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Nutritional Evaluation of Chickens Oil and Its Impact on Type-2 Diabetic Marker in Streptozotocin Induced Experimental Mice

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Abstract

The major goals of the poultry industry are to increase the carcass yield and to reduce carcass fatness, mainly the abdominal fat pad. Chicken meat is generally thought to be health friendly due to its favorable fatty acid composition leading to an antiatherogenic lipidemic status. Beneficial effect of the chicken oil on hyperglycemia has also been claimed through its effect on oxidative stress and insulin resistance. The macro and micronutrient contents of these two chicken species (10 chickens of each species) were also compared. All macronutrients such as ash, moisture, total protein, total carbohydrate and total lipid were found to be high for the hybrid chicken. Lipid content was significantly higher (around 50%) in hybrid chicken than that of domestic chicken. Except for potassium and manganese which were found to be high in hybrid chicken (127.8 and 9.74 µ/kg respectively) than domestic chicken (86.83 and 6.74 µ/kg respectively), all other micronutrients such as iron, calcium, zinc and lead were higher for domestic chicken. Zinc content of hybrid chicken was significantly lower (around 50%) than that of domestic chicken. The average amount if oil content of two species were extracted with soxlet apparatus using n-hexane as an extracting solvent and it was found to be 3.54% for domestic chicken and 4.84 for hybrid chicken. Chemical characteristics of the extracted oil were investigated by analyzing various parameters such as iodine value, saponification value, acid value, peroxide value and percentage free fatty acid. Iodine value and unsaponifiable matter were higher for hybrid chicken (77.92 and 11.14% respectively) than those of domestic chicken (60.56% and 3.5% respectively). Again saponification value, acid value, peroxide value and percentage free fatty acid were higher for domestic chicken than hybrid chicken. This experiment also showed significantly reduction of blood glucose level of diabetic mice (p<0.001).

Keywords: Chicken oil; Free range; Nutrition; Fatty acid; Hypoglycemic activity

Introduction

Bangladesh is an agro-based developing country in the Southeast Asian region and livestock especially poultry is a promising sector for employment generation and poverty reduction in this country [1]. About 30 years back the contribution of poultry to the total animal protein was about 22-27% in the country [2]. Bangladesh is one of the high density countries of the world has a population of 150 million people within the area of 143,000 km². About 80% people of this country still live in villages and are extremely poor. Both the government and a variety of non-governmental organizations are actively promoting poultry development at all levels. Small-scale poultry production has developed in a large number of developing countries around the world as an important source of earning for the rural poor peoples. In the last few years, the recognition of small-scale commercial poultry production helps to accelerate the pace of poverty reduction riding in new height in Bangladesh. The poultry industry has been successfully becoming a leading industry of the country [3]. The sector is also growing rapidly for last two decades though it started farming during mid-sixties in this country. It has already capable to rise at an annual growth of around 20% during last two decades. In Bangladesh, this industry has immense potentialities from the point of view of the economic growth of the country as well as fulfilment of basic needs and to keep the price at a minimum level and ensuring food especially animal protein for the human being. The chicken (Gallus gallus domesticus) is a domesticated fowl, a subspecies of the red jungle fowl. As one of the most common and widespread domestic animals, with a population of more than 24 billion in 2003. Two species of chicken are available in Bangladesh: indigenous (domestic) and broiler (hybrid), of them local chicken meat is the famous one among Bangladeshi people. In addition to broiler and local chicken, now a day's cross-breed Sonali (Fayoumi x Rhode Island Red) getting popular for meat supply in Bangladesh [4]. Identification and characterization of the chicken genetic resources generally require information on their population, adaptation to a specific environment, possession of traits of current or future value and sociocultural importance, which are crucial inputs to decisions on conservation and utilization [5,6]. Indigenous chickens of the tropics are important reservoirs of useful genes and possess a number of adaptive traits [7]. The present study mainly focused on nutritional properties of domestic and hybrid chicken oil and its effect of type-2 diabetic mellitus in experimental mice.

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Materials and Methods

Sources of chemical agents and equipment

All chemical compounds are collected from scientific stores which are available in Sigma USA. The quantification kits which are used in mice experiments are collected (Linear chemicals, Barcelona, Spain) and analysed by automatic Bioanalyzer (Hitachi 7180, Hitachi, Tokyo, Japan).

