Iranian Journal of Fisheries Sciences 17(2) 394-402 2018
DOI: 10.22092/IJFS.2018.115477

Effects of dietary supplementation of date palm (*Phoenix dactylifera*) seed extract on body composition, lipid peroxidation and tissue quality of common carp (*Cyprinus carpio*) juveniles based on the total volatile nitrogen test.

Mohammadi M.[1*]; Soltani M.[2*]; Siahpoosh A.[3]; Shamsaie M.[1]

Received: May 2016 Accepted: June 2016

Abstract

Date palm seed (*Phoenix dactylifera*) (DPS) extract has been used worldwide in pharmaceutical agent due to its constituents. This study aimed to assess the effects of DPS extract as a dietary supplementation at on the body composition, lipid peroxidation and tissue quality of common carp (*Cyprinus carpio*) based on the total volatile nitrogen (TVN) test. Common carp juveniles weighing ca 32 g were cultured in 26°C freshwater for 60 days and fed with diets supplemented with 0.5%, 1%, 2% and 4% of DPS extract. At the end of the trail, the crude protein ($p>0.05$) and crude lipid ($p<0.05$) were higher in fishes fed with diet containing 0.5% DPSthan control group, while their ash ($p<0.05$) and moisture content were lower in than other groups ($p>0.05$). The increase in DPS ratio of diet above 0.5% , resulted in lower crude protein and crude lipid, while the ash and moisture content increased. In addition, the values of TVN and lipid peroxidation in muscle and brain were lower in all treatments fed with supplemented diet compared to the control group. The results indicated that utilization of 0.5% DPS in commercial diet of carp can improve the body composition and antioxidant defense system of fish.

Keywords: Date seed extract, Body composition, Lipid peroxidation, TVN, Carp

1-Department of Aquaculture, Science and Research Branch, Islamic Azad University, Tehran, Iran
2-Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3-Medicinal Plants Research Center and Department of Pharmacognosy, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
*Corresponding authors Email: m40_mohammadi@yahoo.com

Introduction

Herbal medicine is a growing field of alternative medicine. Many active ingredients in manufactured drugs are derived from plant compounds and have a wide range of applications in pharmaceutical industry (Magi and Sahk, 2003). In recent years herbal remedies are being used as a primary health care method with minimal side effects not only in human medicine but also in veterinary medicine worldwide (Ojha *et al*., 2014; Pan *et al*., 2014). The date palm *Phoenix dactylifera* has played an important role in the lives of many people for the last 7000 years due to its remarkable nutritional and economic value (Chandrasekaran and Bahkali, 2013). However, it is only in recent years that dates have drawn greater attention, due to their numerous health benefits obtained from in vitro and in vivo studies worldwide (Chaira *et al*., 2009; Vayalil, 2012). Preclinical studies have shown that date fruits possess numerous and important functions in humans, among which are free-radical scavenging, antioxidant, immunostimulant, antimutagenic, antimicrobial, anti-inflammatory, and anti-cancer compounds as well as and gastro-, hepato- and nephro-protective properties (Dragsted *et al*., 1993). Moreover, fish is a significant source of omega-3 polyunsaturated fatty acids (PUFAs) (Mozaffarian and Wu, 2011; Swanson *et al*., 2012), therefore quality assessment of fishes has more to do with determination of its shelf life or storage life which is the amount of time that they remain edible and palatable. To our knowledge, no previous studies have focused on the effects of dietary administration of palm fruit extracts on fish. Therefore, this study aimed to investigate the effects of date palm seed (DPS) extract as a dietary supplementation agent on the body composition, lipid peroxidation and tissue quality of common carp based on the total volatile nitrogen (TVN) measurement.

Materials and methods

Preparation of date seed extract

The date fruits were collected during the last stage of ripening process in which the dates look dehydrated (Tamar stage), from Shadegan region, south of Iran, and identified by Medicinal Plants Research Center and Department of Pharmacognosy, Faculty of Pharmacy, Jundishapur University of Medical Sciences, Ahvaz, Iran. The isolated seeds from fruits were first soaked in water and then washed to remove any adhered date flesh. The seeds were air-dried under the shade at room temperature and grinded in the form of powder. The powdered seeds were extracted after 72 h of maceration in methanol. The extracted materials were then concentrated under reduced pressure in a rotary evaporator to reach the desired volume. The solvent was removed using freeze dryer (operon). The dried powder was then packed in form of capsules; and was used in fish food in form of a suspension. Phytochemical analysis was carried out by high-performance liquid chromatography (HPLC) to identify the profiles of phenolic and flavonoid

substances (Boligon and Athayde, 2014).

