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RESEARCH PAPER

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Partial Replacement of Plant Sources by Waste Date (*Phoneix dactylifera*) in the Diet of Fingerling Common Carp (*Cyprinus carpio*) on Growth Performance, Feed Utilization, Hematological Parameters and Resistance to Stress

Mehrdad Kamali-Sanzighi[1], Reza Akrami[1,*], Afshin Ghelichi[1], Mahshid Shamloofar[1]

[1]Islamic Azad University, Department of Fisheries, Azadshahr Branch, Azadshahr, Iran.

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Corresponding Author
Tel.: +98.9111772071
E-mail: akrami.aqua@gmail.com

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Abstract

This research was conducted to evaluate the partial replacement of plant sources by date waste meal (DWM) on growth, hematological and resistance of common carp. A total of 168 common carp fingerling (2.44±0.02 g) were randomly assigned to four groups in triplicate and fed for 12 weeks with substitution of plant sources by 5%, 10% and 15% of DWM and control diet. Some growth and feed utilization parameters including WG, SGR, CF, PER and LER were improved in group fed with 10% DWM; but, there were no significant differences (P>0.05) in growth and feeding parameters between different groups. The results showed that replacement of plant sources by DWM in diets did not affect MCV, MCH and all biochemical parameters while the fish treated with 15% WDM exhibited significantly decrease in RBC, Hb and neutrophil compare to the control and other groups (P<0.05). In addition, there were no significant differences (P>0.05) of Hct, MCV, MCH and all biochemical parameters between treatment groups. Resistance rate to acidy and thermal-salinity stress increased significantly in 5% DWM and control group (P<0.05), respectively. The present study indicates that DWM may be included up to 10% as a substitute for plant sources in common carp diet.

Introduction

Agro-industrial by-products in recent years become one of important feed ingredient in aquaculture, poultry and veterinary diets of some religion of the world manly due to the increased competition for the conventional ingredients by human and the food industries (Masoudi, Bajarpour, Chaji, Eslami & Mirzadeh, 2010). To reach a sustainable aquaculture, new alternative ingredient sources including cheaper plant-derived materials is needed to be introduced for stable aquafeed production (Higgs *et al*., 1995). In this condition, it is necessary to develop appropriate complete and supplementary diets using locally available material especially, plant by-products for most of the fish farmers in the world for use in grow-out facilities due to the prohibitive cost and limited availability of fishmeal and other rare material in the world (Obirikorang, Amisah, Fialor & Skov, 2015). In addition, industrial ecology and circular economy are considered the leading principles for eco-innovation focusing on a “zero waste” society and economy where wastes can be used as raw materials (Kasapidou, Sossidou, & Mitlianga, 2015).

Among different agriculture by-products material, date (*Phoneix dactylifera*) is one of the world’s oldest food-producing plants which has always played an important role in the economy and social life of Iran. This country is one biggest producer of date with about capacity of 1 million tons at 185000 hectares in the world (Biglar, Khanavi, Hajimahmoodi, Hassani, Moghaddam, Sadeghi & Oveisi, 2012). Approximately 10-30% of this amount represents the total possible crop of waste date. This value creates bad economic

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and environmental challenge for country and researchers should be find a new valuable usage of this waste by-product (Gaber, Labib, Omar, Zaki & Nour, 2014). While, according to nutrition value, date fruit consist of high energy, carbohydrate, fiber, ash, protein and fat with different percent, depending on the variety of the date fruit (Habib& Ibrahim, *2009*; Abdul Afig, Abdul Rahman, Che Man, Al-Kahtani & Mansor, 2013; Al-Hafedh & Alam, *2013*).

Common carp (*Cyprinus carpio*) is one of the most important cultured fish in the world aquaculture industry. This fish is the third most frequently introduced species worldwide. It is being considered as a potential candidate for commercial aquaculture (Rahman, 2015). In the year of 2014, the world and Iran's common carp production was about ~3.500.000 and ~50.000 tons, respectively (FAO, 2014; Iran's fishery year book, 2016). Carp is an omnivorous species eating plankton, benthos and detritus in the natural conditions. The carp's digestive system is adapted to a diet including more carbohydrates compared with carnivorous species (Mraz, 2011). The economically feasible common carp fish farming can be achieved when it is based on cost effective feed compound of locally available agricultural by-products. Appropriate use of local protein by-products could reduce feed costs and enhance environment and economic sustainability. Therefore, formulation of cheaper diets using locally available ingredients, especially carbohydrate sources, could have a significant impact on common carp farming through cost reduction (Azaza, Mensi, Kammoun, Abdelouaheb, Brini & Kraïem, 2009).