Equipment:

- 1. Centrifuges (Eppendorf, Model 5415D)
- 2. Cryogenic vials (Thermo Fisher Scientific, catalog number: 5000-0012)
- 3. 2 mL Eppendorf tube with locking lid (Thermo Fisher Scientific, catalog number: 02-681-299)
- 4. Spectra Max 384 spectrophotometer (Molecular Devices)
- 5. Balance
- 6. Quartz cuvette
- 7. Scissors

Sample collection and preparation

Collection of chickens: Domestic and hybrid chicken (both sex male and female, weight 1-1.5 kg), ten of each type were collected from the nearby market at Binodpur Bazar, Rajshahi University area. The collected chickens were cleaned, air-dried, packed in polyethylene bag, sealed and stored at 4°C for use in subsequent experiments. Only the meat portion of chicken was used for various experimental purposes.

Extraction of oil: Oil portion of hybrid and domestic chicken were extracted by suitable solvents under the operating condition. Continuous Soxhlet extraction device was used for the extraction of oil [8].

Nutritional analysis

Determination of macronutrients content of domestic and hybrid chicken species

Moisture content: 5-6 g of hybrid and domestic chicken were weighed in a porcelain crucible (which was previously cleaned, heated to about 100°C, cooled and weighted). The crucible with the sample was heated in an electrical oven for about six hours at 100°C. It was then cooled in desiccators and weighted again [9].

Ash content: 5-6 g of hybrid and domestic chicken sample were weighed in a porcelain crucible (which was previously cleaned, heated to about 100C°, cooled and weighed). The crucible with its content was placed in a muffle furnace for about four hours at about 600°C. It was then cooled in a desiccator and weighed again. To ensure the completion of ashing, the crucible was again heated in the muffle furnace for half an hour, cooled and weighed again. This was repeated till two consecutive weights became same and the ash was almost white in color [10]. Per cent of ash content (g per 100 g of hybrid and domestic chicken).

Determination of lipid content: About 5 g of hybrid and domestic chicken samples were first grinded in a mortar and pestle with about 10 mL of distill water. The grinded flesh was transferred to a separating funnel and 30 mL of chloroform-ethanol mixture was added. The mixture was mixed well. It was then kept overnight at room temperature in the dark. At the end of this period 20 mL of chloroform and 20 mL of water were further added and mixed. Generally three layers were

seen. A clear lower layer of chloroform containing the entire lipid, a colored aqueous layer of ethanol with all water soluble materials and a thick pasty interphase were seen. The chloroform layer was carefully collected in a pre-weighed beaker (50 mL) and then placed on a steam bath for evaporation. After evaporation of the chloroform, the weight of the beaker was determined again. The difference in weight gives the amount of the lipid [11].

Calculation: Per cent of lipid content (g per 100 g of hybrid and domestic chicken).

Determination of protein content: Total protein contents of hybrid and domestic chicken were determined by the micro-kjeldahl method [12]. The sample was weighed accurately and transferred to a 250 mL Kjeldahl flask. 1 g of catalyst mixture and 25 mL of conc. H₂SO₄ were added to it. The flask was placed in an inclined position on the stand in the digestion chamber. The flask was heated gently over a low flame until the initial frothing was ceased and the mixture was boiled briskly at a moderate rate. During heating the flask was rotated several times. The heating was continued until the color of the digest was pale blue. The digest was cooled and 30 mL of water was added to 5 mL portion with mixing. The digest was transferred to a 100 mL volumetric flask. The flask was then rinsed 2 or 3 times with water and the washings were transferred to the volumetric flask. The total protein content was determined by Lowry's method of protein estimation, which is based on the formation of a copper-protein complex and the reduction of phosphomolybdate and phosphotungstate present in Folin-Ciocalteau reagent to hetero polymolybdenum blue and tungsten blue, respectively. Bovine Serum Albumin (BSA) (0-100 µg/mL) was used as a standard for preparing the calibration curve.

