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# EVALUATED WHITE CORN *SORGHUM BICOLOR* TREATMENT BY ENZYME LIGNIN PEROXIDASE INSTEAD OF YELLOW CORN ON α-AMYLASE ACTIVITY IN *CYPRINUS CARPIO* L.

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## Abstract

The study was conducted to investigate the effect of replacing the white corn after improving their nutritional value by reducing the tannins by Lignin peroxidase enzyme which locality isolated from bacteria *Sptreotpmyce* ssp. and substituted for yellow corn in *Cyprinus carpio* L. diets on α-amylase activity. The fish average weight (25.12±3.75gm/fish) fed on seven experimental diets contain two substitution levels of white corn for yellow corn, $T_1$ and $T_2$ used white corn without treatment substitution 50% and 100% for yellow corn, $T_3$ and $T_4$ used white corn treatment before grinded by enzyme at same substitution level, $T_5$ and $T_6$ used white corn treatment after grinded by enzyme at same substitution and $T_7$ without white corn for control. The intestinal fish distributed into 3 parts (foreground, middle part, end part). The results of the experiment showed the distribution of the α-amylase enzyme in all the gastrointestinal tract (foreground, middle part, end part), the highest effectiveness was in the foreground and middle part of the intestine which significant different $p \leq 0.05$ with end part, it were before starvation (1.89, 1.77 and 0.55 U/mg protein at the nutrition), and the vale after starvation 5 day 0.546, 0.554 and 0.04 U/mg protein at the nutrition respectively. The results also showed the significant value of enzyme activity in the foreground and middle part compared to end parts after 30 days fish feeding. While there were a significant difference of amylase activity between $T_1$ and $T_2$ compared with all other treatments. The value recorded (1.146, 1.156, 1.397, 1.501, 1.511, 1.498 and 1.498 U/ mg protein at the nutrition) for foreground and (1.158, 1.149, 1. 445, 1.499, 1.498, 1.487 and 1.476 U/ mg protein at the nutrition) for middle part for all treatment respectively. These data suggest, it is possible to replace white corn treatment with lignin peroxidase enzyme up to 100% instead of yellow corn a without any effect on amylase activity.

***Key word :*** enzyme, amylase, digestive, carp, white corn.

## Introduction

The Amylase enzyme belongs to a group of hydrolase enzymes. The amylase enzyme compose of a number of enzymes similar to a-amylase (b-amylase, gluco-amylase, etc.) all of which are involved in the analysis of the glycosidic bond in polysaccharides. However, they differ in the mechanism of action (Kujawski *et al*., 2002). The a-amylase enzyme is highly specialized and it does not analyze cellulose, gums excepted the sugar have glucose which associated with each other with the glycosidic bonds a-1, 4 (Pandy *et al.*, 2000). Animal a-amylase can be obtained from saliva, urine and blood and animal pancreas is the primary source of animal amylase. Kerr (1950). As for omnivorous fish, the enzyme is concentrated in the pancreas and it is found that the amounts of this enzyme vary depending on the type of fish and the way they are fed (Hidalgo *et al.*, 1999). The (Rungkan *et al.*, 2009) work to isolated the a-amylase from the gut of nile tilapia *Oreochromis niloticus*. The researchers said in the field of fish nutrition that the common carp fish *Cyprinus carpio* L. characterized by containing high amounts of a-amylase (Kuzmina *et al*., 2003). The researcher (Chakrabarti *et al*., 1995) noted the enzymes were spread throughout the digestive system and found in most of its parts when studying eleven species of fish. They stressed (Kapoor *et al*., 1976) that the levels of a-amylase enzyme in the digestive system of the common carp fish is much higher than levels in other fish, which can benefit from the carbohydrates. In contrast to the

mammals that produce the enzyme from the pancreatic and saliva glands, the only source of fish is the external pancreas secretion (Krogdahi and Bakke-Mckellep, 2005). The researcher (AL-Gharrawi and Hussein, 2012) study the effect of the type of diet on the secretion of some digestive enzymes in the gastrointestinal tract of the common carp that the α-amylase enzyme is found at different levels along the digestive tract according to the type and percentage of dissolved carbohydrates.