Several researches have reported the beneficial of different form of date as a feed ingredient on tilapia (Yousif, Osman & Alhadrami, 1996; Belal & Al-Jasser, 1997; Belal & Al-Owafeir, 2004; El-Sayed, Hamza & Al-Darmaki, 2006; Belal, 2008; Azaza *et al*., 2009; Gaber *et al*., 2014), African Catfish (Sotolu, Kigbu & Oshinowo, 2014) and common carp (Hoseinifar, Khalili, Rufchaei, Raeisi, Attar, Cordero, & Esteban, 2015; Hoseinifar, Dadar, Khalili, Cerezuela & Esteban, 2017) but there is no documented evidence about the effect of partial replacement date waste meal (DWM) on common carp. Therefore, the objectives of the present study evaluated the effects of DWM on the common carp fingerlings concerning their growth, blood and biochemical profile as well as on the resistant rate of that fish.

Material and Methods

Fish Culture Condition

Common carp fingerling (2.44±0.02 g) were sourced from private center (Golestan province, Iran). The health of the fish was checked and randomly distributed into 12 glass aquaria (50 L) at 14 fish per aquaria density (3 aquaria per treatment) and acclimatize for 14 days before experimental feeding regime. The water quality parameters including temperature, dissolved oxygen and pH were monitored daily and maintained at optimum condition. For this reason, water was changed partially to 50% and siphon once a day before start of first feeding time. The experimental diets were fed three times daily (09:00, 12:00 and 15:00 h) at rate of 5% of body weight for 12 weeks (Sotolu *et al*., 2014).

Diet Preparation and Experiment Design

Date waste meal (DWM with powder form), was purchased from Tabriz's Sepehr Sanat Sahand company (Azarbaijane Sharghi province, Iran). A basal diet was formulated to contain 30% protein and 8% lipid for common carp fingerlings (Table 1). This basal diet served as the control diet and the experimental diets were produced with partial inclusion levels of 5, 10 and 15% DWM respectively (Gaber *et al*., 2014; Belal, El-Tarabily, Kassab, Abdel-Fattah & Rasheed, 2015). The dietary ingredients were blended with water to form a paste, which passed through a meat grinder and pelleted to produce 0.8 mm diet pellets. The diet was air dried at 60 ^{0}C in a vacuum drying oven and was stored in plastic bags at -8 ^{0}C until used (Belal *et al*., 2015). The chemical composition of all experimental diet were determined according to standard methods (AOAC, 2000) (Table 1).

Growth Performance and Feed Efficiency Parameters

Total fish weight in each tank was measured every 15 days at least 12 h after the last feeding to adjust the feeding rate and estimate growth factor. At the end of feeding trial, specific growth rate (SGR %/day), weight gain (WG g), percent of body weight gain (WG %), condition factor (CF), feed conversion ratio (FCR), feed intake (FI %/day), protein efficiency rate (PER), lipid efficiency rate (LER), survival rate (SR) and Biomass gain (g) were calculated according to the follow formulas (Imanpoor, Najafim & Kabir, 2012; Abbass, El-Asely & Kandiel, 2012; Ahmed, 2017).

