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**EFFECT OF DIFFERENT LEVELS OF PROTEIN COMBINATIONS  
AND PROTEIN SOURCES ON SOME BIOCHEMICAL PARAMETERS  
OF TILAPIA (*Oeochromis niloticus*) AND COMMON CARP  
(*Cyprinus carpio*) LIVERS**  
(With Two Tables)

By

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تأثير نسب ومصادر البروتين على بعض الدراسات الأيضية في كبد  
أسماك البلطي والمبروك

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أُستخدِمت أربعة علائق تحتوي على تركيبات مختلفة من البروتين وهي ٢٥ و ٣٠ و ٣٥ و ٤٠ % وذلك لمعرفة تأثير تركيز البروتين في العليقة على محتوى كبد أسماك البلطي والمبروك من البروتين والدهون والفوسفوليبيدات والكوليسترول . ولقد تبين أن زيادة مستوى البروتين في العليقة صاحبه زيادة معنوية بمحتوى الكبد في كل من البروتين والفوسفوليبيدات والكوليسترول بينما نقص محتواه من الدهون الكلية ولم يتأثر معامل تزنج الدهون . كما أُستخدِمت أربعة علائق ذات محتوى بروتيني ثابت وذلك لدراسة تأثير المصادر المختلفة للبروتين على محتوى كبد أسماك البلطي والمبروك من البروتين والدهون والفوسفوليبيدات والكوليسترول وكذا معامل تزنج الدهون . ولقد أظهرت النتائج أن هذا التأثير كان واضحاً على محتوى الكبد من الكوليسترول . وقد أعطيت العليقة المكونة من كسب بذرة القطن ومحقق اللحم أعلى تركيز . كما دلت النتائج أيضاً على أن هناك زيادة في بعض القيم الأيضية في أكباد أسماك البلطي عن أسماك المبروك .

### SUMMARY

Four protein levels (25, 30, 35 and 40%) were used for Tilapia and Common Carp feeding. The results showed that Tilapia fish contained higher total lipids and cholesterol levels than Carp fish. The increment of protein level of the fish ration raised up their total proteins phospholipids and cholesterol and lowered the total lipids contents of both Tilapia and Common Carp fish. The data also revealed that the four formulated diets affected the cholesterol content of both Tilapia and Carp fish livers. The formulated diet (cottonseed cake-meat maeal) gave the highest cholesterol level in the livers of the two examined species. The results also indicated some differences between the two tested species in some parameters which were higher in Tilapia than Carp.

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## INTRODUCTION

The present shortage of animal protein in Egypt is due to the increasing of nation's population while the production of animal proteins can not cover the country's requirements. The fish production accounts for 20% of the protein requirements of Egypt which is extremely low comparing with other countries. The increasing of fish production is one of the main objective of the government to cover the existing gap between production and consumption of animal proteins (ABOU AKKADA and NOUR, 1988).

Fish meal, cottonseed meal, soybean meal and blood meal are traditionally used for feeding monogastric farm animals and believed to be suitable for fish feeds (LIING, 1967 and RANDALL, 1977). Fish meal supply the major protein of the protein in commercial fish rations. It is considered the best protein source in fish feed (TACON et al., 1985). FLOWER and BANKS (1976) mentioned that the rising cost and uncertain availability of fish meal have made it necessary to reduce fish meal content in fish diets. This had led to a search for suitable replacements for fish meal protein sources. They illustrated meat meal is frequently used as an animal protein source in compound fish feed manufacture, although its feed value is generally considered inferior to that of soybean and fish meals. In comparison with meat meal, FOWLER and BANKS (1976) revealed the superiority of blood meal in test diets for salmon.

HEPHER et al. (1970) found that diets containing 22% fish meal were better than those containing less amounts and growth performance of fish were greater on fish meal than other proteins. MACHIELS (1987) reported that fish weight gain decreased when an increasing part of fish meal was replaced by alternative protein sources (blood meal, casein, groundnut, cottonseed and soybean). The same results were confirmed by OMAR (1987) who reported a specific growth rate/day for Tilapia 6.42, 4.79 and 2.56% of fish meal, meat meal and blood meal diets respectively.

Soybean meal is a good source of essential amino acids and is one of a very plant sources rich in lysine. Therefore considerable work has been carried out on the extent of fish meal replacing with processed soybean products as a protein source in fish diets (REINITZ, 1980). VIOLA and ZOHAR (1984) illustrated that cottonseed meal is not commonly used as a protein source in fish feeds. Although it has a high protein content it is rather lacking in lysine, thus it should be used in combination with other materials such as soybean and animal protein sources. They used 20% cottonseed meal supplemented with 7.5% oil replacing by 12.5% fish meal and 15% sorghum grains for Tilapia and carp fish feeding and they found the same growth rates and body composition.

The present work was conducted to study the effect of different levels of proteins from different combinations (plant and animal proteins) and different protein sources at the same level (30% crude protein, the optimum level for maximum growth as obtained by HASSAN, 1989) on some biochemical parameters of tilapia and common carp livers.

