Feeding Preferences of Grass Carp (*Ctenopharyngodon idella*) for Three Species of Aquatic Plants

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Abstract

The present study was conducted to determine the feeding preferences of grass carp (Ctenopharyngodon idella) for three aquatic plants : water fern (Azolla filiculoides), duckweed (Lemna spp.) and water hyssop (Bacopa monnieri), in two different water temperatures (22-24 and 27-29 oC) for the period from 30 Oct.2019 to 12 Dec. 2019. Water hyssop were neglected from the second temperature experiment since no plant consumed in the first trail. The average fish weight in 22 -24 oC was 91.85±12.59g and in 27-29 oC was 89.19±16.18g. Mean daily feeding rates (DFR) of grass carp from water fern and duckweed were not significantly different (P>0.05), but both plants were eaten in significantly (P<0.05) greater quantities than water hyssop plant which was not eaten by grass carp. The consumed plants were 48.00 and 39.77% Body Weight/day from water fern and duckweed in temperature 22-24 oC, while in 27-29 oC it was 47.19 and 41.83 % Body Weight /day respectively. It was concluded from the present study that grass carp equally prefer water fern and duckweed but not water hyssop; this may be associated with the nature of and leaves structure.

Keywords: Azolla filiculoides, Lemna spp., Bacopa monnieri, Grass carp.

تفضيل تغذية الكارب العشبي (*Ctenopharyngodon idella*) لثلاثة انواع من النباتات المائية

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المستخلص:

أجريت الدراسة الحالية لتحديد تفضيل تغذية الكارب العشبي (*Ctenopharyngodon idella*) لثلاثة انواع من النباتات المائية وهي الازولا (*Azolla filiculoides*) وعدس الماء (*Lemna* spp.) و عشبة براهمي (*Bacopa monnieri*)، في مديات درجات حرارة ماء مختلفتين (22– 24 و 27–29 درجة مئوية) للمدة من 30 أكتوبر 2019 إلى 12 ديسمبر 2019. تم استبعاد عشبة براهمي من تجربة درجة الحرارة الثانية إذ لم

يتم استهلاك أي نبات في التجربة الأولى. كان متوسط وزن الأسماك في 22–24 درجة مئوية 91.85 ± 12.59 غم وفي 27–29 درجة مئوية 89.19 ± 16.18غم. متوسط معدلات التغذية اليومية لأسماك الكارب العشبي من الازولا وعدس الماء لم تختلف اختلافا معنويا (P> 0.05)، ولكن كلا النباتين تم تناولهما بكميات أكبر بشكل ملحوظ (P<0.05) من نبات عشبة براهمي الذي لم يأكله الكارب العشبي. كانت النباتات المستهلكة 48.00 و 39.77٪ من وزن الجسم في اليوم من نبات الازولا وعدس الماء في درجة حرارة 22–24 درجة مئوية ، بينما في 27–29 درجة مئوية كانت 47.19 و 41.83٪ من وزن الجسم في اليوم على التوالي. استنتج من الدراسة الحالية أن الكارب العشبي يفضل الازولا وعدس الماء وليس عشبة براهمي وقد يرتبط هذا بطبيعة النكهة وتركيب الاوراق.

الكلمات المفتاحية : *Azolla filiculoides* , *Lemna* spp. , *Bacopa monnieri* , Grass carp.

Introduction:

Fish represent about 17% of the animal protein consumed by the world's population. Moreover, fish provided about 3.2 billion people, approximately 20% of the average per capita animal protein intake (FAO, 2018).

The production of formulated feeds is expensive and lead to an increase in the fish production costs. A major limitation to fish farming is that meals high in protein with high biological value are expensive and often locally unavailable (Asimi *et al*., 2018; Das *et al*., 2018). Plant sources are inexpensive food items for herbivorous fish including grass carp (*Ctenopharyngodon idella)* as low cost and high performance alternatives (Zolfinejad *et al*., 2017). Aquatic macrophytes take much concern as they form dense settlements, above the carrying capacity of the environment which lead to a undesirable impacts to the various uses of water bodies (Cruz *et al*., 2015). A variety of potential alternate proteins have been identified, including: algae protein (Kiron *et al*., 2012) and bacterial protein(Aas *et al*., 2006), poultry by-product (Fowler, 1991); invertebrate and nut meals (Barrows and Frost, 2014), and a variety of plant-based proteins (Gatlin *et al*., 2007).