$$\text{SGR (\% day}^{-1}) = \frac{100 \times (\ln(\text{W2})) - (\ln(\text{W1}))}{\text{t}}$$

$$\text{FCR} = \frac{\text{FI (g)}}{\text{weight gain (g)}}$$

$$\text{WG (g)} = \text{W2 (g)} - \text{W1 (g)}^{***}$$

$$\text{WG (\%)} = \frac{\text{W2}-\text{W1}}{\text{W1}} \times 100$$

$$\text{FI (\%BW/day)} = 100 \times \frac{\text{total dry feed intake per fish} \div (\text{W1}\times\text{W2})\text{\^{}0.5}}{\text{t}} \; ^{***}$$

$$\text{CF} = \frac{\text{weight (g)}}{\text{length (cm)\^{}3}} \times 100$$

Table 1. Formulation and proximate composition of experimental diets

Ingredients	Control	DWM meal		
		5%	10%	15%
Fish meal	15	15	15	15
Soybean meal	22	20	19	17
Wheat meal	22	20	19	17
Corn meal	20	19	16	15
Rice bran	10	10	10	10
Date meal	-	5	10	15
Fish oil	4	4	4	4
Soybean oil	1	1	1	1
Premix *	3	3	3	3
Salt	0.5	0.5	0.5	0.5
Spaghetti meal	2.5	2.5	2.5	2.5
GE (Kcal/kg)	3.98	3.94	3.90	3.86
Ash (%)	12	12	12	12
NFE	45.19	45.54	45.50	45.86
Pr	30	30	30	30
Fat	8.24	8.28	8.29	8.33
Fiber	0.3	0.6	0.9	1.2
Carbohydrate	45.49	46.14	46.40	47.06
Moisture	4.27	3.58	3.31	2.61
Dry matter	95.73	96.42	96.69	97.39

$$\text{PER} = \frac{\text{weight gain (g)}}{\text{protein intake (g)}}***$$

$$\text{LER} = \frac{\text{weight gain (g)}}{\text{lipid intake (g)}}$$

Biomass gain = final biomass (g) – initial biomass (g)***

$$\text{SR (\%)} = \frac{\text{N2}}{\text{N1}} \times 100$$

where, W1= Initial body weight (g); W2= Final body weight (g); BW=body weight; t= duration of experiment in days; N1=Number of fish at the start of the experiment and N2= Number of fish at the end of the experiment.

Blood Sample Collection

At the end of the experiment, five fish were sampled randomly from each tank and were anesthetized with MS222 at 50-75 mg/L, and about 3 mL of blood was drawn from the caudal vein, using a non-heparinized syringe after they were starved for 24 h. Then, blood samples were introduced to both heparinized and non-heparinized tubes in order to perform haematological and immunological studies, respectively. Serum samples were attained after centrifugation (4500 g for 10 min) and stored at -20 °C until analysis for immunological studies (Lewis, Bain & Bates, 2001; Imanpoor & Roohi, 2015; Wu, Zhu, Liu, Chen, Chen & Tan, 2016).

Blood Factors Assays

Red blood cells (RBC) and white blood cells (WBC) were counted using a Neubaur haemocytometer according to Martins, Tavares-Dias, Fujimoto, Onaka and Nomura (2004). Additional parameters were determined: Haemoglobin (Hb) according to Collier (1944); haematocrit (Hct) according to Goldenfarb, Bowyer, Hall and Brosious (1971); and differential white blood cell counts were obtained by preparing panchromatically-stained smears (Klontz, 1994). Differential leukocyte counts (neutrophil, lymphocyte, monocyte and eosinophil) were determined using Giemsa staining method of blood smears using a light microscope. The smears obtained from heparinized sample were first air-dried, fixed in 96 % ethanol for 30 min and stained in Giemsa to determine differential leukocyte counts (Ghiasi, Mirzargar, Badakhshan, & Shamsi, 2010). Other blood indices such as Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular hemoglobin (MCH) and Mean corpuscular volume (MCV) were calculated by using the RBC count and Haemoglobin (Hb) concentration methods (Lewis *et al*., 2001). Aspartate amino transferase (AST), alanine amino transferase (ALT), alkalin phosphatase (ALP), lactate dehydrogenase (LDH), triglyceride, cholesterol, glucose, total protein and albumin content was determined colorimetrically using kits supplied by ZiestChem diagnostics, Tehran, Iran (Fazlolahzadeh, Keramati, Nazifi, Shirin, & Seifi, 2011). Globulin content was calculated by subtracting albumin content from serum total protein content.

