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Diet effects of local and Thai climbing perch, *Anabas testudineus* on plasma biochemical in rats

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Abstract
Local and Thai climbing perch, *Anabas testudineus* are known as koi. These two are important natural and cultured fish, which are available in Bangladesh. This study was carried out to determine the comparative diet effects of local and Thai *A. testudineus* on plasma biochemical in rat. The feeding of rats with 5% powder of local and Thai climbing perch slightly increased the plasma total cholesterol (TC) level by 3.69% and 7.13%, triglyceride (TG) level by 7.2% and 7.95%, LDL/HDL ratio by 0.44 and 0.58 and plasma glutamate piruvate transminase (GPT) level reduced by 5.07% and 7.31% respectively. The effect of local and Thai koi on lipid profile in rats was not significant. However, it had no significant effect on other blood biochemical parameters of liver and kidney functions though Thai variety contain more amount of lipid than local variety. The present study reveals that feeding of 5% fish flesh does not have detrimental effects on the liver and kidneys rather may provide health benefits for the cardiovascular-related complication by decreasing the atherogenic lipid profiles.

Keywords: *Anabas testudineus*, hypercholesterolemic rats, lipid profile, liver and kidney function

1. Introduction
Anabas testudineus, belongs to the order perciformes and family anabantidae [1]. Fish is the major protein source in the diet of the Bangladeshi people. Fish contributes about 60% of the available protein in the diet and the rest 40% protein comes from livestock and poultry. It indicates the importance of fish in contributing to the level of nutrition of the people of Bangladesh [2]. Local koi was more carnivor having comparatively wider mouth gap, larger premaxilla, maxilla and dentari, long oesophagus, short stomach, lesser length of intestine, thus noticed higher amount of protein than Thai koi. Again Thai koi showed two times greater value of intestine having too long folded villi that indicate more absorptive capacity of intestine of the variety than local koi. Thus it showed having greater value of minerals from artificial feeding [3]. Nutritionally both of the varieties are valuable food items with high levels of protein, lipid and minerals. Both the fishes might be good nutrition source for weak and convalescent patients [3].

The most important food components that help to reduce serum cholesterol is polyunsaturated fatty acid (PUFA). Arachidonic acid exacerbates platelet functions, whereas linolenic acid (LNA) acts as a precursor of the physiologically important PUFA, such as eicosapentaenoic acid (EPA; C20:5, ω-3) and docosahexaenoic acid (DHA; C22:6, ω3). There is a considerable data supporting the hypothesis that the health benefit obtained through the lowering of blood cholesterol may be derived from the effects of EPA and DHA [4]. In addition to their roles in the development and function of the central nervous system, these two fatty acids play important role in the physiological functions of the cardiovascular system. Therefore the present study was to generate awareness of the beneficial effects of local and Thai climbing perch (koi), *A. testudineus* on plasma biochemical in rats with a notion to get an over all ideas on other warm blooded animals including human.

Due to the increasing frequency of antihyperlipidemic drug and their common side effects, there is a need to identify natural products with few or no side effects. So, there is a continuous development for highly effective natural ingredients from food, such as fish, that can decrease hyperlipidemia [5]. Previous studies showed that GOT and GPT are typically elevated following cellular damage as a result of enzymes leakage from the cells into blood [6]. Al-Amoudi and Abu Araki [7] found that due to hyperglycemia, the GOT, GPT, and ALP measures increased. However, feeding the diabetic rats with diets containing fish oils reversed these changes.

Maximum reduction of GOT, GPT, and ALP as compared to the control (+) group was found in the thyme oil, organic olive oil, and cod liver oil groups; and in fact, the GOT, GPT, ALP values for the thyme oil group were even lower than those recording for the control (−) group. This finding directly implies that this treatment strategy is highly efficacious in not only ameliorating but even curing the side effects of hyperglycemia concerning liver dysfunction. Therefore, the decreased enzyme activities resulting from treatment with fish flesh might prevent oxidative damage, thus reducing hyperlipidemia.