Azolla filiculoides (Family: Azollaceae) domestication dates back to the 11th century in Vietnam (Sadeghi *et al*., 2013).The aquatic fern *Azolla* is one of the fastest growing nitrogen-fixing plants on earth and therefore considered as a potential source of biomass for bioenergy production (Brouwer *et al*. 2016). It is widely used as a biofertilizer in many rice-growing regions of the world (Senthilkumar & Manivannan, 2016). *Azolla* can be used either directly or indirectly in the fish pond, due to its higher percentage in nutrients composition on dry weight basis (Mosha, 2018). The genus *Azolla* (*Azolla filiculoides*) were identified and recorded for the first time for the aquatic Pteridophyta of Iraq. (Al-Mayah et al., 2016).

Duckweed (*Lemna* spp.) belong to the family Lemnaceae, is small floating aquatic plants, in India, it can easily be grown abundantly with minimum cost, made available in much cheaper than other alternative plant protein sources (Srirangam, 2016; USDA, 2020). Xu *et al*. (2012) considered it a promising feedstock for the production of biofuels, for its advantageous characteristics (Nahar & Sunny, 2020). Newly har-

vested duckweed plants contain up to 43% protein by dry weight and can be used without further processing as a complete feed for fish (Asimi *et al*., 2018). One genus *Lemna*, with four species, *L. gibba*, *L. minor*, *L. perpusilla* and *L. trisulca* have been found in Iraq (Townsend & Guest, 1985).

Bacopa monnieri known as "Brahmi" in Hindi and water hyssop in English is a perennial, creeping herb with small leaves, white or purple flowers belonging to family Schrophulariaceae (Rai *et al*., 2017). It is a highly important medicinal plant, (Tamboli *et al*., 2018; Silpa *et al*., 2019). Das (2016) stated that water hyssop could be used as vegetable successfully grown as hydroponics culture in tropical climate. Bacopa is a herbaceous genus with about 100 species, it is represented by a solitary species in Iraq. *Bacopa monnieri* (Alwash, 2018).

Grass carp was now considered as belonging to the family Xenocypridinae instead of Cyprinidae according to recent phylogenetic studies (Tan & Armbruster, 2018). Grass carp was the most widely cultivated and commercially important freshwater fish species in the world that consist 11% of world production (FAO, 2018). This species is mainly an herbivorous fish that has strong preference for aquatic vegetation (Sangeetha and Rajendran, 2016).

Most studies in Iraq on grass carp deal with laboratory experiments (Jaafar & Ahmed, 2011; Al-Dubakel *et al.*, 2011; Al-Shkakrchy & Ahemed, 2013; Talal, 2013; Al-Maliky, 2017; Taher, 2017; Sayed-Laf *et al*.2018). Whereas limited field studies were conducted (Al-Seyab, 1996; Saleh *et al*., 2008).

The objective of this study was to determine the feeding preferences of grass carp for three aquatic plants : water fern, duckweed and water hyssop in two different water temperatures.

Materials and methods:

The present experiment was conducted in Fish Laboratory of Aquaculture Unit/ Agriculture College/ Basra University from 30th October. 2019 to 12th December. 2019. The experiment test the feeding preferences of grass carp for three aquatic plants : water fern, duckweed and water hyssop which tested with two replicated for each treatment in two different water temperatures (22-24 and 27-29 ^{o}C). Grass carp with average weight of 91.85±12.59 g brought from earthen ponds of Aquaculture Unit located in Al-Hartha Station for Agricultural Researches, north Basrah. Fish were starved for three days prior the data collecting to empty the digestive tract.

Grass carp were maintained (6-7 fish/treat.) in six aquaria with dimensions of 60×40×30 cm provided with aeration and heaters. Plants were selected from a fresh stock for each treatment. The plants were washed and blotted with paper towels, weighed (wet weight), and weighted samples were presented at the start of feeding period. After three hours, all uneaten plants and plant remains were removed at the end of the feeding period, weighed after rinsing and blotting to determine the consumed amount. This producer repeated for three subsequent days were a new amount of known weight of plants were offered to the fishes. All aquariums were cleaned thoroughly, and water changed prior the start of each test.

The following equations were used to display the results according to Toutou et al. (2018)

$$DFR = \frac{FCP\ Wt./d}{F.wt}$$

$$\%\ DM\ = \frac{DMC\ -\ DMU}{DMC} \times 100$$

Where:
DFR = Daily feeding rate.
FCP Wt. = Fresh consumed plant weight.
d = days.
%DM = %Dry matter of plant consumed.
DMC = Dry matter of total plant.
DMU = Dry matter of uneaten plant.