Fish and experimental treatments

A total of 150 common carp juveniles weighting 31.6±3.7g were purchased from a warmwater fish farm in Khouzestan Province, south Iran, and randomly distributed in fifteen 150 L aquaria (10 fish per aquarium) and acclimated for 2 weeks prior to the main experiment. Fish were then weighted individually after being anesthetized by clove oil essence at concentration of 250 μL^{-1}. Fish were fed a commercial common carp feed (FaraDaneh Aquatic Animal Feed Plant, Shahrekord, Iran) (wet basis composition of the food was 11% humidity, 28% protein, 14% lipid ,12% ash, 1% phosphor, and 3.5% fiber) at 3% of the body weight/day, except for 24 h before the initial biometry. Fish were then divided randomly in 5 groups (treatments) each containing 30 fish in 3 replicates. Four of these treatments were fed the above mentioned commercial feed containing 0.5%, 1%, 2%, and 4% of DPS extract for 60 days. The fifth group was considered as the control treatment with no DPS in feed. Water quality parameters consisted of temperature 24±1°C, pH 7.8-8.4, dissolved oxygen 66–87% saturation, NO_2<0.1 mgL^{-1}: NH_3<0.01 mgL^{-1}; and the uneaten feeds /fish excretions were removed daily.

Sample collection and assays

After 60 days of the feeding trial, five fish from each aquarium were obtained and the whole muscle fillets were separated and used for body proximate composition assay. In order to evaluate the effect of antioxidant activity of DPS extract, the muscle fillets and brain were used for lipid peroxidation test. The remaining fish from the same aquarium were ground TVN determination. The proximate compositions of fish body consisting of moisture, ash, crude protein and crude lipid were analyzed by AOAC method (AOAC, 2012). Also TVN was measured using magnesium oxide (AOAC, 2012). Lipid peroxidation was also determined using spectrophotometric analyzer (AOAC, 2012).

Statistical analysis

Data were reported as mean deviation of triplicates and the significant differences (p<0.05) within means were analyzed by one way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test in the R Studio Software Version 0.98.1103 (RStudio, 2013).

Results

Phytochemical analysis

The phytochemical analysis of DPS extract samples are given in Table 1.

Table 1: The phytochemical analysis of date palm seed (DPS) extract analyzed by HPLC and AOAC methods.

DPS phenolic compounds	mg-100g^{-1} DPS
Pyrogallol	59.49
Cinnamic acid	39.24
Benzoic acid	39.02
Elagic acid	38.32
Catechol	24.68
Gallic acid	19.08
Protocatchoic acid	18.05
Syringic acid	17.84
Vanillic acid	8.33
P-hydroxybenzoic acid	6.86
Chlorogenic acid	6.57
Sinapinic acid	6.47
Epicatechin	6.38
Ferulic acid	6.23
Caffeine	6.11
Catechin	5.84
Caffeic acid	3.58
P-coumaric acid	1.82
M-coumaric acid	1.79
O-coumaric acid	1.73
5-o-caffeoylshikimic acid	0.78
4-amino-benzoic acid	0.63
DPS flavonoid compounds	**mg-100g^{-1} DPS**
Hesperdin	19.07
Narengin	9.47
Rutin	6.29
Hespertin	4.15
Quercetin	2.57
Kaempferol	2.46
Luteolin	1.23
Rosmarinic	1.05
7-hydroxy flavon	0.96
Apigenin	0.87
Narenginin	0.52

The major DPS phenolic compounds consisted of pyrogallol, cinnamic acid, benzoic acid, elagic acid, catechol, gallic acid, protocatchoic acid, syringic acid, vanillic acid, p-hydroxybenzoic acid, chlorogenic acid, sinapinic acid, epicatechin, ferulic acid, caffeine, catechin, caffeic acid, p-coumaric acid, m-coumaric acid, o-coumaric acid, 5-o-caffeoylshikimic acid, 4-amino-benzoic acid. Also the main DPS flavonoid substances were hesperdin, narengin, rutin, hespertin, quercetin, kaempferol, luteolin, rosmarinic, 7-hydroxy flavon, apigenin and narenginin.