2. Materials and Methods

2.1 Animals

Fifteen young Long Evan rats (114 ± 12.2 g, 6-week old) were used in the present study. All animals were acclimated to the animal room for 1 week. The rats were housed in an animal room at 23 ± 2 °C under a 12 hours dark-light cycle and relative humidity of 50-60%. Then, they were divided into three feed groups: a basal diet, control group (CG); basal diet with 5% local koi powder (LFDG); basal diet with 5% Thai koi powder (TFDG).

2.2 Diet composition and feeding

The composition of the basal diet was as follows (g/100 g). Wheat flower 50, rice powder 11, wheat bran 19, casein (non fat) 8, egg white 10, soybean oil 1, table salt 0.5, vitamin mixture 0.25 and mineral mixture 0.25. The composition of the vitamin mixture in the diet was as follows (gram/100g vitamin mixture): retinyl acetate 9.5 × 10-4, cholecalciferol 1.2 × 10-3, α-tochoferol acetate 0.05, thiamin hydrochloride 2.4, nicotinic acid 12, riboflavin 2.4, D-calcium pantothenate 9.6, pyridoxine hydrochloride 1.2, folic acid 9.5 × 10-2, vitamin K 0.25, cyanocobalamine 9.5 × 10-3, inositol 47.95 and ascorbic acid 24.0. The composition of the mineral mixture added to diet was as follows (g/100 g of mineral): calcium gluconate 28.5, K2HPO4 17.3, CaCO3 26, MgSO4 12.6, KCl 12.6, CuSO4 0.06, FeSO4 0.3, MnSO4 0.55, NaF 2.5 × 10-4, KI 9 × 10-4, sodium molybdate 3 × 10-4, SeO2 3 × 10-4 and CrSO2 1.5 × 10-3. Rats were feed for 40 days.

2.3 Collection of samples

Samples of local and Thai variety of *A. testudineus* were collected from the different fish market in and around Savar, Dhaka. A total of 30 mature specimens were collected from each variety. After collecting the specimens, the fishes were brought at the laboratory of Limnology and Fishery Sciences, Department of Zoology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh. Colected fish flesh was dried in sunlight and crushed into powder. The powder was mixed with the basal diet.

2.4 Plasma TC estimation

TC was measured enzymatically with a diagnostic kit (CHOLESTEROL: Enzymatic, liquid, Colourimetric test-CHOD/PAP method/ Crescent Diagnostics, Saudi Arabia), using the cholesterol oxidase assay [8]. In this method, cholesterol esterase (ChE) first catalyzed the hydrolysis of cholesterol, which was oxidized by cholesterol oxidase (ChO) to yield hydrogen peroxide. In a coupled reaction catalyzed by peroxidase (POD), quinoneimine dye (red) was formed from hydrogen peroxide, 4-aminoantipyrine and phenol. The absorbance of the dye was measured spectrophotometrically at 546nm, which was directly proportional to cholesterol concentration.

2.5 Plasma TG estimation

TG was measured enzymatically with a diagnostic kit (TRIGLYCERIDE: Enzymatic, liquid, Colourimetric test-GPO-PAP method/ Crescent Diagnostics, Saudi Arabia), using the glycerophosphate oxidase assay [8]. In this method, at first lipase catalyzed the hydrolysis of triglycerides to yield glycerol and free fatty acids. Glycerol concentration was then determined with the Trinder reaction using glycerol kinase, glycerol-3 phosphate oxidase and peroxidase. The end product was the quinoneimine dye (red) of which the absorbance was measured spectrophotometrically at 546nm which was directly proportional to triglyceride concentration.

2.6 Estimation of plasma lipoproteins

High-density lipoprotein cholesterol (HDL) was measured by the same procedure after precipiting low-density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) using magnesium sulfate and phosphotungstic acid, using test kits (Cholesterol, HDL-Cholesterol, Triglycerides/ Crescent Diagnostics, Saudi Arabia).

Low-density lipoprotein cholesterol and very low density lipoprotein cholesterol were calculated, respectively as follows [8].

LDL = {TC – (HDL + TG/5)}

VLDL = {TC – (HDL + LDL)}

2.7 Estimation of plasma total bilirubin

Plasma total bilirubin was measured with a diagnostic kit (Total Bilirubin: Colourimetric test- Jendrassic- Grof method/ Crescent Diagnostics, Saudi Arabia). Bilirubin first reacted with diazotized sulphuric acid (DSA) and in the presence of an accelerator (caffeine) forms a red azo dye, the absorbance, which was measured spectrophotometrically at 546 nm which was directly proportional to bilirubin concentration.