Data from experiment were tested by analysis of variance (ANOVA) to determine the differences between the means and the significant differences were tested by LSD test at 0.5% probability level by SPSS program Ver. 22.

Results

The average and total fish weight for experiment conducted with each temperature 22-24 and 27-29 ^{o}C were shown in Table (1). According to the results of 22-24 ^{o}C temperature water hyssop were neglected from 27-29 ^{o}C experiment since no consumption recorded.

Table 1: Average and total fish weight for experiment conducted with temperature 22-24 and 27-29 ^{o}C.

Treatment (Plant species)	Average fish weight (g)		Total fish weight (g)	
	22-24	27-29	22-24	27-29
A. filiculoides	100.15 a (±4.45)	101.50 b (±9.19)	300.50 a (±13.44)	406.00 b (±36.77)
B. monnieri	99.00 a (±7.07)	00.00 (±0.00)	297.00 a (±21.21)	00.00 (±0.00)
L. spp.	76.35 b (±1.63)	76.88 b (±9.72)	305.50 a (±6.36)	307.50 b (±38.89)

Within rows data with different superscripts are significantly different (P<0.05)

In experiment with both temperatures 22-24 and 27-29 ^{o}C, mean daily feeding rates (DFR) consumption of grass carp from *A. filiculoides* (144.0 and 154.7 g) and duckweed (121.3 and 169.3 g) respectively on wet weight basis which were not significantly different (P>0.05) between days or between the two plants. Both plants were eaten in significantly (P<0.05) greater quantities than water hyssop plant (Fig 1& 2) which was not measurably eaten.

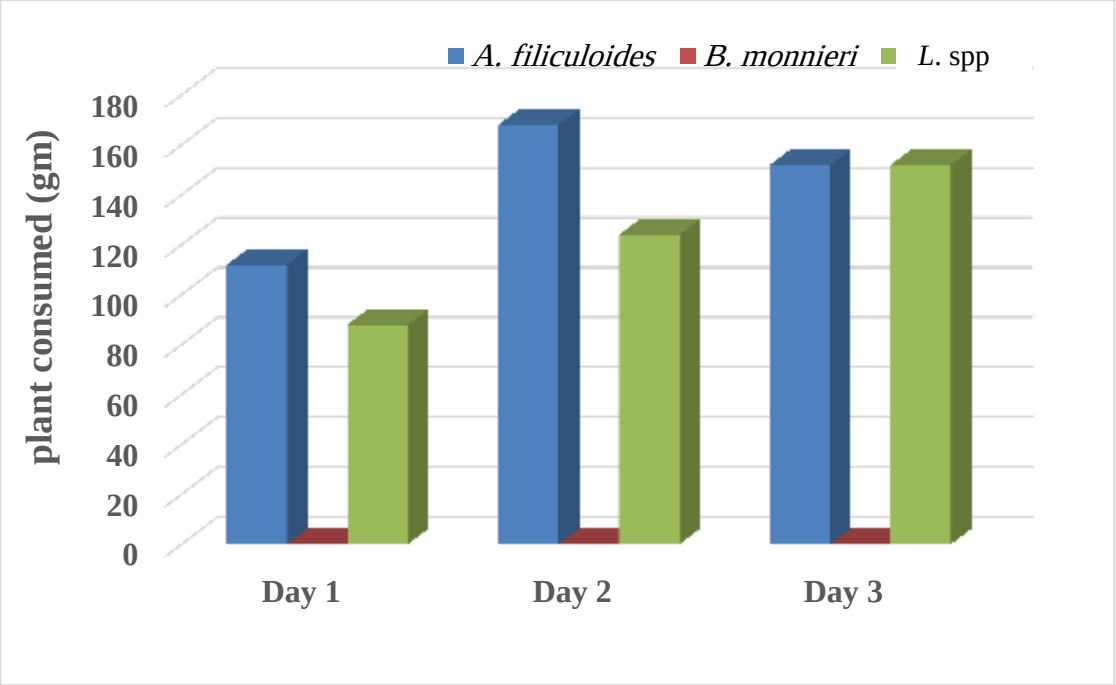

Fig. 1: Daily feeding rates (DFR) of plant consumed (gm) during three days at experiment conducted with temperature 22-24 °C .