Body composition, Lipid peroxidation and TVN

The body composition of fish after 60 days feeding with diets containing different doses of DPS is shown in Table 2. The moisture were

insignificantly lower in treatment fed DPS at 0.5% than treatments fed with 1%, 2% and 4% DPS as well as the control one (p>0.05). With an increase in DPS dosage a higher moisture was obtained in fish fed with 1%, 2% and 4% DPS. Fish treated with 0.5% DPS showed a lower level of ash than the control group (p>0.05). Also, with an increase in the dosage of DPS the ash level was increased compared to both 0.5% DPS and control treatments (p<0.05). The level of crude protein was enhanced in 0.5% DPS treatment compared to the control treatment (p>0.05). However, with an increase in the level of DPS above 0.5% DPS, the crude protein level was reduced with the most reduction seen in fish fed with 4% DPS diet compared to the control treatment (p<0.05). The values of crude lipid were mostly significantly increased in fish fed with 0.5% DPS food, compared to the control one (p<0.05). However, with increasing the dosages of DPS in diet, the levels of crude lipid were measured significantly lower than the control treatment (p<0.05).

The levels of lipid peroxidation in muscle and brain in fishes of treatments with different dosages of DPS exhibited decrease compared to the control treatment, but the highest decrease were found in the muscle (p<0.05) and brain (p<0.05) of fishes fed with 4% DPS (Table 3). The values of TVN were lower in all treatments compared to the than control treatment (p<0.05) and the highest decrease in TVN was seen in treatment with 4% DPS than the control one (p<0.05) (Table 4).

Table 2: The proximate chemical analysis (g kg^{-1} wet basis) of common carp body fed with different concentrations of date palm seed exctract.

Treatments / Body composition	0%	0.5%	1%	2%	4%
Moisture	76.39±2.28^{a}	76.32±2.00^{a}	76.58±3.04^{a}	77.49±3.33^{a}	77.61±3.90^{a}
Ash	1.09±0.02^{cd}	1.07±0.03^{d}	1.11±0.03^{c}	1.19±0.01^{b}	1.22±0.01^{a}
Crude protein	17.06±0.38^{a}	17.37±0.19^{a}	15.18±0.43^{b}	13.50±0.49^{c}	13.03±0.32^{c}
Crude lipid	5.90±0.05^{b}	6.49±0.08^{a}	5.03±0.11^{c}	4.84±0.43^{cd}	4.72±0.22^{d}

All values are expressed as the means ± SD with Tukey test. Numerals refer to significant differences of proximate body composition (g kg^{-1} wet basis) at each date palm seed (DPS) extract treatments (p<0.05) with: Control treatment; 0.5% treatment; 1%treatment; 2% treatment; 4%treatment.

Table 3: The lipid peroxidation (%) of common carp fed with different concentrations of date palm seed exctract.

Treatments / lipid peroxidation	0%	0.5%	1%	2%	4%
Muscle	0.114 ±0.018^{a}	0.098 ±0.027^{ab}	0.095 ±0.023^{ab}	0.088 ±0.011^{bc}	0.067 ±0.009^{c}
Brain	0.141 ±0.035^{a}	0.138 ±0.019^{a}	0.123 ±0.019^{a}	0.118 ±0.016^{a}	0.113 ±0.018^{a}

All values are expressed as the means ± SD with Tukey test. Numerals refer to significant differences of lipid peroxidation (%) at each date palm seed (DPS) extract treatments (p<0.05) with: Control treatment; 0.5% treatment; 1%treatment; 2% treatment; 4%treatment.

Table 4: The total volatile nitrogen (mg N/100g) of common carp fed with different concentrations of date palm seed exctract.

Total volatile nitrogen	0%	0.5%	1%	2%	4%
TVN	1.5±0.24[a]	0.18±0.06[b]	0.16±0.04[b]	0.13±0.02[b]	0.11±0.01[b]

All values are expressed as the means ± SD with Tukey test. Numerals refer to significant differences of total volatile nitrogen (mg N/100g) at each date palm seed (DPS) extract treatments (p<0.05) with: Control treatment; 0.5% treatment; 1%treatment; 2% treatment; 4%treatment.

Discussion

Date seed is a rich source of a wide variety of nutritive and bioactive compounds, including flavonoids, phenolics, anti-hemocyanins and phenolic acids, as well as nutritive compounds such as essential oils, vitamins, and minerals (Chaira *et al*., 2009; Vayalil, 2012). Analysis of date seed composition has revealed many components that can be involved in the nutritional and health properties. For instance, carotenoids (alpha-, beta- and gamma-carotenes), vitamin E (tocopherols and tocotrienols), sterols (sitosterol, stigmasterol and campesterol), phospholipids, glycolipids and squalene are some of the substances available in the date seeds (Wattanapenpaiboon and Wahlqvist, 2003). Owing to its high content of phytonutrients with antioxidant properties, date seeds have some health advantages such as reducing lipid oxidation, oxidative stress and free radical damage. Accordingly, use of date seed or its phytonutrient-rich fractions, particularly antioxidants, may confer some protection to a number of deficiencies and stress condition (Wattanapenpaiboon and Wahlqvist, 2003).