2.8 Estimation of plasma creatinine

Plasma creatinine was measured with a diagnostic kit (CREATININE: End point method- Jaffe reaction/ Crescent Diagnostics, Saudi Arabia), using Jaffe reaction [8]. In Jaffe reaction, creatinine reacts with alkaline picrate to produce a reddish-orange colored complex of which the absorbance was measured spectrophotometrically at 520nm, which was directly proportional to the creatinine concentration.

2.9 Estimation of plasma uric acid

Plasma uric acid was measured with a diagnostic kit (URIC ACID: Enzymatic Colourimetric Test, Uricase/PAP method/ Crescent Diagnostics, Saudi Arabia), using uricase method [8]. Uricase catalyzed the oxidation of uric acid to allantoin and H_2O_2. In the presence of peroxidase, H_2O_2 reacted with 4-aminoantipyrine and 3, 5-dichloro-2-hydroxybenzenesulphonate to form a quinoneimine dye of which the absorbance was measured spectrophotometrically at 520nm, which was directly proportional to the uric acid concentration.

2.10 Estimation of plasma urea

Plasma urea was measured with a diagnostic kit (UREA: Enzymatic method- unease/ Barthelot method/ Crescent

Diagnostics, Saudi Arabia), using Barthelot method [8]. In the Barthelot method, after urea hydrolyzed with urease, the ammonium ion formed was reacted with phenol and hypochloride in alkaline medium to form indophenol. Nitropruside was used to catalyze the reaction. The absorbance of the dissociated indophenol, a blue chromogen, was measured spectrophotometrically at 560nm, which was directly proportional to the concentration of ammonia formed from urea.

2.11 Analysis of plasma enzyme profile

Plasma transaminase GPT and GOT activities were determined with diagnostic kits (GPT and GOT Liquicolor Mono, Human GMBH, Germany) using kinetic method [8]. The oxoacids formed in the transaminase reaction were measured by indirectly by enzymatic reduction to the corresponding hydroxyacids, the accompanying change in NADH concentration was monitored spectrophotometrically at 340 nm.

ALP activity was determined with diagnostic kit (alkaline phosphatase Liquicolor Mono, Human GMBH, Germany). ALP catalyzed the hydrolysis of 4-nitrophenyl phosphate, forming phosphate and free 4-nitrophenol, which in dilute acid solutions was colorless but under alkaline condition 4-nitrophenol was converted to the 4-nitrophenoxide ion, which is an intense yellow color. The absorbance of this color compound was spectrophotometrically at 420nm for the determination of plasma alkaline phosphatase activity.

2.12 Statistical Analyses

The statistical programs used were Microsoft Excel and Statistical Program for Social Science (SPSS 11.5). All parameters for inter group differences were analyzed by one-way ANOVA and then post hoc comparisons, LSD (least significant difference) and DMRT (Duncan's multiple range test) at $P \leq 0.05$ level.

3. Result

3.1 Effect of local and Thai climbing perch (koi), *Anabas testudineus* feeding on the body weight of rats

Fish flesh of local and Thai koi feeding rat increased the body weight as compared to control group (basal diet only) rats. Feeding of local and Thai *A. testudineus* increased body weight in experimental rats by 2.32% and 7.94%, respectively (Table 1). The present study indicates that feeding of 5% local and Thai koi increased body weight as compared to control group.

Table 1: Effect of local and Thai climbing perch (koi), *Anabas testudineus* feeding on the body weight of rats

Rat groups	Initial body weight (g)	Final body weight (g)	Gained body weight (g)
CG	101 ± 0.0	192.5 ± 5.7	91.5 ± 4.6
LFDG	101 ± 0.0	198.4 ± 6.7	99.8 ± 4.2
TFDG	101 ± 0.0	200.8 ± 7.5	97.4 ± 4.5

The results are the mean ± SD; CG- Control group; LFDG- Local fish diet group; TFDG- Thai fish diet group.