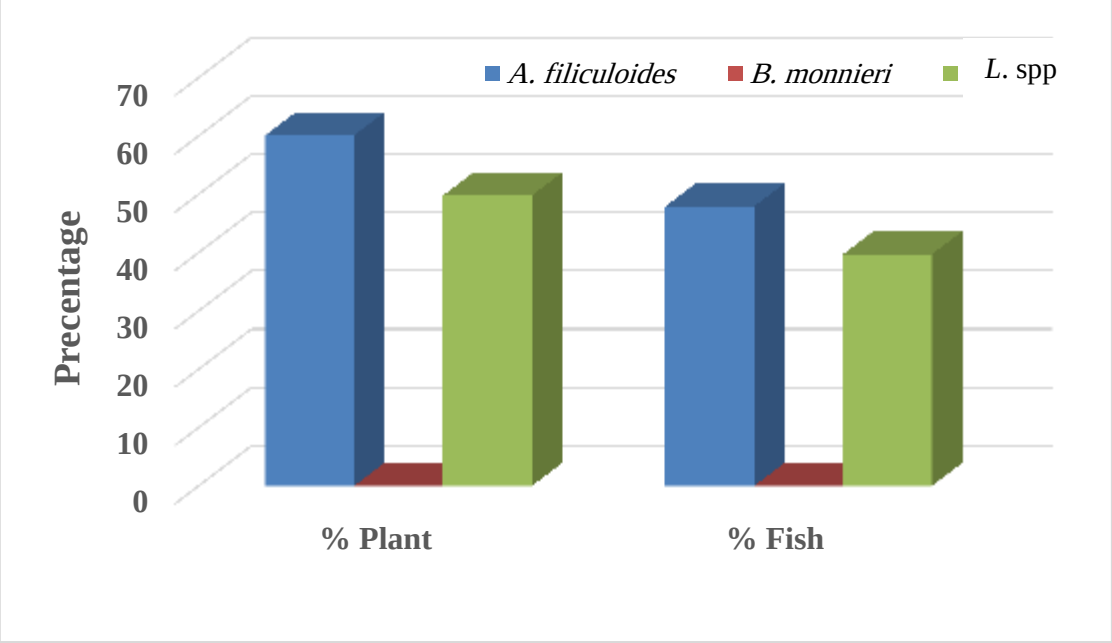

Fig. 2: Average ratio of plant consumed from plant and fish weight during three days at experiment conducted with temperature 22-24 °C.

Grass carp consume 48.00 and 39.77% of Body Weight/day from *A. filiculoides* and duckweed in temperature 22-24 °C, while in 27-29 °C consumed 47.19 and 41.83 %BW/day respectively (Fig 3 & 4). The same figure showed that grass carp consumed 144.00 and 121.33% in temperature 22-24 °C, whereas consume 154.67 and

169.33% in 27-29 °C from *A. filiculoides* and duckweed respectively, all these quantities represent wet weight basis.