The aim of the study was to investigate the effect of replacing the white corn which treatment by Lignin peroxidase with yellow corn on the efficiency of α-amylase in the gastrointestinal tract of common carp *C. carpio*.

## Materials Methods

### Experience fish and experiment design

The feeding experiment was carried out in the laboratories of the Center for Animal Resources and Fisheries for 30 days using 98 common carp (*Cyprinus carpio* L.) at 25.12±2.56 g/fish, brought from a local fish farm. The experiment was initiated by means of glass aquariums. The fish were randomly distributed to 14 glass aquariums with seven fish/aquarium and two replicates per treatment. Seven laboratory experimental diets were prepared to feed the fish (Table 1) as follows:

$T_1$: Use white corn without treatment by substituting ratio of 50% of the yellow corn.

$T_2$: Use white corn without treatment by substituting ratio of 100% of the yellow corn.

$T_3$: Use white corn (treatment before milling with 80 ml/kg of local enzyme) with substitution ratio of 50% for yellow corn.

$T_4$: Use white corn (treatment before milling with 80 ml/kg of local enzyme) with substitution ratio of 100% for yellow corn.

$T_5$: Use white corn (treatment after milling with 80 ml/kg of local enzyme) with substitution ratio of 50% for yellow corn.

$T_6$: Use white corn (treatment after milling with 80 ml/kg of local enzyme) with substitution ratio of 100% for yellow corn.

$T_7$: The comparison diet without white corn.

Samples of experimental diets were analyzed including moisture, crude protein, ether extract, crude fiber and ash determine according to the official methods of analysis (Association of Official Analytical Chemists, AOAC) AoAc (1990). The Nitrogen Free Extract (NFE) was determined by using the equation:

NFE = 100 - (CP%-EE%-Ash%-CF%) (Table 2).

### Enzymes and sample collection

The fish were dissected in the laboratory of nutrition animal-Animal and Fish Resources Center. The digestive tract was collected before the start of the experiment after 5 days of starvation and 30 days of feeding the fish and with two fish from each treatment, cleaned and removed the unwanted parts and cut them into three parts, front, middle and back and froze for 24 hours at a temperature of -18 m until homogeneity.

### Extract enzyme

The enzyme extraction process was performed in laboratories of Al-Ma'moun University. Potassium phosphate solution was used at concentrate 0.2 molar and 6.7% of the pH container 6% of sodium chloride to extract the enzyme from the intestine of common carp fish, extract the enzyme from all studied parts of the intestine after cleaning with cold water and keep it for 24

**Table 1:** Diets components of experimental diets (Dry Matter basis %).

| $T_7$ Control without White Corn | White Corn treatment after milling | | White Corn treatment before milling | | White Corn without treatment | | Ingredient |
|---|---|---|---|---|---|---|---|
| | $T_6$ substitution ratio of 100% for yellow corn | $T_5$ substitution ratio of 50% for yellow corn | $T_4$ substitution ratio of 100% for yellow corn | $T_3$ substitution ratio of 50% for yellow corn | $T_2$ substitution ratio of 100% for yellow corn | $T_1$ substitution ratio of 50% for yellow corn | |
| 10 | 10 | 10 | 10 | 10 | 10 | 10 | Animal protein |
| 25 | 25 | 25 | 25 | 25 | 25 | 25 | Soybean meal |
| 16 | 0 | 8 | 0 | 8 | 0 | 8 | Yellow corn |
| 0 | 16 | 8 | 16 | 8 | 16 | 8 | White corn |
| 22 | 22 | 22 | 22 | 22 | 22 | 22 | Black barely |
| 25 | 25 | 25 | 25 | 25 | 25 | 25 | Wheat Bearn |
| 2 | 2 | 2 | 2 | 2 | 2 | 2 | Vit. And salt |

