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BIOCHEMICAL INFLUENCES OF CONSUMING SOME TYPES OF NILE AND MARINE FISH IN RATS

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SUMMARY

The aim of the present work was to study the effects of consuming different types of Nile and marine fish on serum lipids profile, heamoglobin, protein metabolism(uric acid,urea albumin globulin ,total protein and creatinine),some electrolytes (sodium and potassium) and some levels of hepatic transaminase and alanine -aminotransferase enzymes activity (AST&ALT) in normal male albino rats, weighing 150-155g.The results indicated that consuming diets contained different types of tested fish especially marine types caused significant hypolipidemic and hypocholesterolemic effects .The cholesterol values significantly reduced by about :36.66%,34.13%,15.69% and 10.73 % for staweridia,mackerel ,mabroka and kisher bayaid respectively ,while the lipids level reduced by about :22.90%,18.93%,18.81% and 4.82% for staweridia,mackerel ,mabroka and kisher bayaid respectively .There were no significant difference in serum heamoglobin,tested electrolytes and tested protein metabolites measurements.

INTRODUCTION

Fish meal is the most common and useful source of dietary protein and lipid in formulated compound diets (Tacon,1994). Marine foods such as fish and shellfish are the main dietary sources of long chain n-3polyunsaturated fatty acid, such as eicosapentaenoic acid (EPA or 20:5n-3) and docosahexaenoic acid (DHA or22:6n-3) (Nettleton,1991). The n-3fatty acids of fish and fish oil have great potential for the prevention and treatment of patients with coronary artery disease .The n-3fatty acids promote the synthesis of the beneficial nitric oxide in the endothelium. Experiments in humans indicate a profound hypolipidemic effect of fish oil ,especially lowering of plasma triacylglycerol, (Connor and Connor ,1997). Some of fresh water fish oils contain higher 20:4(n-6)and lower 20:5(n-3) than usual marine fish oils,but their effects on tissue fatty acids are not well known, (Innis, et al., 1995). Mori,et al, (1999) showed that adminstration of a daily fish meal into a weigh loss regimen was more effective than either measure alone at improving glucose-insulin metabolism and dyslipidemia. Cardiovascular risk is likely to be substantially reduced in over weight

loss program incorporation fish meals rich in n-3fatty acids .Fernandez,et al., (1999), showed that the consumption of even relatively small amounts of fish is favorable indicator of the risk of severel cancers ,esecially of the digestive tract. Another study by Clemens, (2000) indicated that eating fatty fish once or more each week or supplementing with fish oils (0.5g./day),has been found to increasa the survival of heart attack patients by 29% .Larsen, et al., (2000), suggested that small fish with bone may be an important source of calcium in human diets ,consumption of small fish in population groups with low intakes of milk and milk products should therefore be encouraged. Mollsten, et al., (2001) reported that high consumers of fish protein had lower odds ratio (Ors) for microalbuminuria than in individuals consuming less fish protein. High intake of fish protein and fish fat showed a reduction in the risk for microalbuminuria.Uhe,et al.,(1992) found that the level satiety was significantly greater after fish meal compared with the beef and chicken meals.Taurine concentration were significantly greater after the consumption of the fish meal compared with the beef or chicken meal .This reflects the higher concentration of this non protein amino acids found in fish, 1.5g.compared with 0.23g.in beef and 0.23g in chicken meals .Plasma methionine concentration was also significantly greater after the fish meal was eaten than after consumption of the beef or chicken meal.Krauss,et al., (2000), reported that the beneficial effects to humans of consuming fish ,particularly oily fish such as salamon, herring and mackerel with a high content of the (n-3) highly unsaturated fatty acids (HUFA) have been documented.

Aim of the work : The present study was aimed to investigate the effect of consuming the whole fish of some marine and nile types on serum lipids profile, protein metabolism and some levels of hepatic liver enzymes activity in normal male albino rats.

MATERIALS AND METHODS

I.Materials: Two types of fish were chosen in this study. Marine fish (mackerel&stawredia fish) and Nile fish , (mabroka&kisher bayaid fish) were selected randomly from different markets in Cairo, Egypt.