Determination of glycogen: The glycogen content of the liver, thigh and breast muscle samples of each domestic and hybrid chicken were determined by indirect acid hydrolysis as described by Passonneau and Lauderdale (1974) [13]. Glycogen content is determined as:

Abs (sample)/Abs (standard) × Concentration (standard) (i.e., 0.5 mM) × Volume (standard) (i.e., 0.01 mL) × Total volume (i.e., 1.0 mL)/ Volume (sample) (i.e., 0.01 mL)/ Weight (sample) (mg) × 1000

Unit=micromoles glucose units/per gram weight.

Total sugar: 4-6 g domestic and hybrid chicken sample were plunged into boiling ethyl alcohol and allowed to boil for 5-10 min (5-10 mL of alcohol was used for each g of domestic and hybrid chicken were). The extract was cooled and crushed thoroughly in a mortar with a pestle. Then the extract was filtered through two layers of muslin cloth and re-extracted the chicken oil for three min in hot 80% alcohol, using 2 to 3 mL of alcohol for each g of chicken sample. This second extraction ensured complete removal of alcohol soluble substances. The extract was cooled and passed through muslin cloth. The extract was filtered through whatmann no-41 filter paper. The volume of the extract was evaporated to about 1/4 of the volume over a steam bath and cooled. This reduced volume of the extract was then transferred to a 100 mL volumetric flask and made up to the mark with distilled water. Then 1 mL of diluted solution was taken into another 100 mL volumetric flask and made up to the mark with distilled water [14]. Aliquot of 1 mL of the chicken oil extract was pipetted into different test tubes and 4 mL of the anthrone reagent was added to this solution and mixed well. The absorbance was measured at 680 nm using the blank containing 1 mL of water 4 mL of anthrone reagent.

Determination of reducing sugars: Aliquot of 3 mL of the chicken oil extract was pipetted into test tubes and 3mL of DNS reagent added

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to each of the solutions and mixed well. The test tubes were heated for 5 min in a boiling water bath. After the colour has developed, 1 mL of 40% Rochelle salt was added when the contents of the tubes were still worm. The test tubes were then cooled under a running tap water. A reagent blank was prepared by taking 3 mL of water and 3 mL of DNS reagent in a tube and treated similarly. The absorbance of the solutions was measured at 575 nm in a colorimeter [15].

Measurement of K and Ca: Transfer 10 mL diluted filtrate into a 50 mL volumetric flask using a pipette. The flask was made to volume with water and mixed. The content of K and Na were measured by flame photometer. If the reading is higher than the reading of the highest standard solution, it is need to a larger dilution, e.g., 5 mL volumetric flask. In this case 0:100 diluted HNO₃ must be added to the volumetric flask to make the total volume of 1:100 diluted HNO₃ and filtrate equal to 10 mL [16,17].

Measurement of Fe, Mn, Zn and Pb: The content of these elements were measured by Atomic Absorption Spectrometer (AAS) directly in the undiluted filtrate [18-20]. Additional dilution is made before the transfer to the 50 mL volumetric flask; the result is multiplied with the dilution factor. Fe, Mn, Zn and Pb.

Mg per kg chicken material=
$$\frac{d \times 100}{c}$$
Where

d=mg/L Fe, Mn, Pb, Cu, as and Zn measured on atomic absorption spectrometer or spectrophotometer.

c=g material weighed into the digestion tube.

Study on chemical characteristics of domestic and hybrid chicken oil

Determination of iodine value of: The iodine value of oil was determined by the Hanus method using the formula [21].

Iodine value=
$$\frac{(V_1 - V_2) \times 0.127 \times 100}{W}$$
Where

 $\rm V_1=Volume$ in mL of sodium thiosulphate solution required for blank experiment.

V₂=Volume in mL of sodium thiosulphate solution used.

S=Strength of sodium thiosulphate.

W=Weight of the oil in gram.

A blank experiment was performed exactly in the same manner without the oil.

Determination of saponification value: Saponification value was determined by conventional methods [22].

Saponification Value (SV)=
$$\frac{(V_1 - V_2) \times N \times 56.1}{W}$$

Where

 V_1 =mL of acid used for blank test.

 V_2 =mL of acid used for the test experiment.

N=Strength of acid used.

W=Weight of oil.