The phytochemical analysis carried out on the DPS extract used in this study revealed that it contains several phenolic and flavonoid substances. These compounds possess multiple protective functions such as antioxidative activity and probably can act as protectants (Rusak *et al*., 2005).

In the present work the inclusion of DPS at 0.5% in common carp feed gave a better quality of body composition in compariso to both control and higher DPS dosages after 2 months feeding. This positive trend in crude protein and lipid could be in part due to a suitable balance in 0.5% DPS ingredients for improving the digestibility of feed in the fish alimentary tract.

When fish were fed with DPS extract at higher than 0.5% DPS concentration, the body composition was reduced to some extent as was also shown by decreased changes in the crude protein and lipid levels. Also, an increase in the levels of moisture and ash of fishes in treatments with DPS extract above 0.5% indicates a better nutrient balance provided in feed containg lower dose of DPD extract i.e. 0.5%. The medicinal herbs have favorable effects on digestion, stimulating effect on bile secretion and the activity of pancreatic enzymes (Platel *et al*., 2002). Moreover, adding plants extracts can affect the fish's food finding ability by stimulating their sense of smell and encouraging them to eat more than normal (Adams, 2005). Therefore, an increase in crude protein and lipid of

DPS at 0.5% dosage, may be attributed to its phytochemical substances including fatty acids, amino acids, minerals, vitamins, phenolic substances and flavonoids, which may play a balance role in body composition via enhancement in nutrient digestibility, increasing the efficiency of nutrient absorption and utilization of feed by the fish. Similar results were seen in the body composition of Nile tilapia (*Oreochromis niloticus*), juvenile Vundu catfish (*Heterobranchus longifilis*) and rainbow trout (*Oncorhynchus mykiss*) fed with alfalfa (*Medicago sativa*), soybean (*Glycine max*) meal, cottonseed (*Gossypium herbaceum*) and yellow lupin meal (*Lupinus luteus*) (Ali *et al.*, 2003; Glencross *et al*., 2004; Toko *et al*., 2008). The levels of lipid peroxidation in muscle and brain in fishes treated with different dosages of DPS extract exhibited a decreasing trend compared to the control treatment; and by increasing the dose, the highest decrease were found in muscle and brain with the highest decrease level seen in fishes fed with 4% DPS extract indicating an increase in antioxidant activity of DPS supplementation. Probably, such antioxidant activity may be in part due to phenolic and flavonoid compounds of date seed. Natural phenols are reactive species towards oxidation, notably the complex mixture of phenolic substances found in fillet, can undergo autoxidation during the ageing process. Phenolic compounds prevent the formation of free radicals by releasing hydrogen and protect the tissues to rancidity development as also was mentioned by Farahi *et al.* (2012) who showed that phenolic compounds (flavonoids, proanthocyanidin) have strong antioxidant activity for the removal of free radicals and oxidative reactions are completed.

The values of TVN was lower in all treatments compared to the control one and the highest decrease was observed in fishes fed with 4% DPS indicating an increase in antioxidant activity at higher concentrations of DPS extract.

In conclusion, the results of this study clearly showed that date palm seeds in the form of extract can improve the body composition properties and antioxidant defense system of common carp via preventing the lipid peroxidation. Such protection can be provided via the prevention of formation of free radicals by releasing hydrogen ions and also, preventing the putrefaction of amino acids, the process that leads to increase in the TVN levels in the presence of microbial community. However, the best result was obtained in treatment fed with 0.5% DPS extract feed. As date seed is mainly a waste product in the date plants and is available in a large scale with a very low price, therefore from the aquaculture practice point of view, it can be added to the commercial carp feed at 0.5% for improving the body composition and antioxidant defense system of fish. However, the use of this product in other fish species require more research works.

Acknowledgement

Authors would like to acknowledge the Medicinal Plants Research Center and

Department of Pharmacognosy, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran and Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. Authors also thank FaraDaneh Aquatic Animal Feed Plant, Shahrekord, Iran for supplying the common carp commercial feed.