Preparation of samples: The fish was washed with tap water, the heads, tails and vessels of fresh fish were removed to yield the net meat, then washed again carefully with tap water. The fish meat was cooked in boiling water for 20 min. without salt, then strained and left to stand for 3 hrs in the strainer, and then the bones were removed. Fish were cut into small pieces, spread out in single layer on filter papers and allowed to dry at room temperature for 3 hrs, after that it was dried in preheated air oven with fan at 100°C for 15min., then temperature was lowered at 50°C for 24 hrs. After drying, fish samples were grounded. The dry powder samples of marine fish were soaked in fat solvents mixture (petroleum and diethyl ether a ratio of 1:1 by volume) untill defatted according to Abd-El-Razek, et. al., (1983). Dry powdered from fish samples were kept in plastic containers at 20°C. Biochemical analysis and biological experiment were carried out.

II. Experimental animals design:

Animals:

Thirty male albino rats of Sprague Dowley strain, weighing between 150-155 g. were used. They were bought from Vaccine and Immunity Organization, Helwan Farm, Cairo, Egypt. The rats were divided into five groups, each of six rats. Animals were housed individually in wire cages. Food and water were provided ad-libitum for 4 weeks.

III.Diet:

The diets were prepared using different types of dry fish meal powder, which was added as a protein level at 14% according to (Reeves, et al., 1993) Sunflower oil was added to fish oil to reach 10% in diet depending on the percentage of oil in different types of fish as shown in table (1). All groups fed on the basic diet which contains of 14% protein,10% oil, 3.5% salt mix.,1% vitamine mix.,0.3 D-l methionine and 0.2 choline chloride ,but there are differences in proteins and oils sources- which differ according to types of fish and the percentage of oil in each type. The animals were divided into five groups as the following :

Group (1) : Control:fed on basic diet(14% casein (85% protein)+10% sunflower oil .

Group (2): Fed on 14% mackerl fish protein +10% mackerl fish oil .

Group (3): fed on 14% staweredia fish protein +5% staweredia fish oil +5% sun flower .

Group (4): fed on 14% mabroka fish protein +2% mabroka fish oil+8% sun flower oil .

Group (5):fed on 14% kisher bayaid fieh protein +0.5%kisher bayaid fish oil +9.5% sunflower oil . The diets were completed with starch to reach 100 g.

At the end of experimental period , rats were sacrificed under ether anesthesia. Blood samples were collected from hepatic portal vein; serum was separated by centrifugation at 1300g for 15min, and stored in glass vials at -20°C until analysis.

Table (1): Composition of diets for rats experiments (g/100g diet).

Groups / Ingrediants	(1) Basic diet (control)	Marine fish		Nile fish	
		(2) Mackerl	(3) Stawe-ridia	(4) Mabroka	(5) Kisher bayaid
Procin** (14%) Oil** (10%)	16.67 10g Sunflower oil	28.43 10g Mackerel fish oil	18.59 5g Staweridia fish oil+5g Sunflower oil	17.92 2g Mabroka fish oil+ 8g Sunflower oil	18.19 0.5g Kisher bayaid fish oil + 9.5 Sunflower oil
Salt mix. (3.5%)*	3.5	3.5	3.5	3.5	3.5
Vit. Mix. (1%)*	1	1	1	1	1
D-1 methionine (0.3%)	0.3	0.3	0.3	0.3	0.3
Choline chloride ((0.2%)	0.2	0.2	0.2	0.2	0.2
Starch	68.33	56.57	66.41	67.08	66.81

* Basic diet ,salt mix. and vit .mix .,were prepared according to Reeves,et al.,(1993).
**Protein and oil % of dry fish samples are shown in table (2).

Chemical analysis: Ash contents, fat contents using Soxhelt method and Protein content by Kjeldahl method were determined according to A.O.A.C. (1985).

Biochemical analysis: Biochemecal colormeteric measurents were used the Bio-kits of Biocon made in Germany by using Spectrophtometer, (SPEKOL 11).