Determination of peroxide value: The peroxide value is expressed in terms of mille equivalents of active oxygen per kilogram of oil. Peroxide value was calculated using the following formula [23]. Peroxide value= $\frac{(S-B) \times N \times 1000}{\text{Weight of sample}}$

S=mL of thiosulphate required in the test experiment

B=mL of thiosulphate required in the blank experiment

N=Strength of sodium thiosulphate

Determination of acid value: The acid value of oil oils were measured by Hanus method. The acid value was calculated using the following formula [24].

Acid value=
$$\frac{56.1 \times N \times V}{W}$$

Where

N=Strength of alkali

V=mL of sodium hydroxide solution used for the oil

W=Weight of oil in gram

Determination of free fatty acid (% FFA) of the oil: The percentage of free fatty acid (as oleic acid) was calculated using the following formula [25].

Percent of free fatty acid=
$$\frac{\text{Acid value}}{1.99}$$

Determination of unsaponifiable matter: This test method is intended for use in the determination of the unsaponifiable, non-volatile matter contained in sulphated oils for the purpose of quality assurance. This includes sterols, higher alcohols, pigment, and hydrocarbons. The amount of unsaponifiable matter present in the oil was determined using the method as described [26].

The quantity of unsaponifiable matter present in 100 g of oil was calculated from the formula.

U.M.=
$$\frac{\text{Weight of unsaponifiable matter}}{\text{Weight of oil taken}} \times 100$$

Hypoglycemic activity of domestic and hybrid chicken oil (animal studies)

Animals care: 42 both male and female Wister strain mice weighing about 100-150 g age about 21-28 days were collected from Chittagong BCSIR to carry out the experiment. All the animals were kept and maintained under laboratory conditions. They were individually housed in polypropylene cages in well-ventilated rooms, under hygienic conditions, humidity (45.75%) and 12 h day: 12 h night cycle; and were allowed free access to food (standard pellet diet) and water ad libitum. The animals were divided into seven groups of six mice each and provided with standard diet for 21 days in animal's house.

Induction of diabetics: Diabetes mellitus was induced by single intraperitoneal injection of freshly prepared streptozotocin (65 mg/ kg body weight) in a 0.1 M sodium citrate buffer (pH-4.5). Diabetes was developed and stabilized in this streptozotocin treated mice over a period of 16 h. After three days of streptozotocin administration, plasma glucose levels of each mouse were determined. Mice with a fasting plasma glucose range of 280–350 mg/dL were considered diabetic and included in the study, bloods were collected every week from the eyes of mice with highly sterilized capillary tube. Blood (3-4 mL) samples were kept in capped, air tight, plastic sterile test tubes.

Blood collection: Blood samples from all groups were collected on day 7, day 14 and day 21 in a fasting state from mice marginal ear vein by 26 G needle and syringe [27]. Blood glucose level was determined by "Humylazer 2000" analyser (Human, Germany). The values was expressed as mean \pm S.E.M, Statistical analyses were performed by SPSS-16 one-way Analysis of Variance (ANOVA), followed by post-hoc Tukey's test for multiple comparisons. p<0.05, p<0.001 were considered as significant.

Experimental animals grouping and treatment: The animals were divided four grouped. The animals were treated for 3 weeks as follows:

Group-1: Normal (non-diabetic mice).

- **Group-2:** Control (streptozotocin induced diabetic mice without treatment).
- **Group-3**: The diabetic mice treated with diabetic+domestic chicken oil (1% of total diet), diabetic+hybrid chicken oil (1% of total diet) extract solution dose for 21 days.

Group-4: Diabetic+glibenclemide. (0.5 mg/kg b.wt).

Results

Table 1 showed the macronutrient contents of domestic and hybrid chickens were compared. The results showed that, the moisture and ash contents of domestic chicken were 73.89% and 2.25%, respectively. On the other hand moisture and ash content of hybrid chicken were 75.22% and 2.4%, respectively. Again total lipid content of domestic and hybrid chicken were 2.82% and 8.78%, respectively. Chicken meat is a very good source of protein. Result showed that protein content of domestic chicken was 12.84% whereas that of hybrid chicken was 13.75%. Glycogen, free sugar and reducing sugar content of domestic chicken were 0.039%,0.1%, 0.002% whereas that of hybrid chicken were 0.041%,0.13% and 0.002%, respectively. So, all macronutrients such as ash, moisture, total protein, total carbohydrate and total lipid were found to be high for the hybrid chicken. Lipid content was significantly higher (around 50%) in hybrid chicken than that of domestic chicken.

