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EFFECTS OF SUPPLEMENTATION OF DATES KERNEL MEAL (DKM) AS A FEED ADDITIVE ON THE ACTIVITIES OF DIGESTIVE ENZYME, IN THE DIETS OF COMMON CARP (*CYPRINUS CARPIO* L.)

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Abstract

The present study was conducted to examine the utilization potential of Date Kernel Meal (DKM) as a feed additive in the diet of *Cyprinus carpio* for improved fed utilization and activities of digestive enzyme. A total of 160 fingerlings (weight 21.47 ± 0.84 g / fish) were divided into ten groups. The experiments were conducted for 90 days; diets formulae were identical in all feeding trials except for variation in (DKM) constituents. Non – DKM diet was used at 0% (control), and other levels were (33.3 %, 66.67 % and 100%). (T_2, T_3 and T_4) treatments respectively without treated by yeast bread; (33.3 % 66.67 % 100) (T_5, T_6, and T_7) treatments respectively treated by yeast bread; (33.3 %, 66.67%, 100 %) for (T_8, T_9, T_{10}) treatments respectively treated by diets enzyme (protease, amylase and cellulase). Overall performance of enzymatic activities indicated that, diets containing (33.3 % , T5) and (66.67 % T6) DKM (treated by bread yeast) recorded the highest enzyme activities for protease and amylase in the fish intestine, compared with other levels, including control diet. The whole intestine recorded the highest enzyme activities for protease and amylase among other parts. Values recorded (1.76, 1.26 and 1.89, 1.86 U/ ml g protein in T_5, T_6) for protease and amylase, respectively. Based on the results of the present study, it is concluded that DKM supplementation positively influence growth performance and feed utilization of common carp *(Cyprinus. carpio*) as well as ensuring their healthy status.

Key words : Enzyme, Date Palme Seed, amylase, protease, supplementation, feed additive.

Introduction

In general, feeding cost of carp culture is more costly than other fish and animal cultures. In recent reports, it is indicated that only 1.3 % farmer's use commercial carp feed, whereas 65.4% farmers use only mash feed (Ramakrishna *et al.,* 2013). Plant cells are strengthened by cell walls that are mainly indigestible by vertebrate intestinal enzymes. The long fiber- like molecules of cellulose are cemented by pectin hemicellulose and lignin. None of the cell wall components can be hydrolyzed by the vertebrate digestive enzyme amylase. However, celluloses, hemicellulose and pectin can be hydrolyzed by a complex of microbial enzymes known as (cellulases hemicellulose and pectinases). At the absence of cellulase, the plant cell walls cannot be digested. Furthermore, the encased cell components cannot be exposed to digestive enzymes; this will result in the reduced digestibility of plant based feeds (Chesson, 1993; Dudley – Cash, 1997; Cunningham and Klein, 2007). There is an evidence suggesting that, soluble high molecular weight (non-starch polysaccharide) contained within plant cell walls increases digesta viscosity, thereby reducing digestive enzyme access to other nutrients (Bedfored, 2000; Francis *et al.,* 2001). This would result in reduction feed of efficiency and lowering growth rates in fish (Watanabe, 2002). Supplementation of fish feed with exogenous enzymes appears to be an ideal strategy to improve the nutrient value, nutrients containing cellulases, hemicellulases, etc. ; It has shown the beneficial effects in improving the growth /feed conversion rate in different domestic animals,

including fish (Carter, *et al.,* 1994; Kolkovski, *et al.,* 1993). These enzymes are also widely used to reduce the anti – nutritional effect of non- starch polysaccharide in animal feeding, including fish, which has been successful in breaking down phytate to increase mineral and nutrient digestibility that turn improves the growth performance of fish (Kiarie, *et al.,* 2013; Adeoye, *et al.,* 2016). Non-starch polysaccharide degrading enzymes (e.g cellulase) are capable of disrupting plant cell wall integrity, this would enhance rapid digestion by reducing viscosity in the gut (Bedford & Cowieson, 2012). The aquaculture nutritionists are in a continuous search to reduce the use of fish meal and look for more cost effective substitutes. The process of digestion determines the accessibility of nutrients needed for all body functions and enzymatic activity, which is the basic tool to observe feeding acceptability and its contribution towards.