Serum triacylglycerol was determined according to Fossati and Principe, (1982). Serum cholesterol was determined according to Thomas (1992). Total lipids were determined by chemical method using sulpho- phosphovanillin reagent according to Knight, et al., (1971). Serum total protein was determined using Biuret reagent according to Josephson and Gyllensward (1975). Serum albumin was determined by Bromcresol green dye using kits according to Doumas and Giggs (1971). Serum uric acid was determined according to Trinder, (1969). Serum creatinine was determined according to Henry, (1974). Blood haemoglobin was determined according to Van Kampen and Zijlstra, (1961). Serum liver enzymes activity , aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Reitman and Frankel (1957). Serum sodium and potassium were determined according to Henry et al., (1974).). These parameters were determined by (kits of Bicon-made in Germany).

Statistical analysis: Analysis was conducted using the one way classification F test and least significant differences (LSD) according to Ronald, (1981).

Results AND DISCUSSION

The chemical composition of fish are presented in table (2). The results showed that marine fish contains high level of fat compared to nile fish, while nile fish contains high level of protein than mackerel. Marine fish contains high level of fat and this results agreement with the findings reported by Abd El Razek et al., (1993).

Serum Lipids Profile:

Table (3) illustrated the effect of different types of fish on serum triacylglycerol, total cholesterol and total lipids. The results showed that there were no significant differences in serum triacylglycerol (TAG) between groups of rats fed on nile and marine types of fish diet when compared with control group.Stawredia (marine fish)and kisher bayaid (nile fish)increased TAG signifcantly higher than mabroka fish,which showed the lowest concentration of TAG.

The level of total cholesterol in rats fed on marine fish was decreased more than those fed on nile fish , and the differences were significant at $P< 0.01$. The reduction of cholesterol was 34.14%, 36.67%, for marine fish (mackerel& staweridia)and 15.69,10.73% for nile fish (mabroka &Kisher bayaid) respectively compared with control group.

Fish oil have a great effect on lowering TAGs.Consumption of fish diet induced a highly significant ($p<0.01$) fall in TAGs level and a strong decrease was observed by Von Lossonczy et al., (1978) .Treatment with fish oil showed a re-

duction in serum fasting TAGs 30-40% consistent with that in hyperlipidemic humans (Harris, 1989). An inhibition of hepatic fatty acid synthesis by EPA and DHA and impaired TAGs synthesis (including very low density lipoprotein assembly and secretion) are among some of mechanisms proposed for the plasma TAGs lowering effect of dietary fish oil (Harris, 1989; Yeo and Holub, 1990). The beneficial effects to humans of consuming fish, particularly oily fish such as salamon, herring and mackerel with a high content of the n-3 highly unsaturated fatty acids (HUFA), eicosapentaenoic acid (EPA, 20:5 (n-3) and docosahexaenoic acid (DHA, 22:6(n-3)), have been well documented (Krauss, et al., 2000). Arachidonic acid is a long-chain n-6 PUFA that is found in meat, fish and plants or is synthesized from linoleic acid. Arachidonic acid and marine lipids both serve as key intermediates for eicosanoids like thromboxanase and prostacyclins, which are important for platelet and vessel wall physiology,(American Heart Association, "A.H.A". 1996). Sugano et al., (1982), showed that serum TAG levels tended to rise with increasing the amount of lysine supplementation. The opposite tends was obtained with arginine supplementation, where as arginine/ lysine was more effective in regulation serum TAG than serum cholesterol..

The reductions of cholesterol values by marine fish consumption may be due to its high content of marine oil, which contain large amounts of w–3 fatty acids, while Nile fish contain high level of protein. Gibney, (1983) found that animal protein was generally considered to be of more cholesterolemic effects than vegetable protein, and the amino acids composition may be responsible for the different response of serum cholesterol, Nagaoka et al., (1990), showed that excess dietary intake of tyrosine, caused hypercholesterolemia. Singer et al., (1985), found that serum TAGs and total cholesterol were significantly decreased by 28% and 9% respectively after mackerel diet, these results are in agreement with previous finding (Bronsgeest et al., 1981 and Mortensen et al., 1983), and increased HDL cholesterol was found by (Saynar and Gillott., 1981). Several marine fish species are rich in n-3 PUFA such as EPA or DHA. This is attributed to the lipid composition of plankton. There is a strong evidence suggesting that consumption of fish containing high levels of these fatty acids is favorable for human health and has a particularly beneficial effect in preventing cardiovascular diseases. Fresh water fish species can also serve as a valuable source of essential fatty acids. Fresh water fish contains, in general,higher level of C-18 polyunsaturated fatty acid (PUFA) compared with marine fish species. Therefore the ratio of total n-3 to n-6 fatty acids is much lower for fresh water fish than for marine fish, ranging from 1 to about 4 and can be beneficial for human nutrition (Steffens, 1997). The daily fish consumption and increased the concentration of n-3 PUFAs lowered serum TAGs and total cholesterol levels (Torres, et al., 2000). Das, (2000), showed that low rates of coronary heart disease was found in Greenland Eskimos and Japanese who are exposed to a diet rich in fish oil. They suggested mechanisms for this cardio protective effect focused on the effects of n-3 fatty acids on eicosanoid metabolism, inflammation,