3.2 Effects of local and Thai climbing perch (koi), *Anabas testudineus* on plasma lipid profile

Plasma lipid profile concentrations in CG, LFDG and TFDG rats after 5% local and Thai koi fish feeding, plasma TC, TG, LDL, and VLDL, in LFDG and TFDG rats increased by 3.8% and 7.7%; 7.8% & 8.6%; 14.8% and 40.7%; and 7.7% and 8.7% respectively compared with levels in CG rats (Table 2). The results of HDL levels of CG, LFDG, and TFDG are almost similar (Fig. 1), whereas, other parameters showed raising trend between the experimental varieties. In CG rats, this ratio increased by 15.2% and 40.7%, respectively compared with LFDG and TFDG rats.

Table 2: Effect of local and Thai climbing perch (koi), *Anabas testudineus* feed on plasma lipid profiles of rats.

Parameters (mg/dl)	CG	LFDG	TFDG
TC	57.3±5.3	59.5±4.3	61.7±5.2
TG	91.4±6.8	98.5±7.6	99.3±8.5
HDL	30.9±4.2	30.5±2.5	30.4±1.9
LDL	8.1±2.7	9.3±1.8	11.4±2.5
VLDL	18.3±1.6	19.7±1.6	19.9±1.6

The results are the mean ± SD; CG- control group; LFDG- local fish diet group; TFDG- Thai fish diet group; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

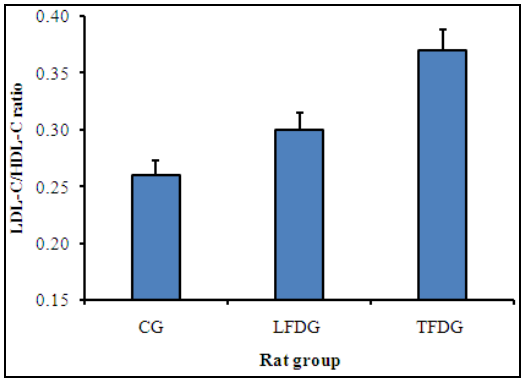

Fig 1: Effect of local and Thai climbing perch, *Anabas testudineus* feed on plasma LDL/HDL ratio of rats. CG- control group; LFDG- local fish diet group; TFDG- Thai fish diet group.

3.3 Effects of local and Thai climbing perch, *Anabas testudineus* on plasma biochemical profile

The results of plasma biochemical concentrations i.e. bilirubin, creatinin, uric acid, and blood urea nitrogen in control group rat (CG), local koi fish diet group rat (LFDG), Thai koi fish diet group rat (TFDG) have been presented in Table 3. Bilirubin, creatinin, uric acid, and blood urea nitrogen contents of LFDG and TFDG were higher as compared to control group rats. Moreover, estimated all biochemical parameters of TFDG rat were slightly increased than LFDG rats.

Table 3: Effect of local and Thai climbing perch, *Anabas testudineus* feed on plasma biochemical profiles of rats

Parameters	CG	LFDG	TFDG
Bilirubin (mg/dI)	0.29±0.07	0.31±0.08	0.33±0.1
Creatinin (mg/dl)	0.55±0.17	0.59±0.17	0.61±0.17
Uric acid (mg/dl)	3.9±0.5	4.2±0.3	4.3±0.3
Urea (mg/dl)	17.8±1.2	18.9±0.9	19.6±1.4

The results are the mean ± SD. CG- control group; LFDG- local fish diet group; TFDG- Thai fish diet group.

3.4 Effects of local and Thai climbing perch, *Anabas testudineus* on plasma enzymes profile

The effects of local and Thai *Anabas testudineus*-feed rats on plasma enzymes profile is shown in Table 4. There was no significant difference in the activities of rat plasma GOT, GPT, and ALP among CG, LFDG and TFDG rats. After 5% local and Thai koi fish feeding rat plasma enzymes profile slightly decreased as compared to control group.