Stress Challenge

At the end of experiment, four fish were randomly sampled for an environmental stress (high temperature and salinity, high and low pH) trial. The fish were exposed to high temperature (33 °C), salinity (40 ppt), acidity (pH = 2, HCl) and alkalinity (pH = 12, NaOH) according to the methods of Akrami, Rahnama, Chitsaz and Razeghi Mansour (2015). Then mortality time measured in seconds.

Statistical Analysis

Values were expressed as Mean ± standard division (SD). Growth performance, haematological, biochemical parameters and resistant rate were tested using one-way ANOVA and Duncan's multiple range test was used for comparison of the mean values at the 5% level of significance using software SPSS (Version 16.0).

Results

Growth Performance and Feed Utilization

The result of partial replacement of plant sources by DWM on growth performance and feed utilization of common carp is presented in Table 2. There were no significant differences in All of growth and feeding parameters between fingerling fed control and DWM supplementation diet, (P>0.05). All group treated with DWM led to reduction in mortality after 12 weeks.

Haematological and Biochemical Parameters

The hematological and biochemical parameters of fingerling carp fed the experimental diets are presented in Table 3 and 4. Statistical analysis of data showed that, there were no significant differences in value of RBC, Hct, MCV and MCH components between the treatment groups (P>0.05), but control group showed a significant increase (P<0.05) in RBC, Hb and Hct compared with other treatment (Table 3). There were no significant differences in eosinophile, lymphocyte, neutrophil and monocyte count between the DWM treatments and control group (P>0.05). The highest value of lymphocyte and neutrophil were in DWM 15% and control group respectively (P<0.05) (Table 3). Changes in blood biochemical parameters on day 90 are shown in Table 4. Replacement of plant sources by DWM led to negliglibe effect on the glucose, cholesterol, triglycerides, total protein and albumin of plasma of the treatment group (P>0.05) (Table 4). Also, a non-significant differences of AST, ALT, ALP and LDH was found between treatment and control group on week 12 (P>0.05) (Table 4).

Stress Challenge Parameters

Results from the stress test include temperature, salinity, alkalinity and acidicty challenges are presented in Table 5. Resistance rate to thermal-salinity and acidy stress increased significantly in control group and 5% DWM (P<0.05) respectively. Also a non significant elevation of alkalinity stress was observed in the fish fed with different experimental diets (P>0.05).

Discussion

Effective use of locally waste products from agriculture industry in biofloc and other technologies to obtain multi-layers benefit and better fish growth performance as waste minimization and water treatment in aquaculture industry. In this condition, aquaculture industries are suffering from high input cost, low return and environmental pollution threats (Deb, Noori & Rao, 2017). In the present study, the fish readily accepted the experimental diets. The results showed improvement of fish growth and feed

Table 2. Growth and nutritional performance of fingerling common carp fed with different experimental diets (means±SD)

Parameters	Control	DWM 5%	DWM 10%	DWM 15%	P-value
Initial weight (g)	2.43±0.29[a]	2.43±0.25[a]	2.44±0.32[a]	2.48±0.34[a]	0.697
Final weight (g)	4.95±0.89[a]	4.59±0.48[a]	5.26±0.89[a]	4.75±1.09[a]	0.802
Final length (cm)	7.21±0.27[a]	7.02±0.17[a]	7.17±0.36[a]	7.08±0.49[a]	0.901
Survival rate (%)	74.44±10.65[a]	93.15±7.16[a]	84.19±6.73[a]	93.33±6.67[a]	0.318
SGR (%day)	0.29±0.08[a]	0.26±0.04[a]	0.31±0.08[a]	0.26±0.08[a]	0.766
WG (%)	103.67±37.61[a]	88.30±18.74[a]	116.39±39.49[a]	91.04±38.13[a]	0.745
WG (g)	2.52±0.90[a]	2.15±0.46[a]	2.82±0.92[a]	2.27±1.02[a]	0.784
CF ($g.cm^{-3}$)	1.25±0.12[a]	1.31±0.03[a]	1.37±0.04[a]	1.28±0.04[a]	0.266
Biomass (g)	44.17±12.82[a]	61.69±13.62[a]	62.96±11.91[a]	59.18±7.37[a]	0.360
Feed Intake (%/day)	3.61±0.35[a]	4.40±0.31[a]	3.96±0.98[a]	4.15±0.90[a]	0.569
FCR	5.46±1.19[a]	7.23±1.45[a]	5.62±1.98[a]	7.24±3.19[a]	0.604
PER (g/g)	0.63±0.15[a]	0.46±0.09[a]	0.65±0.28[a]	0.53±0.27[a]	0.709
LER (g/g)	2.29±0.57[a]	1.71±0.33[a]	2.39±1.05[a]	1.94±1[a]	0.715

* Same letters are not significant different (P>0.05).

