these diets is a progress research trend especially protein metabolism as the highest price of feed components (Goda *et al.*, 2019). Also, improving protein metabolism is necessary to control the nitrogenous waste in aquaculture systems to meet effluent standards (Kelly & Kohler, 2003). Furthermore, the good aquatic animal health is a result of high quality feed and suitable water condition, at the same time, maintaining the animal healthy is a prominent factor for better growth and feed utilization (Trichet, 2010 and Mansour *et al.*, 2017 and Mansour *et al.*, 2018 and Wang *et al.*, 2018).

The effect of YE dietary supplementation at different level (0, 0.25, 0.5 and 1 g kg<sup>-1</sup> diet) on growth performance of European sea bass *D. labrax* were investigated and the results showed an improvement of final weight by 8.16, 26.02 and 36.98% with 0.25, 0.5 and 1 g YE kg<sup>-1</sup>, respectively compared to the control. In consistence with the present findings, channel catfish, *Ictalurus punctatus*, fry fed on YE supplemented diets at dose of 1 g kg<sup>-1</sup> diet exhibited the higher significant weight gain (Kelly & Kohler, 2003). Also, growth performance of Nile tilapia, *O. niloticus*, improved significantly with feeding YE (El-Saidy & Gaber, 2004) not only but also

YE at level of 0.75 mg kg<sup>-1</sup> diet improved the growth of fish fed plant protein based diets to equalized fish fed fish meal based diets (Gaber, 2006). In addition, growth of striped catfish, *Pangasianodon hypophthalmus*, improved significantly with *Y. schidigera* and *Y. saponaria* extract dietary supplementation (Güroy *et al.*, 2014). The effect of YE on growth was confirmed in several animal species including, white shrimp, *Litopenaeus vannamei*, (Yang *et al.*, 2014) Arbor Acres broilers (Su *et al.*, 2016) growing rabbit (Amber *et al.*, 2004 and Földešiová *et al.*, 2017).

Regarding the feed and protein utilization, the current findings showed an improvement of feed intake in YE supplemented group, which illustrated that YE did not negatively affected the diet palatability. Also, the increase in feed intake in the present study may due to the hormonal regulation of YE as reported by (Kucukkurt & Dundar, 2013), where YE increased leptin and insulin level in plasma of treated rats.

Furthermore, FCR improved significantly with YE supplementation at the higher two doses (0.5 and 1 g kg<sup>-1</sup> diet) by 39 and 55% compared to non-supplemented group, respectively. Also, the PER increased significantly with 0.5 and 1 g YE kg<sup>-1</sup> diet by 43 and 70%, respectively. In the same sense, YE improved feed and protein utilization of channel catfish, *Ictalurus punctatus*, (Kelly & Kohler, 2003), Nile tilapia, *O. niloticus*, (El-Saidy & Gaber, 2004) (Gaber, 2006), striped catfish, *Pangasianodon hypophthalmus* (Güroy *et al.*, 2014), white shrimp, *Litopenaeus vannamei*, (Yang *et al.*, 2014) and broilers and rabbit (Amber *et al.*, 2004 and Su *et al.*, 2016 and Földešiová *et al.*, 2017). The nitrogen utilization improved by 24.3% in rabbit fed YE (0.25 g kg<sup>-1</sup>) supplemented diet than the control group (Amber *et al.*, 2004). In contrast, the present findings did not showed any improvement of PER with the same level of YE supplementation (0.25 g kg<sup>-1</sup>) and the PER improved with the higher level of YE supplementation, this may be attributed to the different experimental animals and the completely difference of digestive system.

The improvement of growth performance, FCR and PER in the present study could attributed to the YE active components which include steroidal and glyco-components saponin fractions (Cheeke, 2000 and Ayasan *et al.*, 2005). The presence of steroidal saponin can improve the absorption of nutrients (Yang *et al.*, 2014) by changing enterocytes membrane structure and reducing surface tension (Goetsch & Owens, 1985) and increasing the permeability of intestinal membranes to dietary nutrients (Francis *et al.*, 2002). In addition, YE can improve the intestinal wall integrity via increasing the thickness of intestinal mucosa and preventing the invasion

of certain kinds of viruses, improving the animal immunity and suppress the intestinal bacterial growth (Huang *et al.*, 2005).

Furthermore, the improvement of protein utilization with increasing YE in the present study may attributed to the improvement of digestive enzymes activity (Liu *et al.*, 2005). Also, the protein retention could due to the reduction of ammonia excretion and fecal nitrogen concentrations in channel catfish (Kelly & Kohler, 2003) and reduced blood urea and caecal ammonia concentrations in rabbit (Amber *et al.*, 2004), whereas, YE active components have a great adsorption capacity volatile compounds, including ammonia and hydrogen sulfide (Cheeke, 2000). The effect of YE on hematological and immunological status of European seabass were studied and presented in Fayed *et al.* (2019).

### Conclusion

It can be concluded that, dietary supplementation of YE significantly improved growth performance in groups fed on 0.50 and 1 g YE kg<sup>-1</sup> diets compared to the control. The feed conversion ratio and protein efficiency ratio improved significantly with the highest two level of YE compared to the control. From the above mentioned, YE can be used at level of 1 g YE kg<sup>-1</sup> diet as feed additive in sea bass fingerlings diet for improving growth performance and FCR and protein metabolism and maintaining survival rate.

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## تأثير التذعيم الغذائي بمستخلص نبات اليوكا علي معدلات النمو والإستفادة من الغذاء والبروتين في إصبعيات اسماك القاروص الاروربي

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### الملخص العربي

تم إستخدام ١٨٠ إصباغية قاروص اوربي بمتوسط وزن ٥ جم لكل سمكه، لدراسة تأثير التغذية علي علائق مدعمة بمستويات مختلفة من مستخلص نبات اليوكا علي معدلات النمو والإستفادة من الغذاء والبروتين والإعاشة. استخدم مستخلص اليوكا بأربعة مستويات وهي ٠ و ٠,٢٥ و ٠,٥ و ١ جم لكل كجم علف لمدة ٤٥ يوم. تمت رعاية الأسماك في ١٢ حوض زجاجي سعه ٧٠ لتر ( بأبعاد ٧٠\*٤٠\*٣٠) بمعدل تسكين ١٥ سمكه لكل حوض. تم إمداد الاحواض بالتهوية مع معدل تغير مياة يومي ١٥٪ بمياة جوفية مالحة ٣٢٪. أظهرت النتائج تحسن معنوي في الوزن النهائي والوزن المكتسب ومعدل النمو النوعي في المعاملات المغذاه علي علائق مدعمة بمستخلص اليوكا بمعدل ٠,٥ و ١ جم لكل كجم علف. ارتفع معدل التغذية بصورة عديدة مع كل المعاملات المغذاه علي العلائق المدعمة بمستخلص اليوكا. تحسن أيضا معدل التحول الغذائي ومعدل كفاءة البروتين معنويا في المعاملات المغذاه علي المستويات المرتفعة من مستخلص اليوكا بالمقارنة بالكنترول. لذلك يوصي باستخدام مستخلص اليوكا كإضافة علفية بمعدل ١ جم لكل كجم علف لتحسين معدلات النمو والإستفادة من الغذاء وميتابوليزم البروتين في إصباغيات أسماك القاروص الأوربي.