beta oxidation, endothelial dysfunction, cytokine growth factors, and gene expression of adhesion molecules, but non of these mechanisms could adequately explain the beneficial actions of n-3 fatty acids. One attractive suggestion is a direct cardiac effect of n-3 fatty acids on arrhythmogenesis. n-3 fatty acids can modify Na+ channels by directly binding to the channel proteins and thus, prevent ischemia-induced ventricular fibrillation and sudden cardiac death. Studies by Malasonos and Stacpoole (1991) showed that the biological effects of ω -3 fatty acids in diabetes mellitus had a great effect for reducing serum lipids. Kowale and Misra (1976) found that quality and quantity of protein may influence the concentration of lipid fraction in serum. Iritani et al., (1979), suggested that shellfish (Carbicula Japonica meat) is a hypolipidemic food, where as serum and liver TAGs levels significantly reduced by feeding Carbicula Japonica meat.

Marine and Nile fish resulted in a significant reduction in total lipids at $P < 0.05$ level compared with control group. These reductions were 18.93%, 22.90% , 18.81% and 4.82 % for Mackerel, Staweridia , Mabroka and Kisher bayaid fish, respectively.It was found that Kisher bayaid recorded high value of total lipids (290mg/dl) when compared with other types.

Serum Protein Metabolism:

The effect of different types of fish studied on blood heamoglobin and serum protein metabolism are shown in table (4). It was found that consumption of both marine and Nile fish studied had no effect on blood heamoglobin levels. Also there were no significant differences on serum protein metabolism (uric acid, urea, albumin, globulin, total protein and creatinine) between groups fed on different types of fish studied when compared with control group. Serum creatinine level showed slightly increase in group of rats fed on staweridia fish diet (0.90±0.19) and the lowest value showed in group of rats fed on kisher bayaid (0.59±0.07).

Table (2): Proximate analysis of dry fatted fish samples g/100g.(mean ± SE).

Parameters	Marine fish		Nile fish	
	Mackerl	Stawridia	Mabroka	Kisher bayaid
Protein	49.24 ± 1.54	75.30 ± 1.60	78.13 ± 0.89	79.98 ± 0.98
Fat	43.61 ± 0.57	18.27 ± 0.42	11.25 ± 0.24	2.43 ± 0.07
Ash	2.86 ± 0.19	3.63 ± 0.01	3.45 ± 0.08	3.19 ± 0.14

Table (3) The effect of different types of fish (marine &nile) on serum triacylglycerols, total cholesterol and total lipids (Mean±SE mg/dl).

Parameters	Control	Marine fish		Nile fish		P<0.05
		Mackerl	Stawredia	Mabroka	Kisher bayaid	
Triacyl-glycerols	36±2.83	36.02±3.61	42.52±3.28	32.88±2.74	42.39±0.93	8.265
Total cholesterol	163*.58±2.27	107.74*±6.07	103.60±7.30	137.92*±5.22	146.03*±3.4	15.601
Total lipids	304.69±20.98	247*3.72	234.91*±8.81	247.37*±13.16	290*±5.81	35.421

Table (4) :The effect of different types of fish (Marine and Nile) on blood heamoglobin and some serum protein metabolism parameters.(The data presented are mean values ± SE).