Micronutrients contents of domestic and hybrid chicken are presented in Table 2. Iron, calcium, zinc and lead content of domestic chicken were found to be high that of hybrid chicken. Iron, calcium, zinc and lead content of domestic chicken were 32, 105.2, 17.84 and 38.28 ppm/100 g. Whereas, that of hybrid chicken were 27.0, 97.5, 9.11 and 37.17 ppm/100 g, respectively. Again potassium and manganese which were found to be high in hybrid chicken (127.8 and 9.74 ppm/100 g, respectively) than domestic chicken (86.83 and 6.83 ppm/100 g, respectively). Zinc content of hybrid chicken was significantly lower (around 50%) than that of domestic chicken.

Table 3 showed Feed for the hybrid chicken was collected from CP-Bangladesh, a famous industry for the production of poultry chicken feed in Bangladesh, and analysis of nutrient content was done by investigating various parameters such as moisture, total protein, fat, total sugar, calcium and phosphorus. Feed of hybrid chickens contain 12% moisture, 17% total protein, 5% fat, 0.34% total sugar, 1mg /kg calcium and 0.70mg/kg phosphorus.

Table 4 showed Chemical characteristics of the Domestic and Hybrid Chicken oil. The average amount if oil content of two species were extracted with soxlet apparatus using n-hexane as an extracting solvent and it was found to be 3.54% for domestic chicken and 4.84% for hybrid chicken. Chemical characteristics of the extracted oil were investigated by analysing various parameters such as iodine value, saponification value, acid value, peroxide value and percentage free fatty acid. Iodine value and unsaponifiable matter were higher for hybrid chicken (77.92 and 248.6 respectively) than those of domestic chicken (60.56 and 303.15 respectively). Again saponification value, acid value, peroxide value and percentage free fatty acid were higher for domestic chicken than hybrid chicken by 22%, 15%, 14% and 15%, respectively.

Figure 1 showed the hypoglycemic activity of Domestic and Hybrid Chicken oil. In 21 days the level of glucose decreases significantly. Comparing the blood sugar level in Streptozotocin induced diabetic mice, Domestic and Hybrid Chicken oil administered subject showed significant reduction of blood glucose level which is as near as glibenclamide administered subject at (p<0.001). After first week of chicken oil treatment the domestic and hybrid chicken oil and chicken flesh, had no significant effect on blood glucose level of mice, But after 3rd week of treatment there was a significant hypoglycemic effect had seen, comparing the blood glucose level in Streptozotocin induced mice. The blood glucose levels were decreased by 12% and 10% for

Parameters	Domestic chicken	Hybrid chicken	% deviation
Moisture	73.89	75.22	± 2
Ash	2.25	2.406	± 9
Total lipid	2.835	4.783	± 40
Total protein	12.84	13.75	± 6.6
Glycogen	0.039	0.041	± 25
Total sugar	0.1	0.13	± 23
Reducing sugar	0.002	0.003	± 22

Values are mean ± S.D. of triplicate analyses.

Table 1: The macronutrients content of Domestic and Hybrid Chicken species (g %).

Name of minerals	Domestic chicken	Hybrid chicken	% deviation
Iron	32	27	± 20
Calcium	105.2	97.5	± 9
Potassium	86.83	127.8	± 34
Manganese	6.83	9.74	± 28
Zinc	17.84	9.11	± 50
Lead	38.28	37.17	± 3

Values are mean \pm S.D. of triplicate analyses.

 Table 2: The micronutrients content of Domestic and Hybrid Chicken species (ppm/100 g).



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Name of parameter	Amount(g/100 g)	
Moisture	12 g	
Total protein	17 g	
Fat	5 g	
Total sugar	0.34 g	
Calcium	1 mg/kg	
Phosphorus	0.70 ma/ka	

Values are mean ± S.D. of triplicate analyses.

Table 3: The macronutrients content of Hybrid Chicken feed.