Table 3. Haemetalogical parameters of fingerling common carp fed with different experimental diets (means±SD)

Parameters	Control	DWM 5%	DWM 10%	DWM 15%	P-value
RBC (10^6/ml)	1.75±0.07[a]	1.66±0.11[ab]	1.56±0.11[ab]	1.45±0.07[b]	0.097
WBC (10^3/ml)	15.30±1.13[ab]	16.23±0.05[a]	14.04±0.64[b]	14.75±0.77[ab]	0.037
Hb (g/dl)	8.45±0.07[a]	8.33±0.37[a]	7.23±0.49[b]	6.85±0.07[b]	0.007
Hct (%)	50.45±0.45[a]	47.66±0.61[a]	50.20±0.11[a]	46.35±2.95[a]	0.140
MCV (10^{-1}.µm^3)	288.60±10.80[a]	286.63±7.46[a]	321.53±13.13[a]	320.70±31.40[a]	0.286
MCH (10^{-2}.pg)	48.30±1.10[a]	50.26±3.18[a]	46.20±0.25[a]	47.30±1.30[a]	0.546
MCHC (%)	16.75±0.25[ab]	17.50±0.65[a]	14.40±0.55[c]	14.85±1.05[bc]	0.039
Lymphocytes (%)	80±1[b]	84±0.57[ab]	80.66±0.66[b]	85±3[a]	0.095
Neutrophils (%)	17±0[a]	13±1.15[ab]	16±0.57[ab]	11.50±2.50[b]	0.073
Eosinophiles (%)	2±1[a]	1.66±0.33[a]	1.33±0.33[a]	2±1[a]	0.833
Monocyctes (%)	1±0[a]	1.33±0.33[a]	2±0[a]	1.50±0.50[a]	0.179

* Same letters are not significant different (P>0.05); Different letters are significant different (P<0.05).

Table 4. Biochemical of blood parameters at fingerling common carp fed with different experimental diets (means±SD)

Parameters	Control	DWM 5%	DWM 10%	DWM 15%	P-value
Glucose	279.50±94.04[a]	107.50±50.20[a]	129±21.21[a]	225±76.36[a]	0.160
Cholesterol	170±27.37[a]	185.50±7.77[a]	180±43.94[a]	265.50±13.43[a]	0.793
Triglycerides	492±229.92[a]	243.50±159.09[a]	324.50±217.08[a]	387.50±238.29[a]	0.339
Total protein	17.08±13.73[a]	18.25±8.13[a]	22.85±6.37[a]	20.45±1.06[a]	0.981
Albumin	5.68±2.47[a]	5.15±2.89[a]	6.75±2.12[a]	7.70±0.28[a]	0.860
AST	200±121.62[a]	191.50±133.34[a]	118.50±53.23[a]	129±90.50[a]	0.566
ALT	36±22.62[a]	63±16.97[a]	39.50±34.64[a]	48±28.28[a]	0.753
ALP	1541.50±171.82[a]	1530±169.70[a]	1525±148.49[a]	1545±35.35[a]	0.909
LDH	449±171.34[a]	400.50±113.43[a]	589.50±154.92[a]	326±196.57[a]	0.723

* Same letters are not significant different (P>0.05).