The growth and maintenance of the fish body (Caruso *et al.,* 2009). Metabolic adaptations to changes in feed ingredients and in enzymatic secretion, gives better result feed utilization (Coruso, *et al.,* 2009). Digestive enzyme activities varied in different fish species, which may due to differences in digestive potential and feeding habits. The study of enzyme function is helpful in fish and changes of ambient environment (Sund, *et al.,* 2004; Chakrabarti and Sharma, 2005). Protease and amylase enzyme activities disclose the ability of different fish species to use protein and carbohydrates (Hidalgo, *et al.,* 1999). Therefore, the examination of digestive physiology is of a major concern to evaluate upon the type and function of the digestive enzymes. The comparative studies of these enzymes and their activities in different fish species are well documented (Xavier, *et al.,* 2012). Studies of proteolytic enzyme in fish have to provide knowledge for improving protein utilization and established the importance of proteases as key enzymes for food utilization and growth that have to improve role in the processes of protein digestion (Rungruangsak-Torriessen, *et al.,* 2006). Various commercial enzyme mixture are available and routinely supplemented in fish feed. These products, contain (protease, amylases, celluloses, etc.), which hydrolyze the bonds of (proteins, carbohydrates, cellulose) product, respectively, within the digestive tract. The aim of this study is to investigate the effectiveness of Endofeed (commercial multienzyme product used in fish farms) on the activities of digestive enzymes in common carp intestinal tract.

Materials and Methods

Adaptation of fish and condition of experimental and maintenance

The common carp *Cyprinus carpio* L. Fingerlings were obtained from a local fish farm (dealer) transported to the laboratory of AL- Mammon University College. Fish were acclimatized in glass aquariums for 10 days and fed with commercial diet containing 10% protein. The fish were washed with salt solution (3% NaCl) for 3 minutes to get rid of parasites. 160 fingerlings (21.47 ± 0.84 g / fish) were randomly distributed in 20 glass aquaria at the rate of 8 fish per glass aquarium, each diet treatment was twice replicated. Any aquarium was supplied with air pump. Fish were feed twice daily (in a rate of 3% body weight) per day for 90 days. Water of the aquarium was changed partially in a daily base.

Diet formulation

The different ingredients were purchased from local markets, and date kernel was obtained from date moles plant in Babylon Governorate. The dates kernel crushed and grinded into powder form by a private mill in the local market. Each ingredient was grinded alone, by grinder and mixed together to homogenize. Two treatments were prepared for the date kernel meal 300 mg of yeast bread / kg of the date kernel meal was added and covered for 48 h at laboratory temperature. The second formula was prepared by adding commercial diet enzyme (enzyme type LABAZYME prepared by Korea' s New Pharm) to the date kernel meal at a ratio 2.5 g / kg, the diet enzyme containing:

Protease (More than 2.750 Colony Starch Unit (CUS)

Amylase (More than 5.500 Starch Laysis Uint (SLU)

Cellulase (More than 27.5 Filter Paper Uint (FPU)

The different ingredients used in the experiment were prepared, and each ingredient was weighed alone by electronic balance and formulated into 10 experimental diet preparation as follows (table 1) :

(T_1) Without dates kernel meal for control.

(T_2) Add dates kernel meal without treatment at 8.34 % and substitution ratio 33.3% of the yellow corn.

(T_3) Add dates kernel meal without treatment at 16. 67 % and substitution ratio 66.6% of the yellow com.

(T_4) Add dates kernel meal without treatment at 25 % and substitution ratio 100% of the yellow corn.

(T_5) Add dates kernel meal treatment by yeast of bread at 8.34 % and substitution ratio 33.3% of the yellow com.

(T_6) Add dates kernel meal treatment by yeast bread at 16.67 % and substitution ratio 66.6% of the yellow corn.

(T_7) Add dates kernel meal treatment by yeast bread at 25 % and substitution ratio 100% of the yellow com.

(T_8) Add dates kernel meal treatment by diet enzyme at 8.34 % and substitution ratio 33.3% of the yellow corn.

(T_9) Add dates kernel meal treatment by diet enzyme at 16.67% and substitution ratio 66.6% of the yellow com.

(T_{10}) Add dates kernel meal treatment by diet enzyme at 25% and substitution ratio 100% of the yellow com

Chemical analyses

Chemical analyses were conducted for each diet formula; dates kernel meal and yellow corn (which were used for nutritional treatments) in the laboratories of the center for animal resources and fisheries / Ministry of Science and Technology Baghdad. The analyzes were carried out using the standard methods, adopted in AOAC (2000). The soluble carbohydrate were calculated according to the formula mentioned by (Wee and Shu, 1989).

Dissolved C. H. O = 100 – (protein % + ether extract % + Ash % + fiber %).

Metabolic energy is calculated by (Hepher and Prugining, 1981). (Table 1, 2).