References

Adams, C., 2005. Nutrition-based health. *Feed international*, 2, 25–28.

Ali, A., Al-Asgahn, N.A., Al-Ogaily, M.S. and Ali, S., 2003. Effect of feeding different levels of Alfalfa meal on the growth performance and body composition of Nile tilapia (*Oreochromis niloticus*) Fingerlings. *Asian Fisheries Science*, 16, 59–67.

AOAC, 2012. Official Methods of Analysis of AOAC International, 19th Edition.

Boligon, A.A. and Athayde, M.L., 2014. Importance of HPLC in analysis of plants extracts, *Austin Chromatography*, 1, 1–2.

Chaira, N., Smaali, M.I., Martinez-Tomé, M., Mrabet, A., Murcia, M.A. and Ferchichi, A., 2009. Simple phenolic composition, flavonoid contents and antioxidant capacities in water-methanol extracts of Tunisian common date cultivars (*Phoenix dactylifera* L.). *International Journal of Food Sciences and Nutrition*, 60, 316–29.

Chandrasekaran, M. and Bahkali, A.H., 2013. Valorization of date palm (*Phoenix dactylifera*) fruit processing by-products and wastes using bioprocess technology review. *Saudi Journal of Biological Sciences*, 20(**2**), 105–20.

Dragsted, L.O., Strube, M. and Larsen, J.C., 1993. Cancer-protective factors in fruits and vegetables: biochemical and biological background. *Pharmacology and toxicology*, 72, 116–35.

Farahi, A., Kasiri, M., Sudagar, M, Soleimani Iraei, M. and Zorriehzahra, S.M.J., 2012. Effect of dietary supplementation of Melissa officinalis and *Aloe vera* on hematological traits, lipid oxidation of carcass and performance in rainbow trout (*Oncorhynchus mykiss*). *Online Journal of Animal and Feed Research*, 2(**1**), 01–05.

Glencross, B., Evans, D., Hawkins, W. and Jones, B., 2004. Evaluation of dietary inclusion of yellow lupin (*Lupinus luteus*) kernel meal on the growth, feed utilization and tissue histology of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 235, 411–422.

Magi, E. and Sahk, M., 2003. Use of herbal medicine principle in local conditions. *Journal of Agricultural Science*, 14, 172–178.

Mozaffarian, D. and Wu, J.H., 2011. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *Journal of the American College of Cardiology*, 58(**20**), 2047–67.

Ojha, M.L., Chadha, N.K., Saini, V.P., Damroy, S., Chandraprakash and Sawant,

P.B., 2014. Effect of ethanolic extract of *Mucuna pruriens* on growth, metabolism and immunity of *Labeo rohita* (Hamilton, 1822) fingerlings. *International Journal of Fauna and Biological Studies*. 1 (**5**), 1–9.

Pan, S.Y., Litscher, G., Gao, S.H., Zhou, S.F., Yu, Z.L., Chen, H.Q., Zhang, S.F., Tang, M.K., Sun, J.N. and Ko, K.M., 2014. Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. *Evidence-Based Complementary and Alternative Medicine*, 2014, 1–20.

Platel, K., Rao, A., Saraswahi, G. and Srinivasan, K., 2002. Digestive stimulant action of three Indian spices mixes in experimental rats. *Nahrung*, 46(**6**), 394–398.

RStudio and Inc. shiny, 2013. Web Application Framework for R. R package version 0.5.0.

Rusak, G., Gutzeit, H. and Ludwig-Muller, J., 2005. Structurally related flavonoids with antioxidative properties differentially affect cell cycle progression and apoptosis of human acute leukemia cells. *Nutrition Research*, 25, 143–155.

Swanson, D., Block, R. and Mousa, S.A., 2012. Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Advances in Nutrition*, 3(**1**)**,** 1–7.

Toko, I.I., Fiogbe, E.D. and Kestemont, P., 2008. Growth, feed efficiency and body mineral composition of juvenile vundu catfish (*Heterobranchus longifilis*, Valenciennes 1840) in relation to various dietary levels of soybean or cottonseed meals. *Aquaculture Nutrition*, 14(**3**), 193–203.

Vayalil, P.K., 2012. Date fruits (*Phoenix dactylifera* L.): an emerging medicinal food. *Critical Reviews in Food Science and Nutrition*, 52, 249–71.

Wattanapenpaiboon, N. and Wahlqvist, M.L., 2003. Phytonutrient deficiency: the place of palm fruit. *Asia Pacific Journal of Clinical Nutrition*, 12, 363–368.