Table 5. Stress challenge value of fingerling common carp fed with different experimental diets (means±SD)

Stress parameters	Control	DWM 5%	DWM 10%	DWM 15%	P-value
Temperature	254±11[a]	89.66±51.85[b]	133.66±61.61[b]	95±12[b]	0.004
Salinity	2215±15[a]	1292.50±552.50[b]	1485±205[b]	1810±40[ab]	0.023
Acid pH	584±212[bc]	991±26[a]	490±169[c]	791±30[ab]	0.008
Alkalinity pH	1019.50±153.50[a]	863.50±250.50[ab]	625.50±127.50[b]	1068±155[a]	0.059

* Different letters are significant different (P<0.05).

utilization by replacing 10% DWM, while higher substitution levels could not provide further enhancement. It seems that positive effect may be attributed to the palatability, presence a range of relevant level of fiber and unsaturated fatty acid such as oleic acid (Akbari, Razavizadeh, Mohebbi & Barmak, 2012), digestive enzymes (Pascual, Fernandez, Diaz, Garces & Rubert-Aleman, 2000; Sotolu *et al*., 2014) and possibly the availability of nutrients especially amino acids (Yeong, 1983; Onwudike, 1986) in waste date seed and bodies meal. The fiber in date pits (DP), according to Babatunde, Fetuga, Odumosu and Oyenuga (1975), has lingocellulosic structure; therefore, it can be reducing digestive enzyme action on DP protein and availability of the protein (Masoudi, Chaji, Bojarpour, & Mirzadeh, 2011). Also, in our research combination of date pits and flesh cause to improved to this structure without any negative effect of growth performance and feed utilization. Al-Qurawi, Ali, Al-Mougy and Mouse (2003) mentioned that date body structure enzymes such as amylase, protease and phytase would enhance growth performance consequent to higher nutrient digestibility and effectiveness of gastrointestinal activities. Yousif *et al*. (1996) reported that, the inclusion of the wet date did not influence on tilapia fry fish growth. Increasing body protein and decreasing body fat was observed of fish fed on diets supplemented with date pits meal due to estrogenic action of date pits was hypothesized as direct cause of this phenomenon. Different research on agriculture by product as interesting results, for example Luo, Zhao, Chang, Feng and Tian (2014) found that gonad flavor of sea urchins (*Strongylocentrotus intermedius*) fed banana peel was significantly better than that of individuals fed other specielize diet. Deka, Sahu and Jain (2003), reported that, better growth rate recorded in group fed by pineapple waste may be due to better utilization of pineapple waste diet but, it was not statistically significant from other groups. Though, highest proteolytic activity was found in this group. It may correlate with the presence of a proteolytic enzyme 'bromelain' in pineapple, which might be the factor for better utilization of protein (Deka *et al*., 2003). In contrast, Chatzifotis, Esteban and Divanach (2006), who studied different plant meal substitutions, obtained inversely proportional results with statistical differences between treatments and observed that high plant meal substitution resulted in worse FCR values. Common carp similar to tilapia and African catfish is an omnivore that is capable of efficiently digesting and utilizing diverse feeds and ingredients of both plants and animal origin (Sotolu & Faturoti, 2011). For this reason, addition of DWM can help to common carp fish fed diets with more carbohydrates composition. Belal and Al-Jasser (1997) who noted improved weight gain, feed conversion, specific growth rate and protein efficiency ratio after feeding tilapia with diets containing date (15, 30 and 45%). Belal and Al-Owafeir (2004) observed that fish WG, FCR, SGR and PER of tilapia did not be affected by different level of date pits as a replacement for corn starch.

But, in another experiment mention that sprouted date pits did not affect on growth and body composition, whereas nonsprouted date pits negatively influenced fish performance. On the other hand, El-Sayed *et al*. (2006) found that date pits based diets replaced up to 75% wheat bran resulted in reduced growth rates and feed utilization efficiency of Nile tilapia. They reported that, fungi degraded pits (DDP) could replace 300 g/kg dietary corn. This result revealed that, despite the sharp retardation in the performance of Nile tilapia fed raw date pits, the expected reduction in the cost of DP-based diets may justify the use of this by-product in Nile tilapia feed. However, they mention to further work is urgently needed to improve the quality of DP for tilapia, using proper processing and treatment techniques. While, Belal (2008) found that, growth performance of Nile tilapia fed sprouted date pits diets not differ from control diet. All of previous results were confirm of current research. It means that, the addition of DWM to diet compare with control diet had no significant effect on growth performance. Azaza *et al*. (2009) noted that, no feed-related mortality was observed during the entire experimental period. Final body weight and specific growth rate in the different treatments were statistically not significantly different ($P>0.05$). While, waste date meal could be substitute with soybean meal up to 300 g/kg^{-1} without compromising growth of Nile tilapia. Hosseinifar *et al*. (2015 & 2017) reported that feeding carp fry by date palm fruit were significantly improved growth performance, immune efficacy and increase the antioxidant activity ($P<0.05$) in early stages of common carp culture (Hoseinifar *et al*., 2015; 2017). This report was contrary with our research. In this research experimental diets prepared by date extract, but in our research we used from waste date meal (Powder form) product.