Sample preparation

At the end of each experiment, fish were collected from each aquarium. Dissected, intestine were immediately removed. The whole procedure was conducted in ice – cold condition. Homogenized samples were centrifuged at 10,000 g for 30 mint at 4°C.

The supernatant was collected in 5ml tube and stored in deep freezer (-18°C) for enzyme assay. Suitable dilutions of the sample were prepared as required.

Protease activity

Protease activity in the intestinal tissue was determined by the Casein digestion method (Drapeau, 1974). The enzyme reaction mixture (consist of 1% Casein in 0.05 M tris- phosphate buffer pH 7.5) incubated for 10 min at 37 °C; then tissue homogenate was added to the enzyme mixture. After the 10 min reaction was stopped by adding 10 % TCA followed by filtration of the whole concentrate. The blank reagent was made by adding tissue homogenate just before stopping the reaction and without incubation. One unit of enzyme activity was defined as the amount of enzyme needed to release acid soluble fragments equivalent to 0.001 A_{280} per mint at 37 °C and pH 7.5.

Amylase activity

The reducing sugars produced due to the action of gluco amylase and amylase on carbohydrate was estimated using Dinitro- salicylic acid (DNS) method (Rick and Stegbuar, 1974). The reaction mixtures consist of 1% (w/v) soluble starch solution, Phosphate buffer pH 6.9 and the tissue homogenate. The reaction mixture was incubated at 37°C for 30 min. DNS mixture was diluted with distilled water and absorbance was measured at 540 nm. Maltose was used as the standard. Amylase activity was expressed as Mol of maltose released from starch per min at 37°C.

Statistical analysis

Data were subjected to two-way analysis of variance. The following model was used:

$Yijk = \mu + Ti + Lj + eijk$

Where, Yijk the individual observation; μ=The overall mean; Ti=The treatment (i=10); Lj=The location effect (j=3) (1: Foregut, 2: Midgut, 3: fullgut; eijk=The random error associated with experimental unit (NID, $\sigma^2 e$). Duncan's multiple range tests was used to compare the differences among means (in columns and rows). All statistical analysis by ANOVA procedure was carried out by SAS (2012) program.

Results

Amylase activities

Table 3 showed significant differences in amylase activity as noticed among the 3parts of intestinal tract, in treatments (T_2, T_3 and T_5). Non – significant differences in amylase activity in the same intestinal among treatment groups (T_1, T_4, T_6, T_7, T_8, T_9 and T_{10}) at ($P< 0.05$). Highest amylase activity (1.66 U / ml g protein) was observed treatment (T_2) ; While it was as low as (0.67 U/ ml g protein) in treatment (T_{10}) in the foregut part ; And the highest amylase activity (1.08 U / ml g protein) in treatment (T_1). (T4) showed the lowest amylase activity (0.54 U / ml g protein) in the midgut part ; Whereas the highest amylase activity (1.76 U / ml g protein) in the treatment (T_5) ; And the lowest amylase activity (0.68 U / ml g protein) observed in the whole gut of the intestine.

Protease activities

The protease activity was found significantly different throughout the intestinal tract in (T_1, T_3) treatment groups ($P < 0.05$). Non – significant protease activity noticed in all parts of intestinal, among treatment groups ($P < 0.05$) (Table 4). The treatments (T_2, T_7, T_{10}) showed the lowest protease activity (1.42, 1.42.1.42 U / ml g protein)

Table 1: Formulation and proximate composition diets (%).

Ingredients (%)	Control	Dates kernel meal without treatment			Dates kernel meal treatment with yeast bread			Dates kernel meal with diet enzyme		
Treatments	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
Fish meal	12	12	12	12	12	12	12	12	12	12
Soybean meal	30	30	30	30	30	30	30	30	30	30
Sesame meal	10	10	10	10	10	10	10	10	10	10
Yellow corn	25	16.67	8.33	0	16.67	8.33	0	16.67	8.33	2
DKM	0	8.33	16.67	25	8.33	16.67	25	8.33	16.67	25
Black barley	11	11	11	11	11	11	11	11	11	11
Wheat bran	10	10	10	10	10	10	10	10	10	10
Vit	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100	100	100	100	100
Ingredient composition of experimental diets (% DM basis										
CP	28.15	27.24	27.08	27.11	27.67	27.87	27.92	27.13	27.58	27.72
EE	6.08	5.52	5.64	5.36	5.87	5.41	5.68	5.87	5.74	5.68
CF	7.21	11.08	12.14	13.19	8.61	8.42	9.13	8.29	8.52	9.23
ASH	7.82	8.44	8.82	8.92	8.31	8.31	8.40	8.22	8.35	8.38
C.H.O	50.74	47.72	46.32	45.42	49.54	49.99	48.87	50.49	49.81	48.99
ENERGY (kcal / 100 g	438.24	414.59	408.57	402.16	428.31	427.18	424.96	429.52	427.81	424.38

Table 2: Analyses of nutrient composition of Dates kernel meal and yellow corn (% of DM basis).