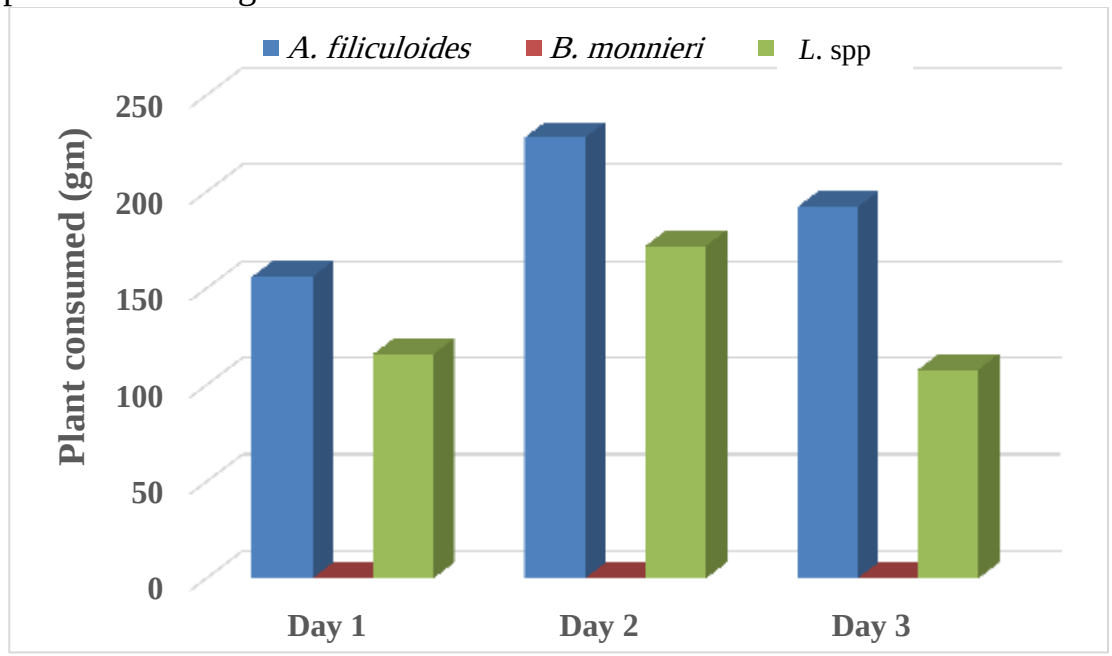

Fig.3: Daily feeding rates (DFR) of plant consumed (gm) during three days at experiment conducted with temperature 27-29 °C .

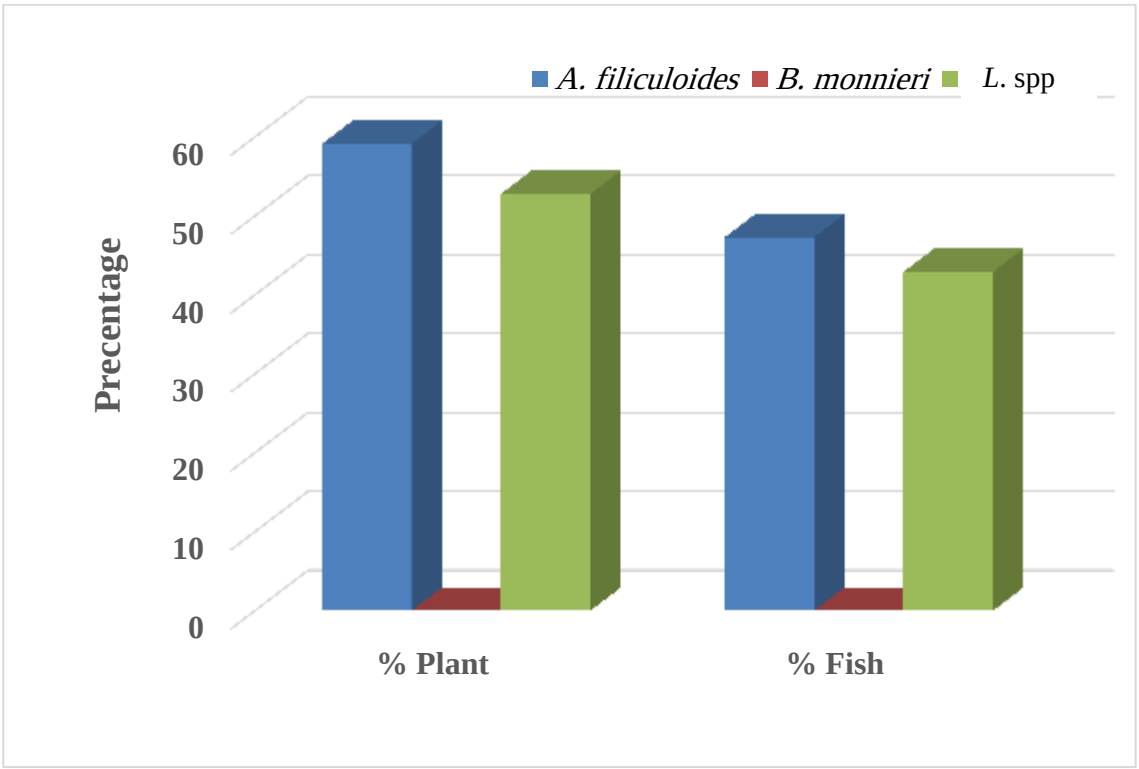

Fig. 4: Average ratio of plant consumed from plant and fish weight during three days at experiment conducted with temperature 27-29 °C.

On dry matter basis (Moisture content were 93.24 and 90.00 % for water fern and duckweed respectively) as presented in Table (2), the mean daily plant consumption from the two eaten plants were 4.80 and 3.98 % (22-24 ^{o}C), however 27-29 ^{o}C temperature recorded 4.72 and 4.18 % respectively. No significant differences (P>0.05) observed between the two plants or temperatures.