**Table 2:** Chemical composition for experimental diets (Calculated by dry matter %).

| $T_7$ | $T_6$ | $T_5$ | $T_4$ | $T_3$ | $T_2$ | $T_1$ | Nutrition constituents for diets |
|---|---|---|---|---|---|---|---|
| 28.14 | 28.51 | 28.22 | 28.74 | 28.34 | 29.04 | 28.38 | Crud Protein |
| 6.73 | 6.57 | 6.88 | 6.29 | 6.66 | 6.75 | 6.81 | Ether Extract |
| 7.66 | 6.19 | 6.47 | 6.65 | 6.35 | 6.22 | 6.12 | Ash |
| 7.41 | 7.49 | 7.58 | 7.32 | 7.19 | 7.84 | 7.21 | Crud fiber |
| 50.06 | 51.24 | 50.85 | 51.00 | 51.46 | 50.51 | 51.48 | *NFE |
| 1445.61 | 1463.19 | 1462.75 | 1454.83 | 1469.12 | 1466.05 | 1472.11 | ** Metabolic EnergyKJ/Kg |

hours and at temperature of -18 m. The fatty layer was removed the parts mixed with the extraction solution of potassium phosphate solution at ratio (1:4, weight: volume), homogeneous by electric mixture for 2 minute, the mixture transfer to glass container and mix by using a magnetic stirrer at $4^0$C for 6 hours. The mixture was mediated by a piece of filler to remove the excess parts. The cold centrifugal process was performed at a speed of 10000 rpm for 30 minutes to dispose of the remaining parts, the supernatural was separated from the precipitate and the stink is obtained and the enzymatic efficiency estimated by the spectrophotometer at wave length of 540 nano meters. AL-Quraishi (2005).

**Statistical Analysis**

The statistical analysis was carried by using the steps of the general statistical model (SAS) Statistical Analysis System (SAS). Sas (2012) and determined the effect of the coefficients of the studied properties using the complete randomized design (CRD). The averages were compared to each characteristic using a Duncan test at a probability level of $P \leq 0.05$ to determine mean differences between average transactions. Duncan (1955).

## Results and Discussion

The results of the study to estimate the enzymatic efficacy of α-amylase in the crude extract of the gastrointestinal tract of *C. carpio* showed that the enzyme was spread along the gastrointestinal tract after starvation and feeding of fish. The studied parts recorded enzymatic efficiency at starvation for 5 days and were 0.546, 0.554 and 0.04 mg/mg protein, while those values in fish fed on the diet increased to 1.89, 1.77 and 0.55 mg/mg for the studied front, middle and back parts respectively. The results of the statistical analysis showed no significant differences between the front and middle parts, which differed significantly $P \leq 0.05$ with the back part (Table 3).

Table 4, showed the enzymatic efficiency of the amylase enzyme for the gastrointestinal tract extract after 30 days of feeding the fish on diets containing different percentages of white corn with different treatments. The values were significantly higher than the values recorded in starvation, which differed significantly $P \leq 0.05$ (Table 3). The results of statistical analysis showed significant differences $p \leq 0.05$ between $T_1$ and $T_2$ for all treatments for the foreground and middle sections of the enzyme activity were 1.498 and 1.498 units/mg of protein. The values of 1.146, 1.156, 1.397, 11.50, 1.511, 1.498 and 1.498 ($P \leq 0.05$) with the end for all the treatments (Table 4).

The difficulty of studying the efficiency of digestive enzymes in fish was strongly related to the method of collection and efficiency evaluation. Therefore, the results differed according to these methods. Some researchers used the entire digestive tract, others divided it into parts, others the lining of the intestines and the difference of the substrate AL-Aldibikl (1996). In general, α-amylase activity increases in diets with high carbohydrate levels in general and starch in particular (Fountoulaki *et al*., 2005). The results of the current study showed the prevalence of α-amylase along the gastrointestinal tract in all studied parts (foreground, middle and end), the highest in the foreground and middle part compared to the end part (Table 3). These results were consistent with the results of (Frais *et al*., 1981) studies in the study of the distribution of digestive enzymes of catfish *Ameiurus nebulosus* and common carp *C. carpio* L. that the enzyme was spread along the gastrointestinal tract and that the activity of the enzyme increases with the increase of intestinal filling of food, the enzyme's effectiveness in feeding evaluated it in starvation. The amylase enzyme was associated with the chyme produces by mechanical and chemical effects of the leaf, consisting of partially digested food, water and various digestive enzymes, the chyme pass slowly through the pyloric sphincter, the intestine will digest food to the chyme in any where ranges from 40 minutes to 3 hours at most and is associated with the lining of the intestinal wall of