Table 4: Effect of local and Thai climbing perch, *Anabas testudineus* feed on plasma enzyme profiles of rats

Parameters (U/l)	CG	LFDG	TFDG
GOT	37.5±2.7	35.8±3.3	36.2±4.2
GPT	35.2±4.1	33.5±3.6	32.8±3.4
ALP	122.4±6.8	118.6±7.8	120.5±5.6

The results are the mean ± SD. GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; ALP, alkaline phosphatase. lues are mean ± SD of 5 samples

4. Discussion

Feeding of dry fish flesh of local and Thai *A. testudineus* increased body weight in experimental rats by 2.32% and 7.94%, respectively as compared to control group (basal diet only) rats. So, the present study indicates that feeding of 5% local and Thai koi increased body weight as compared to control group. It is generally known that fat diet is one of the major factor causing obesity. The present study indicates that Thai koi diet group of rats increased body weight more as compared to both local koi diet and basal diet group of rats. Protein in fish is a main component of tissue and organ, they are precursors of other nitrogen compounds and constitute an important energy source. Thus, a consistent intake of protein or amino acids is required, since they are continually used by the fish to build new proteins. Inadequate protein levels in the diet results in a reduction of growth and loss of weight [9, 10].

Plasma lipid profile concentration in LFDG and TFDG rats slightly increased as compared to CG rats. Moreover, Thai fish feeding plasma TC, TG, LDL, and VLDL, inreased by 7.7%, 8.6%, 40.7%, and 8.7%, respectively compared with levels in CG rats. The results of HDL levels of CG, LFDG, and TFDG are almost similar. In CG rats, this ratio increased by 15.2% and 40.7%, respectively compared with LFDG and TFDG rats. The increase in plasma cholesterol in rats in the present study was comparable with that reported by Bobek *et al*. [11] Several researchers have suggested that compared with dietary fish protein better reduces plasma cholesterol concentrations in rats [12, 13]. It was proposed that dietary fish oil may interfere with the production of vitamin K-dependent coagulation factors [14]. Earlier, this hypothesis was challenging in animal studies, indicating that fish oil also lowers the vitamin K-independent factor V and, further, that the anticoagulant effect of fish oil is not sensitive to vitamin K depletion [15]. In the present human intervention studies, we again found a reduction in factor V by fish oil, particularly in subjects with high triglycerol concentrations. Despite of this, factor V was not associated with the concentrations of vitamin K-dependent factors (prothrombin, factor VII, and factor X) at baseline, suggesting that its steady-state concentration in plasma is regulated separately. The higher level of plasma HDL indicates that more cholesterol from peripheral tissues was returning to the liver for catabolism and subsequent excretion. Plasma VLDL is the major transport vehicle for the TG from the liver to extrahepatic tissues, whereas LDL is not secreted as such the liver; rather, it seems to be formed from VLDL after partial removal of TG by lipoprotein lipase [4].

Bilirubin, creatinin, uric acid, and blood urea nitrogen contents of LFDG and TFDG were higher as compared to control group rats. Moreover, estimated all biochemical parameters of TFDG rat was slightly increased than LFGD rats. In contrast no significant difference was found for plasma bilirubin, creatinin, uric acid, and blood urea nitrogen among the experimental treatments. The glucose-lowering effect of propionate is associated with gluconeogenesis and the regulation of serum lipid levels [16]. Antonov *et al*. [17] reported that plasma biochemical contents were significantly increased in hypertensive rats. Impaired function of Na, K-ATPase, Na-H antiport, which is typical of arterial hypertension, may promote an increase in plasma electrolytes. The present study reveals that feeding of 5% local and Thai *A. testudineus* dry fish powder does not have detrimental effects on the liver and kidneys rather may provide health benefits for the cardiovascular related complication by decreasing the atherogenic lipid profiles.

After 5% local and Thai koi fish feeding rat plasma enzymes profile slightly decreased as compared to control group. Lower plasma GOT, GPT, and ALP concentrations were observed in LFDG-fed rats than TFDG rats. The feeding of rats with 5% powder of local and Thai climbing perch (koi) showed slightly increased in plasma TC, TG, LDL/HDL ratio

but decreased plasma GPT level. The effect of local and Thai koi on body lipid profile in rats was not found significant. Though, The Thai variety contained more lipid than local variety but it had no significant detrimental effect on other blood biochemical parameters of liver and kidney functions.

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