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## MATERIAL and METHODS

**Materials:**

**Fish:** Two separated experiments were carried on tilapia (Oreochromis niloticus) [N 40 g weight] and common carp (Cyprinus carpio L) [60 g weight] at the experimental fish farms, Faculty of Agriculture, water was obtained from the irrigation canal (a branch of El Mahmodia Canal). Water temperature ranged from 23.5 to 29.5°C, dissolved oxygen ranged between 5.5 to 8.7 ppm, total alkalinity 283-294, chlorosity 1.5-2 g/l and pH 7.6 : 7.8.

**Experimental net enclosures:** Thirty six net enclosures were used for rearing fish in the present work. Each measured 100 cm X 100 cm made of cotton net with a mesh size of 100 mm. It fixed with nylon rope on ordinary wood stocks which fixed on the bottom sediment of the pond.

**Feeding Regime:** Fish in all nets were fed the experimental diets (two net enclosures for each diet) for 112 days on 3% of the total biomass of fish daily. Two experiments were conducted as the following:

1- Effect of different levels of protein combinations (plant and animal proteins) on some biochemical parameters in tilapia and common carp livers. The two species were separately fed on four tested diets contained 25, 30, 35 and 40% protein from a combination of fish meal, meat meal, soybean meal and cottonseed meal.

2- The effect of different protein sources at same level of protein on some biochemical parameter of tilapia and common carp. Four diets contain (30% CP) were formulated from the following combinations:

- a. Soybean meal and fish meal.
- b. Soybean meal and meat meal.
- c. Cottonseed cake and fish meal.
- d. Cottonseed cake and meat meal.

The control in each experiment was of two groups of fish reared on the natural feed without any artificial feeding.

The different treatments were collected and brought alive to the laboratory in nylon bags under continuous aeration. The apparent examination showed that all fish samples were somatically healthy and parasite free. The fish were kept alive in well aerated free running tap water aquarium (18-20°C) until used. Twenty five liver samples were used for each species.

**Methods:**

The fish samples were killed by vertebral rupture, then the livers were rapidly removed. The livers were cleaned from accessory connective and adipose tissues and weighted. A part of the liver tissues was homogenized with 0.64% sodium chloride for total protein and lipid peroxidation determinations. The retained part was homogenized with mixture of chloroform and methanol for the other determinations.



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**Total proteins:** The methods of LOWERY et al. (1951) was followed. Bovine serum albumin was used for calibration curve preparing. Total lipids were determined according to the method of FOLSH et al. (1957), using a mixture of chloroform and methanol (2:1 v/v) as an extractant. Phospholipids were colourimetrically determined according to the method described by KATES (1972) who precipitated the proteins with trichloroacetic acid and digested the precipitate containing the phospholipids with sulphuric-perchloric acids mixture. The developed blue colour was measured after 15 minutes at 700 nm using spectronic 20 Bush & Lamb.

**Cholesterol:** The method mentioned by ZLATKIS et al. (1953) was followed. The reagent (Conc. sulfuric acid and ferriochloride) was added to the tube containing the tested samples with glacial acetic acid. The developed violet colour was measured after 20 minutes at 570 nm using Spectronic 20 Bush & Lamb.

**Lipid peroxidation:** The malondialdehyde (MDA) was used as an indication for lipid peroxidation. It is determined by the method described by PLACER et al. (1966). A calibration curve was prepared by using malondialdehyde-diethylacetate (Merck, F.R.G.).

**Statistical analysis:** The data of the present work were subjected to the analysis of variance as calculated by SNEDECOR and COCHRAN (1967).

## RESULTS

Are presented in tables 1 & 2.

## DISCUSSION

Table (1) shows the effect of different protein levels in diets on some metabolic parameters in the livers of tilapia and common carp. Concerning the effect of species on the level of some metabolites, the results showed that the protein and phospholipids contents of the livers of tilapia and common carp fish were not significantly differed, however significant differences were found in the total lipids, cholesterol and lipid peroxidation.

The protein contents of the livers of the two examined fish species were gradually increased with the increase of the protein ration. The raising up of protein ration caused highly significant increment of phospholipids and cholesterol levels of the livers of tilapia and carp fish, however the total lipids of livers of examined fish were significantly decreased.

The data also revealed different correlations between the total proteins and the lipid characteristics. Positive correlations between total proteins and both of phospholipids and cholesterol were found ( $r = 0.42$  and  $0.44$ , respectively). However, the correlations between the total proteins and each of total lipids and lipid peroxidation are negative ( $r = -0.39$  and  $-0.64$ , respectively). On the other hand the total lipids

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are positively correlated with lipid peroxidation ( $r = 0.64$ ). It was also found a negative correlation between total lipids and phospholipids contents ( $r = 0.75$ ). These relations are due to the high amounts of amino acids in the high protein diets, which in turn increase the structural lipids (phospholipids and cholesterol) and prevent the degradation of their fatty acids (CHAMPAN and WALLACH, 1968 and BITTMAN and BLAU, 1972).

