



Nutritional Analyses of Domestic and Poultry Chickens and Evaluation of the Subsequent Effects on Lowering Blood Glucose Levels in Alloxan-Induced Wister Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAI designed and supervised the study. Author MK conducted the experiments and analyzed the data. Authors MU and MM assisted in the laboratory experiments. Authors MGA, IH and MHK analyzed the data of the study. Author MMR wrote the manuscripts and extensively analyzed the data. All authors read and approved the final manuscript.

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ABSTRACT

In the recent decades, peoples concentrate more on reduced-consumption of diets containing saturated fatty acids and replace them with essential polyunsaturated ones, including omega-3 and omega-6 fatty acids, due to their promising nutritional benefits. Therefore, the production of chicken meat having lower fat compositions, and riches in pro-healthy fatty acid and other macro-and micronutrients deserves great attention in the food industry. The research aimed to characterize

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meat oils of two commercially available, domestic and poultry, chickens in the Northern part of Bangladesh (Rajshahi), followed by the investigation of their nutritional compositions and hypoglycemic effect in vivo. In this work, the oil was isolated from the dried meat sources by Soxhlet extractor and purified using a rotary evaporator. We subsequently characterized meat oil in terms of various parameters, such as iodine value, saponification number, unsaponifiable matter, free fatty acid (FFA) contents, peroxide value, and acid value. Besides, the nutritional parameters of chicken oil were investigated as percentages of essential fatty acids and several other micro-and macronutrients using gas-liquid chromatography and other conventional methods. Finally, Alloxan-induced Wistar rats were fed with oil and chicken flesh to investigate the hypoglycemic effects. The experimental analyses show that chicken oil possesses a significant proportion of omega-3 and omega-6 fatty acid, macro-and micronutrients. Besides, oil and flesh were found to lower blood glucose levels in diabetic rats despite flesh could not significantly show hypoglycemic properties.

Keywords: Domestic chicken; poultry chicken; fatty acid; quality control; nutrients; diabetes.

1. INTRODUCTION

The production of animal meat with low-fat content as well as healthy fatty acid profiles and other essential nutrients is a matter of huge interest in the food industry [1–3]. A US-based survey demonstrates that about 60% of meat consumers prefer to have meat with low animal fat content and 40% of consumers have reduced beef meat consumption since the beef meat contains a higher quantity of unhealthy constituents, including triglycerides and low-density lipoprotein (LDL)-cholesterol [4]. Meats from the various animal kingdom are characterized mostly by their essential fatty acid content, and other macro-and micronutrient compositions such as vitamins, minerals, etc [5].

In the last few decades, consumption of both poultry and domestic chicken meats has been boosted significantly, and it is assumed that it would continue to increase in the near future, especially in the developing nations [6]. Meats consumers are generally interested in chicken diets especially from poultry skins as it is, cost effective, low fat content (5g/100g raw meat without skin), valuable sources of healthy fatty acids, proteins (20g/100g raw meat without skin) fat-soluble vitamins and inorganic minerals [7]. It has been reported that poultry chicken meat with low-saturated fat content may assist to resolve major global public health complications including diabetes [8,9]. Feskens *et al.* have summarized interesting correlations among insulin resistance, hyperinsulinemia and the ingestion of saturated fats from animal sources [10].

In developing countries diabetes is prevalent in adult and has become one of the major causes

of death due to its association with elevated risk of cardiovascular diseases.

In 2000, it was reported that diabetes had affected about 171 million people worldwide, and it was anticipated that by 2030 this figure would cross 366 million [11]. A previous report has mentioned that, in Bangladesh, the figures of diabetes would increase from 3.2 million to 11.1 million from 2000 to 2030 [12]. The consumption of red meats with high-fat content may block glucose uptake, leading to the development of insulin resistance as well as diabetes [13]. Compare to white meat, red meat contains higher heme-iron, a pro-oxidant that triggers oxidative stress, free radicals, and inflammation in the body. Heme-iron plays dual roles in the case of glucose metabolism, it disturbs glucose storage in the tissues, and influences hepatic glucose production which results in elevated blood glucose levels and pancreatic β -cells destruction. [14]. Moreover, red meat contains high levels of harmful cholesterol, responsible for the blockage of arteries and puts the diabetic subjects at high risks of developing coronary heart disease [15–17]. This phenomenon has influenced to replace red meat diets with low-saturated fat-containing white meat diets like chicken [18]. However, for many peoples in developing countries, consuming domestic chicken meat is much difficult due to its high prices in the local market. For this reason, a large number of people in the underdeveloped countries depend on poultry meat to replenish the high-quality nutrients in the body. The step-up demand for poultry meat has exerted pressure on farmers and, breeders to increase breast-meat production [6].