Blood indices are reflection of dietary treatments effects on the fish health condition and its physiological, biochemical and metabolic necessities. In this research, some haematological parameters were no significant differences in value of RBC, Hct, MCV, MCH and differential leucocyte count (DLC) among different treatment diets ($P>0.05$). The findings show that these blood cells were not significantly affected by DWM diet. The observed improvement in the values of most haematological parameters assessed (RBC, Hct, MCV, MCH) although, non-significant differences between them, it could serve as indication that it can be included in fish diet to improve and prevent to anaemic condition. But other parameters including WBC, Hb and MCHC were significant between all experimental diets ($P<0.05$) (Table 3).

The WBC serves as one of the first lines of body defense and their numbers increase sharply when infections arise (Vahedi, Hasanpour, Akrami, & Chitsa, 2017). In our experiment, WBC counts increased significantly in 5% DWM group in comparison with other group. This may explain the efficacy of 5% DWM in terms of the immunestimulatory response and anti-infection properties. The haemoglobin (Hb) content in the blood plays a vital role and serves as transportation element of oxygen to body tissues (Vahedi *et al*., 2017). In the current study, the Hb and MCHC content was significantly higher in 5% DWM treated groups than the control and other groups, which demonstrates that oxygen supply increases consequently, reflecting beneficial health effect on fish (Vahedi *et al*., 2017). This is in agreement with Zheng, Wen, Han, Li, and Xie (2012), who reported significant decrease in haemoglobin level in grass carp fed with a high cottonseed meal (CM) diet (48.94%) were significantly lower than those of the fish fed with low CM diets. In channel catfish, the dietary free gossypol (FG) of 900 mg/kg decreased the hematocrit (Hct) value, hemoglobin content, plasma protein, and the dietary FG of 1200 mg/kg further reduced the number of RBC (Yildirim, Lim, Wan, & Klesius, 2003). The decreased Hb and Hct resulting from the high level of dietary CM or FG were also reported in rainbow trout (Blom, Lee, Rinchard, Dabrowski, & Ottobre, 2001; Rinchard, Lee, Czesny, Ciereszko, & Dabrowski, 2003) and tilapia (Yue & Zhou, 2008). In another study on channel catfish, high level of dietary CM (55%) did not affect the Hct value, Hb content and the number of RBC (Barros, Lim & Klesius, 2002). Wang *et al*. (2014) found that, increase in dietary cottonseed meal (CM) level from 0% to 45%, the RBC and Hct value increased, but the Hb content showed little change, which indicated that the Hb and RBC content significantly decreased. In the current study, the decrease in the Hb level of fish resulting from the 10% DWM may be related to the reaction of date with iron, which further reduced the iron concentration in the body (Al-Orfe *et al*., 2012). The reason for different results between different values of DWM in diet might be attributed to difference in the effect of active compound of date and immune system reaction (Binaii *et al*., 2014). Dates are not a rich source of protein, they considered as a reasonable source of vitamins, essential amino acids, mineral elements, dietary fiber, phenolics and polyphenolic (Al-Orfe *et al*., 2012). Vitamins play role as antioxidants and phenolics can scavenge free radicals help regulate and stimulate the immune function, maintenance of cell function for growth and reduce morbidity of infectious diseases (Masoudi *et al*., 2011). Minerals are generally important as constituents of hemoglobin, soft tissues, bones, body composition, and nerve cells. Date is also a good source of iron, sodium, potassium, magnesium and calcium. Iron is an important source for Hb synthesis, and a deficiency in iron can result in a decrease in Hb level, which has been extensively reported in livestock animals (Wang *et al*., 2014).