Parameters	Domestic chicken	Hybrid chicken	% deviation
Oil content Iodine value	3.54 60.56	4.84 77.92	± 26 ± 23
Acid value	32.47	28.24	± 15
Saponification value	303.15	248.6	± 22
Unsaponifiable matter	3.5	11.14	± 67
Peroxide value	81.62	71.87	± 14
Percent of free fatty acid	16.25	14.55	± 15

Values are mean ± S.D. of triplicate analyses.

Table 4: Chemical characteristics of the domestic and hybrid chicken oil.

Domestic and hybrid chicken oil administered subject whereas 8% and 9% for Domestic and hybrid chicken flesh administered subject which is as near as glibenclamide administered subject (16%).

Discussion

This study evaluated food and macro and micronutrient intake in a sample of adults with T2DM, and found that this group made different food choices to adults with metabolic disorder. These choices resulted in high nutritional properties, lipid profile and protein are remarked which are beneficial for human being. The moisture content of domestic and hybrid chicken oil has shown antimicrobial activity and oxidative activity [28]. The domestic and hybrid chicken oil has essential properties of life as like protein, carbohydrate, etc. Moisture also showed essential for most of the physiological activity and making of animal tissue. The fat contents of the chicken samples investigated in this study showed high amount of lipid and therefore not considered a good source of lipid [29]. Hybrid chicken investigated higher amount of lipid than domestic chicken. The protein constituents of chicken oil are responsible for making structure of body involved in metabolism during growth, development and maturation [30]. The domestic and hybrid chicken oil contain a large amount of carbohydrate molecule which plays an important role on the physiological activities of both in animal and plant. Glucose and glycogen serves important sources of energy for vital activities. Some carbohydrates have highly specific functions [31]. The storage carbohydrate in animal body is known as glycogen [32]. The sugar element of domestic and hybrid chicken oil depends upon the sugars present in the Fructose and glucose constitutes the primary sugars in all chicken oil samples of good quality the fructose molecule [33]. The inorganic substances which are known as minerals are very small amounts for their growth and maintenance of functional activities. Minerals are responsible for the teeth and bone formation in living organism. Various regulatory compounds such as vitamins, enzymes and hormones are enhancing activity of minerals compound [34]. Some enzymes require calcium for their activities such as lipase and succinate dehydrogenases. Iron is required for the enzymatic activities of several enzymes such as ferredoxin catalase, indophenol oxidase, aldehyde oxidase etc. Minerals also present in both extra cellular and intra cellular spaces [35]. Zinc is involved in the several enzyme systems and is a constituent of insulin. It is also essential for growth, reproduction and support immunity. Zinc acts as a cellular second messenger in the control of insulin signaling and glucose homeostasis [36]. Hybrid chicken is rich in unsaturated fatty acid which is contributed mostly by long chain fatty acid because iodine value was higher for hybrid chicken than domestic chicken by 23% which was indication of the presence of higher amount of unsaturated fat in hybrid chicken. The unsaturated fatty acids in the triglyceride molecules of domestic and hybrid chicken oil showed iodine values activity [37-39]. The saponification value of domestic and hybrid chicken oil showed inversely proportional to the average molecular weight or chain length of the fatty acids in fat or oil [40]. The glibenclamide was used compared with, a standard hypoglycemic drug of plasma glucose lowering activity [41]. The result indicates that domestic and hybrid chicken oil administered group had more significant effect on reduction of blood glucose level.

Conclusion

Both domestic and hybrid chicken meats are very popular in our country. But meat of domestic chicken is still now considered as more health friendly in comparison to hybrid chicken meat, though the production rate of hybrid chicken is increasing day by day due to its increased rate of consumption. The study aimed to investigate the meat composition of both type of chickens mentioned above. From the study, it may be concluded that: The protein and lipid contents of the hybrid chicken were higher than the domestic chicken. All other macronutrient contents were also found to be high in the hybrid chicken as they were fed with highly nutritious artificial feeds. Except potassium and manganese, all the other micronutrients were found to be high in domestic chicken. On the other hand, zinc content of hybrid chicken was found to be lower than that of domestic chicken. From the antidiabetic activity studies it was found that crude ethanol and methanol extracts of domestic and hybrid chicken oil showed significant activity against alloxan induced diabetic mice.

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