**Table 3:** The level of effective of α-amylase enzyme before and after starvation.

| Significantly level | (Mean±standard deviation) After Starvation for 5 Dsys | (Mean±standard deviation) Before Starvation | Part of gastr-ointestinal tract |
|---|---|---|---|
| * | 0.546± 0.04 b A | 1.89± 0.08 A a | Foreground part |
| * | 0.554± 0.04 b A | 1.77± 0.06 a A | Middle part |
| * | 0.04± 0.00 a B | 0.55± 0.02 B a | Back part |

**Table 4:** Effected of the level and treatment of white corn and the section (gastrointestinal tract) The efficiency level of α-amylase enzyme.

| Signif-icantly level | Control without White corn | The efficiency, Unite/mg protein (after 30 days from the start nutrition) | | | | | | The part of gastroin-testinal tract |
|---|---|---|---|---|---|---|---|---|
| | | White corn treatment by enzyme after milling | | White corn treatment by enzyme before milling | | White corn without treatment | | |
| | $T_7$ | $T_6$ | $T_5$ | $T_4$ | $T_3$ | $T_2$ | $T_1$ | |
| * | 1.498±0.07aA | 1.498±0.05aA | 1.511±0.02aA | 1.501±0.02aA | 1.397±0.05aA | 1.156±0.03bA | 1.146±0.05Ab | Foreground part |
| * | 1.476± 0.05Aa | 1.487±0.03Aa | 1.498±0.02Aa | 1.499±0.02Aa | 1.445±0.05aA | 1.149±0.05Ab | 1.158±0.07bA | Middle part |
| * | 0.432±0.03Bb | 0.522±0.06Bb | 0.501±0.04Bb | 0.533±0.04Bb | 0.543±0.03Bb | 0.345± 0.04Bb | 0.364±0.05Bb | End part |

the mucus membrane (Hoehne *et al*., 2001). This explains the distribution of the enzyme along the gastrointestinal tract in this study. The results of this study were similar to the results of the study (Miriam *et al*., 2002) when starving and re feeding of the sturgeon *Acipenser naccric* and trout *Oncorhynchus mykiss* that the effectiveness of the enzyme was spread along the digestive tract and the highest value was in the foreground and middle of the digestive tract. The results of the current study (Chakarabarti *et al*., 2006) confirmed that the enzyme amylase is spread along the gastrointestinal tract of the silver carp *Hypophthalmichthys molitrix* and higher in the front and middle part. In his study, the researcher noted the prevalence of the digestive enzymes of *C. carpio* L. along the gastrointestinal tract at higher rates in the foreground and middle part of the gastrointestinal tract compared with the end part.

The effect of replacing white corn with yellow corn in this study when feeding common carp *C. carpio* L. on diets containing different percentages of white corn, there was a significant difference $P \leq 0.05$ in the enzyme efficiency values of the first and second treatments with all other treatments and the control treatment (Table 4). This may be due to equal the carbohydrate ratios (Table 2), that support the α-amylase activity depend on carbohydrate levels in general and starch in particular. The results of this study were confirmed by (Salah *et al*., 2016) when they studied the effectiveness of α-amylase for three species of fish, *Lucius vorax* (carnivores) and *Liza abu* (omnivores) and blue tilapia *Oreochromis aureus* (herbivores) in southern of Iraq (Hor al-Jabbaysh) that the efficiency of α-amylase was clear in tilapia about other fish because it was a herbivores. In their study of the functions of digestive enzymes and their relationship with food in the silver carp *Hypophthalmichthys molitrix* and big head carp *Hypophthalmichthys nobilis*, the enzyme was the highest in fish fed on high carbohydrate diets. The (Baohua *et al*., 2009) explained when feeding common carp *C. carpio* L. on three diets containing 35% protein supplemented with 1.5, 2.1 and 3.2 mg/kg of xylolgo saccharides (XOS) for three treatments, the efficiency of amylase was significantly higher when addition 3.2 mg/kg of XOS compared to the control treatment without supplementation XOS. The efficiency of α-amylase did not have any significant effect when substituting soybeans with grass pea seed *Lathyrus sativus* L. which heat-treated in common carp diets and may be due to the approximate carbohydrate ratios in the used diets (AL-Gharrawi *et al*., 2016).

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