The results from blood biochemical parameters, showed no significant differences between groups (P>0.05) (Table 4). This is in agreement with the investigation of Al-Maiman (2005) that observed favourable effects of fibers of date palm seed on plasma lipids in rats. Addition of DWM to treatment diets resulted in a linear insignificant increase (P>0.05) of blood glucose concentration (Table 4). However, it agreed with the findings of Onifade (1997) who reported that glucose was accentuated high when fibrous diet was fed to broilers. It seems that this effect of fibrous material contributed to increase in gluconeogenesis activity (Masoudi *et al*., 2011). Since date pit (DP) has a fibrous structure which supports low glucose than maize, maybe some effect of date pith on blood parameters contributes to its effect on increment of lipid metabolism for supporting the energy requirements of common carp fish (Masoudi *et al*., 2011). Zhou, Lin, Ji and Yu (2016) reported that the blood serum total protein, albumin, glucose, high and low density of lipoprotein and total antioxidant capacity in green tea waste (GTW) group were significantly higher than control group and GTW supplementation has positive impact on health of fish without affecting the growth of fish. Liver plays an essential role in transforming and clearing metabolites and xenobiotics, and is susceptible to the toxicity from these agents. Liver enzymes include of ALT, AST, ALP and LDH are important indicators reflecting liver function. An increase in the activities of serum AST, ALT and ALP might indicate a damage of liver function (Wang *et al*., 2014). As differences liver enzymes were not observed in the experimental groups, the consumption of DWM did not appear to induce liver toxicity in fish, Also the group tested with 10% DWM decreased liver enzyme activity for 12-week compared to the control. It could be explained that mechanism of hepatoprotective effects of date flesh and pits is possibly related to vitamin C, polyphenolic compounds (flavonoids and phenolic acids), β-sitosteroland trace elements (selenium, zinc, copper and manganese) (Al-Orfe *et al*., 2012). Likely that the mechanism of antioxidants in DWM may be related to the ability of its active compounds to detoxify free radicals and to inhibit lipid peroxidation in the liver and protein oxidation (Al-Orfe *et al*., 2012).

Stress resistance of the fish in different life period is affected by levels of salinity, temperature, environment and nutrition (Jalali, Hosseini & Imanpoor, 2008; Gholami, 2010). The results of present study showed that resistance rate to temperature-salinity stress significantly increased in fish fed control diet (P<0.05) than those fish fed with the DWM groups, but responses to alkalinity stress was insignificant (P<0.05). Resistance to acidity stress increased significantly in fish fed diets supplemented with 5% DWM (P<0.05) (Table 5). It seems that greater resistance of control group to stress challenges might also be due to low potential of digestibility of date, higher and lower value of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) and high antiprotease activity, which may increase protective function of body resistance of fish, but studies are required to test this speculative hypothesis. Antiprotease contributes to the innate immunity of fish by its benefit alimentary tract bactericidal community and antiinflammatory properties (Guardiola, Porcino, Cerezuela, Cuesta, Faggio & Esteban, 2016).

In this research, addition of DWM alone enhances several of growth and feed utilization parameters, while maybe for enhances the stress resistance or expression of immune-related genes when is administrated in combination with other substance such as probiotic. Therefore, the mixture this diet could potentially be considered of great interest as immunostimulant diet to use as food additive for farmed fish.

Hwang *et al*. (2013) found that the stress recovery time differed significantly between the control and the 3 and 5 % green tea extract (GTE) diet groups and the mortality of black rock fish (*Sebastes schlegeli*) fed on the GTE diet was significantly lower than that of the control group. This research showed that the addition of GTE to the fish diet improved stress recovery in a dose-dependent manner. However, more studies are still required to determine the effective of DWM concentration in diet and to understand the specific mechanisms involved in stress control by DWM in fish diets.

Conclusion

To conclude, DWM up to 10% as a substitute for plant sources could improve growth and blood variables in common carp. Further research is needed to clarify the action mechanisms of DWM, as well as the immune response and feeding period in common carp.

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