Ingredient	Yellow corn	Dates kernel meal without treatment	Dates kernel meal with yeast breads (300 mg / kg)	Dates kernel meal with diet enzymes (2.5 g /kg)
DM	87.8	90.21	90.24	90.49
CP	8.7	8.21	11.08	9.82
EE	3.6	2.64	2.04	2.16
CF	3.4	26.25	19.13	21.24
ASH	1.9	6.41	5.11	5.27
C.H.O	71.85	56.49	62.64	61.51
ENERGY (kcal / 100 g)	401.224	321.315	359.12	348.19

respectively ; Whereas (T_1, T_5, T_6) showed the highest protease activity (1.89, 1.99, 1.89 U / ml g protein) respectively, in the foregut intestine ; Moreover the treatment (T_5) showed the highest protease activity (1.87 U ml g protein), Meanwhile the lowest protease activity (1.01 U / ml g protein) showed in treatment (T_4) in the midgut intestine. In the whole gut the highest protease activity (1.99 U / ml g protein) in the treatment (T_1) ; And the lowest activity (1.45 U / ml g protein) in the treatment (T_{10}).

Discussion

Digestive processes in fish are not well known as in mammals, although the data obtained in fish so far showed that, the protease and amylase were qualitatively similar to those observed in other vertebrates. Enzymes such as amylases, proteases, etc. Enhance growth performances consequent to higher nutrient digestibility and effectiveness of gastrointestinal activities as, earlier stated by (Al- Qurawi, *et al.,* 2003). Feed and its constituents are the factors which influence the activities of digestive enzyme in fish (Fernandez, *et al.,* 2001).

In the present study, diet formulation and supplementation of exogenous enzymes (proteases, amylases and cellullase), and DKM with or without treated with bread yeast , appeared to have beneficial effect on nutrient utilization, growth performance and enzyme activities of common carp. The study showed that when fishes were fed with DKM treated with yeast bread, the enzyme activities (amylase and protease) were improved more than fish fed with untreated DKM. However, the enzyme activities and nutrient utilization of DKM based

Table 3: Effect of different Dates kernel meal levels on intestinal amylase activity(U/m l g protein) of common carp.

Sections / Treatments	Mean ± SE			Level of Sig.
	Foregut	Midgut	Whole gut	
T_1	1.09 ± 0.02 B a	1.08 ±0.03 A a	1.26 ± 0.06 B a	NS
T_2	1.67 ± 0.02 A	0.66 ± 0.03 B	0.71 ± 0.04 C	*
T_3	1.66 ± 0.06 A a	0.60 ± 0.05 B b	0.61 ± 0.03 C b	*
T_4	0.61 ± 0.02 C a	0.54 ± 0.03 B a	0.50 ± 0.03 C a	NS
T_5	1.08 ± 0.03 B b	1.07 ± 0.02 A b	1.76 ± 0.06 A a	*
T_6	1.09 ± 0.01 B a	1.10 ± 0.03 A a	1.26 ± 0.04 B a	NS
T_7	0.78 ± 0.01 C a	0.67 ± 0.02 B a	0.87 ± 0.03 BC a	NS
T_8	0.76 ± 0.03 C a	0.73 ± 0.04 B a	0.75 ± 0.03 BC a	NS
T_9	0.78 ± 0.02 C a	0.74 ± 0.02 B a	0.71 ± 0.04 C a	NS
T_{10}	0.67 ± 0.03 C a	0.67 ± 0.02 B a	0.68 ± 0.03 C a	NS
Level of Sig.	*	*	*	.....