Table 2: Average daily percentage of plant dry matter /fish wt. of for experiment conducted in temperatures 22-24 and 27-29 ^{o}C.

Days	Temperature (^{o}C)	Plants		
		A. filiculoides	*B. monnieri*	*L.* spp.
Day 1	22-24	3.70 a (±1.34)	0.00 (±0.00)	2.87 a (±1.05)
	27 -29	3.76 b (±1.75)	0.00 (±0.00)	3.74 b (±0.45)
Day 2	22-24	5.56 a (±1.26)	0.00 (±0.00)	4.07 a (±1.38)
	27 -29	5.66 b (±0.93)	0.00 (±0.00)	5.42 b (±2.81)
Day 3	22-24	5.14 a (±3.62)	0.00 (±0.00)	4.99 a (±1.21)
	27 -29	4.74 b (±0.15)	0.00 (±0.00)	3.39 b (±1.96)
Mean	22-24	4.80 a (±2.07)	0.00 (±0.00)	3.98 a (±1.22)
	27 -29	4.72 b (±0.94)	0.00 (±0.00)	4.18 b (±1.74)

Within rows data with different superscripts are significantly different (P<0.05).

Discussion:

The observed preferences of studied plants indicated that *Azolla* and duckweed were similar without significant differences in respect to daily feeding rates (DFR) in both ranges of temperature. This results were corresponding with almost all previous finding deal with the same plants, Masser (2002) in his ranks arrangement of grass carp preference for aquatic plants give seven degree for both, while *Hydrilla* get one degree.

Feed consumption in the present study (144.0, 154.7, 121.3 and 169.3 g plant / day) respectively from both plants (*Azolla* and duckweed) in the two studied temperatures resemble to the study of Toutou et al. (2018) when feeding grass carp four aquatic plants including duckweed.

El-Sayed (1992) recorded 7 to 28 g/fish/day in fingerlings and adult of *Oreochromis niloticus* and Majhi *et al*. (2006) noted 14.6 g/fish/day in grass carp fingerlings fed fresh *Azolla*. Cassani & Caton (1983) stated that mean daily consumption of grass carp from duckweed reached 178.2 g/fish.

Daily consumption of grass carp from *Azolla* and duckweed within the range of other fishes consumed both plants, Thai Silver Barb *Barbonymus gonionotus* (Das *et al*., 2018) as fish consumed 15–20% fresh *Azolla* at the time when provided, but all the plants taken gradually. Zolfinejad *et al*. (2017) found duckweed utilized more by grass carp and higher decreases were recorded (91.5%) after delivery fish to each pond.

Consumption of grass carp of fresh plants as percent of body weight (39 – 48 %) in the present study, recorded in *Tilapia zillii* fed *Azolla* (Abdel-Tawwab, 2008) and duckweed in polyculture pond (Kabir *et al*., 2009). Grass carp fed on duckweed have higher feed conversion efficiency, conversion rate and protein efficiency ratio (Al-Shkakrchy & Ahemed, 2013).

The palatability of examined plants was different for grass carp. Water hyssop not accepted, and record nearly zero consumption. The reason for this may be related to its taste and leaves structure. Devendra *et al*. (2018) found that saponins are the major compounds in this plant that has a strong herbal taste and a bitter aftertaste. In addition, it is a nonaromatic herb, succulent and oblong leaves approximately 5mm thick (Vishnoi *et al*., 2016). While fresh *Azolla* was crisp and juicy, without much flavor but tasting somewhat of earth, and grass, duckweed flavor is similar to sweet cabbage (Sjödin, 2012)

Herbivorous fishes, including grass carp and tilapia, seems to favor the softer, more easily broken up and digestible macrophytes, while plants with rigid structure and high cellulose content have low palatability for these fishes, however grass carp showed higher growth performance for alfalfa compared to other plants such as duckweed, *Azolla* and formulated diets (Filizadeh, 1996; Nekoubin.& Sudagar, 2013; Zolfinejad *et al*., 2017).

Conclusions:

Water fern (*Azolla filiculoides*) and duckweed (*Lemna* spp.) seems to be preferred to grass carp and could be used as feeding sources for this species. In addition, these plants shows to have a positive effect on feed consumption, this may reduce the cost of feeding from other sources and could be incorporated in the diet for grass carp. However, water hyssop (*Bacopa monnieri*) not desired by grass carp due to its structure and flavor, which reduce palatability to fish.

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