Parameters	Control	Marine fish		Nile fish		P<0.05
		Mackerl	Stawredia	Mabroka	Kisher bayaid	
Heamoglobin (g/dl)	13.90±0.84	16.71±1.64	13.63±0.86	16.84±1.36	15.41±2.15	N. S.
Uric acid (mg/dl)	1.70±0.28	1.45±0.15	1.74±0.38	1.80±0.46	1.86±0.17	N. S.
Urea (mg/dl)	26.98±1.93	21.43±1.88	26.45±2.16	22.95±2.12	22.89±1.93	N. S.
Albumin (g/dl)	3.1±0.24	3.76±0.35	3.55±0.51	3.47±0.51	3.64±0.30	N. S.
Globulin (g/dl)	3.26±0.72	2.65±0.67	2.94±0.39	1.82±0.77	3.57±0.78	N. S.
Total protein (g/dl)	6.36±0.64	6.42±0.89	6.49±0.53	5.29±0.88	7.20±0.90	N. S.
Creatinine (mg/dl)	0.69±0.03	0.76±0.08	0.90±0.19	0.76±0.08	0.59±0.07	0.276

Table (5): The effect of different types of fish on serum sodium and potassium content (The data presented are mean values mean ± SE).

Parameters Meq/L	Control	Marine fish		Nile fish		P<0.01
		Mackerl	Stawredia	Mabroka	Kisher bayaid	
Sodium	128.36±4.40	119.155±1.12	124.65±3.99	121.64±4.32	118.93±5.44	N S
Potassium	2.995±0.12	3.145±0.20	3.18±0.26	2.86±0.25	2.81±0.09	N S

Table (4), declains that consumption of both marine and Nile fish studied had no effect on blood heamoglobin levels and serum protein metabolism .The results are agreement with Singer et al., (1985), who reported that serum uric acid and creatinine remained unchanged after Mackerel or Herring diet.

Serum Electrolytes (Sodium & Potassium):
The effect of different types of fish (marine and Nile fish) on serum electorlytes sodium and potassium levels is presented in table (5). It is clear that there were non-significant differences in serum sodium and potassium values between all groups fed different types of fish compared to control group.

These results may be due to the short period of the experiment. The results agreed with that of Singer et al., 1985, who reported that blood pressure remained unchanged after mackerel or herring diet ,Connor and Connor, (1997), showed that fish oil has a mild blood pressure-lowering effect in both normal and mildly hypertensive individuals. Also studies by Connor (1995), found that fish oils exert their protective effect by lowering blood pressure. Blood pressure of spontaneously hypertensive rats fed a diet supplemented with 10% silver carp oil for eight weeks was lower than in the control group (Wirth et al., 1990). In a clinical test, 14 hypertensive patients were put in a two diet of 100 g silver carp meat/ day and Mackerel. The results showed a significant drop in systolic blood pressure and diastolic blood pressure in patients were put in two diets (Wirth et al., 1990a; Steffens , 1997). Demaison et .al., (2000), found that fish oil preventing hypertension and cardiovascular diseases.

Liver enzymes activity (AST&ALT): Table (6), represented the effect of different types of fish consumption on serum enzymes activity, (AST & ALT). This table showed that liver enzymes activity recorded highly significant decrease for AST enzyme in groups fed on different types of marine or nile fish ,when compared with control group . For ALT enzyme the results indicated that there were non-significant difference between groups of rats fed mackerel, mabroka and control group. On the other hand group of rats fed on staweridia and Kisher bayaid recorded significant low level (8.88u/l and 8.83 u/l) compared with control group (12.85 u/l).

Table (6): The effect of different types of fish (marine and Nile fish) on liver enzymes activity ALT & AST (The data present ed are mean values ±SE.).

Liver enzymes u/I	Control group	Marine fish		Nile fish		P<0.05
		Mackerl	Stawredia	Mabroka	Kisher bayaid	
AST	33.86±2.05	16.36±1.3	17.87±1.3	18.75±1.9	15.70±1.3	4.56
ALT	12.85±0.88	12.81±0.97	8.88±1.64	11.34±0.45	8.83±1.39	2.60

CONCLUSION:

1. Nile fish contains protein more than marine fish.
2. Marine fish contains fish oil more than nile fish.
3. Marine and nile fish had hypocholesterolemic and hypolipidemic effects, and marine fish showed more effect.
4. Nile and marine fish had no effect on serum protein metabolism.

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