* (P<0.05), NS: Non-Significant.Means having with the different big letters in same column and small letters in same row differed significantly

on diet with treated bread yeast, were very much comparable with fish fed with commercial carp diet. The highest enzyme activities for (T_5, T_6) treatments as compared to other treatments were obvious for protease and amylase), it was apparent that, the supplementation of bread yeast to DKM ; enzyme activities were improved in (T_5 and T_6) treatments as compared to other experimental treatments of DKM (untreated with bread yeast) . Also, a higher amylase and protease activities were enhanced in the whole intestine of fish as recorded in the treatments (T_5 and T^6) (containing DKM treated with bread yeast at (8.34 % and 16.67 %) respectively, and substitution ratio (33.3 % and 66.7%) of yellow corn respectively. Several studies concerning the activities of digestive proteolytic and amylolytic enzymes, performed with different revealed that, the capacity of different species to utilize proteins and carbohydrates for growth performance (Chan, *et al.,* 2004) pointed out that, adaptation of the digestive system of different species (*Pagrus pugrus, Pagellus erythinus, Pagellus bogaraveeo, Boops boops, and Diplodus annlaris)* exhibit closer correlation with their diet rather than on their taxonomic category. Lazzari, *et al.,* (2010) observed variations in amylase activity in different parts of intestine fed with different diets. While De- AL meida *et al.,* (2006) found higher amylase activity in the posterior part of the intestine in tambaqui (*Clossoma macropomum*). A higher amylase secretion in foregut part of intestine when Cyprinus. *carpio* and *Tenopharyugodan idella* where fed with animal origin feed stuffs were observed (Fisher, 1973) , Whereas Khalid, *et al.,* (2015) noticed highest amylase activities in whole intestine, when Juvenile(*Labo rohita*) fed with (guar meal and cottonseed meal). Moreover, Akeem, *et al.,* (2014) observed that overall growth performance and subsequent fish quality assessment indicated that, diet containing of 1.5 % date palm seed, recorded the best performance in fish compared with other supplementation including the control diet. The same study concluded that, date palm seed supplementation positively influenced growth performance and feed utilization of African catfish as well as ensuring their health. AL- Tameemi, *et al.,* (2010) noticed that omnivorous common carp collected from fish ponds, showed a significantly (P<0.01) higher value of α – amylase specific activity, reached to 1.92 U/ ml g protein, when compared with values recorded in common carp collected from Garma River, which reached to 1.33 U / ml g protein. This might be due to differences in their food components a higher proportion of carbohydrate compared with other naturally fed carp. Al – garrawi, *et al.,* (2017) found high enzymes activities for proteases in the foregut and midgut , Also they found that, the replacement of white corn meal (treated with lignin peroxidase enzyme) instead of yellow corn up to 100% replacement, has no effect on growth and protease enzyme activity.

Conclusion

The significant role of DKM treated with bread yeast for the nutrition of common carp, as DKM treated with 8.34 % and 16.67 % bread yeast substituted instated of yellow corn (33.3% and 66.7 % respectively) were studied. The intestinal digestive enzyme activities of amylase and protease) were significantly improved. This study promotes the application value of DKM in aquaculture feed. Further investigation, would be needed, in order to study clarify and the possible morphological and proteolytic changes in fish intestine, liver, muscle, in

Table 4: Effect of different Dates kernel meal levels on intestinal protease activity (U/ml g protein) of common carp.

Sections / Treatments	Mean ± SE			Level of Sig.
	Foregut	Midgut	Whole gut	
T_1	1.89 ± 0.03 A a	1.77 ± 0.05 A a	1.99 ± 0.05 A a	NS
T_2	1.42 ± 0.05 B a	1.39 ± 0.03 BC a	1.45 ± 0.02 C a	NS
T_3	1.01 ± 0.03 C b	1.10 ± 0.02 C b	1.44 ± 0.02 C a	*
T_4	0.93 ± 0.01 C b	1.01 ± 0.02 C ab	1.34 ± 0.01 C a	*
T_5	1.99 ± 0.06 A a	1.87 ± 0.04 A a	1.89 ± 0.06 A a	NS
T_6	1.89 ± 0.04 A a	1.79 ± 0.05 A a	1.88 ± 0.04 A a	NS
T_7	1.42 ± 0.03 B a	1.41 ± 0.05 BC a	1.53 ± 0.03 BC a	NS
T_8	1.61 ± 0.03 AB a	1.63 ± 0.04 AB a	1.71 ± 0.04 AB a	NS
T_9	1.53 ± 0.05 B a	1.49 ± 0.02 BC a	1.55 ± 0.03 BC a	NS
T_{10}	1.42 ± 0.02 B a	1.38 ± 0.02 BC a	1.45 ± 0.03 C a	NS
Level of Sig.	*	*	*	.....

* ($P<0.05$), NS: Non-Significant. Means having with the different big letters in same column and small letters in same row differed significantly

response to dietary with DKM instead of protein and carbohydrate.

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