The results of the effect of the different protein sources on the protein content and lipid characteristics of tilapia and carp fish livers are shown in (Table 2). The different protein sources had no significantly effect on the total proteins, total lipids, phospholipids contents and also on the lipid peroxidation. Only they had significant effect on the cholesterol content comparing with the standard and the examined rations. The cottonseed meal meat admixture gave the highest cholesterol contents of the livers of both tilapia and carp fish. The data also show that tilapia fish fed on the different protein sources had significantly higher phospholipids and cholesterol contents than carp fish.

**In conclusion:** The raising up of protein level in fish diet affected the protein content and lipid characteristics of the livers of tilapia and carp fish which in turn affect their growth rate. On the other hand the variation in protein source of fish diet has affected on the cholesterol content.

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Table 1: Effect of the different protein levels on the protein content and lipids characteristics of the livers of Tilapia and common carp fish.

Species Treatment	Total proteins g/m %			Total lipids g/m %			Phospholipids g/m %			Cholesterol g/m %			Lipid peroxidation mM/g		
	Tilapia	Carp	Mean	Tilapia	Carp	Mean	Tilapia	Carp	Mean	Tilapia	Carp	Mean	Tilapia	Carp	Mean
Control.	5.06	5.75	5.41 <sup>b</sup>	15.84	12.90	14.37 <sup>a</sup>	0.559	0.494	0.527 <sup>c</sup>	0.066	0.038	0.052 <sup>c</sup>	60.37	37.29	48.83
25% Protein.	6.14	5.79	5.97 <sup>b</sup>	14.69	11.41	13.05 <sup>ab</sup>	0.694	0.610	0.652 <sup>bc</sup>	0.084	0.049	0.067 <sup>b</sup>	47.13	33.10	40.12
30% Protein.	6.24	5.85	6.05 <sup>b</sup>	12.63	9.17	10.90 <sup>cb</sup>	0.797	0.632	0.715 <sup>b</sup>	0.091	0.050	0.070 <sup>b</sup>	45.22	32.30	38.76
35% Protein.	6.94	5.88	6.41 <sup>b</sup>	10.55	8.50	9.53 <sup>dc</sup>	0.804	0.762	0.783 <sup>b</sup>	0.094	0.059	0.077 <sup>b</sup>	34.41	27.78	31.10
40% Protein.	7.69	7.44	7.57 <sup>a</sup>	8.04	7.97	8.01 <sup>d</sup>	0.945	0.974	0.960 <sup>a</sup>	0.119	0.095	0.107 <sup>a</sup>	33.96	27.28	30.62
Mean.	6.41	6.14		12.35	9.99		0.760	0.676		0.091	0.058		44.22	31.55	
F Calculated for Treatment.	9.07				13.74			18.48			33			4.81	
Species.	1.31				14.35			6.44			108			17.2	

P Table:  
for treatment  
at P > 0.05 = 6.39.  
at P > 0.01 = 15.94.

for species:  
at P > 0.05 = 7.71.  
at P > 0.01 = 21.2.



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Table 2: Effect of different protein sources on the protein content and lipid characteristics of the livers of Tilapia and Common Carp Fish.

Treatment	Species		Total proteins		Total lipids		Phospholipids		Cholesterol		Lipid peroxidation				
	<i>g/m %</i>	<i>g/m %</i>	<i>g/m %</i>	<i>g/m %</i>	<i>g/m %</i>	<i>g/m %</i>	<i>g/m %</i>	<i>g/m %</i>	<i>g/m %</i>	<i>g/m %</i>	<i>nm/g.</i>				
	Tilapia	Carp	Mean	Tilapia	Carp	Mean	Tilapia	Carp	Mean	Tilapia	Carp	Mean			
Control.	5.06	5.75	5.41	15.84	12.90	14.37	0.559	0.494	0.527	0.066	0.038	0.052 <sup>b</sup>	60.37	37.29	48.83
Soybean meal fish meal.	5.39	6.74	5.93	14.50	11.41	12.96	1.158	0.515	0.837	0.054	0.043	0.049 <sup>b</sup>	21.76	14.23	18.0
Soybean meal meat meal.	6.61	3.67	5.14	15.16	12.11	13.65	1.147	0.328	0.738	0.060	0.035	0.048 <sup>b</sup>	30.30	28.08	29.19
Cottonseed cake fish meal.	9.68	7.19	8.44	15.50	15.17	15.34	1.183	0.596	0.890	0.070	0.064	0.067 <sup>b</sup>	31.90	23.38	27.64
Cottonseed cake meat meal.	5.23	4.31	4.77	18.53	19.69	19.11	1.137	0.554	0.846	0.096	0.092	0.094 <sup>a</sup>	33.38	45.15	39.27
Mean.	6.39	5.53		15.91	14.26		1.037	0.497		0.069	0.054		35.54	29.63	
P Calculated for Treatment.	2.39				6.08		1.07				12.86	*		3.54	
Species.	1.04				3.56		18.29	*			9.17	*		1.11	

P Table :  
for treatment  
at  $P > 0.05 = 6.39$ .  
at  $P > 0.01 = 15.94$ .

for species  
at  $P > 0.05 = 7.71$ .  
at  $P > 0.01 = 21.2$ .