From these perspectives, consumers are conscious about the labeling of food with proper

nutritional profiles [19] One effective way to meet consumers' demand is the analyses of nutritional properties of marketed meat followed by processing and proper labeling, which needs effective analyses with efficient methods. Secondly, there is a strong belief among the common people that domestic chickens are much more beneficial for animal health when compared to hybrid chickens. Therefore, specific and comparative studies are required to understand their nutritional composition as well as other pharmacological properties of these chicken species. For this purpose, the authors aim to determine and compare the nutritional properties of two commercially available meat resources such as, domestic and poultry chickens, in the Northern part of Bangladesh (Rajshahi). Besides, the chemical properties of isolated fats from the chicken oils have been investigated in details. As an essential composition, the fatty acid content of both domestic and poultry chicken oils has been determined. Finally, we comparatively analyzed the effects of chicken oils and flesh of both domestic and poultry chickens on blood glucose levels in alloxan-induced Wister rats.

2. MATERIALS AND METHODS

2.1 Sample Collection

Domestic and poultry chickens, ten of each, were purchased from the Binodpur Bazar, Rajshahi, Bangladesh. The chickens were immediately cleaned, air-dried, labeled, and stored in a deep fridge for future experiments. For analyses, only the meat portion of chicken was used.

2.2 Extraction of Oil

Oils were extracted from both poultry and domestic chickens using solvents under Soxhlet extractor [20]. Briefly, fresh selected chickens were sun-dried for removal of moisture and then crushed into pests. The oil was extracted as follows: the sample was placed in a porous thimble covered with cotton wool and the weight of the sample was measured before it was further placed in an inner tube of the apparatus and fitted to a round bottom flask containing n-hexane solvent. The sample was then heated and boiled for 1 hour. While heating, the solvent inside the flask was started boiling within 5 minutes and water begins to drop from the top to the sample in the thimble. When the solvent reached the top of the tube, it siphoned over into the flask and then removes the oil portion

extracted in the refluxing process. The solvent used in a round bottom flask of the Soxhlet apparatus and the oil extracts were collected and measured (Fig. 1). The extract was evaporated under reduced pressure (rotary evaporator) to obtain the oil.

2.3 Purification of Crude Oil

About 100 g of oil was taken in a separating funnel, followed by the addition of 100 ml of water, 200 ml of ether, and 25 ml of saturated sodium chloride solution. The solution was then stirred and kept at room temperature until two distinct layers appeared. The aqueous layer was discarded and the remaining solution was stirred with 100 ml of distilled water followed by the addition of 25 ml of sodium chloride. The ether layer was separated in a conical flask and dried over anhydrous sodium sulfate. The extract was then evaporated by a rotary evaporator at 40°C to get the purified oil [21].

2.4 Quality Control

The iodine value of oils was measured by the Hanus method [22]. Saponification value, unsaponifiable matter, acid value, peroxide value, and percentage of FFA were determined according to Viswanathan [23]. The fatty acid composition of extracted oil was determined by Gas-liquid chromatography [24]. GC-2025 gas chromatograph with AOC-20i Auto-Injector (Shimadzu Co, Japan) was used for quantitative analysis of chicken oil under condition (temperature; 280°C, pressure; 175.4KPa, total flow; 165 ml/min, purge flow; 3ml/min, column temperature; 270°C). The area of fatty acids contents was expressed as the relative percentage of fatty acids, which were calculated by the following formula:

The relative percentage of fatty acid =

$$\frac{\text{Area of fatty acid} \times 100}{\text{Total area of detected fatty acids}}$$

2.5 Nutrients Content Measurement

Moisture and ash contents were determined according to ICOMR [25] and AOAC method [26], respectively. Lipid, total sugar, reducing sugar, glycogen, total protein, and mineral contents were determined by Blight and Dyer method [27], Anthrone method [28], Dinitrosalicylic acid [29], Anthrone method [28], micro-Kjeldahl method [26] and atomic absorption spectrophotometer [30], respectively.

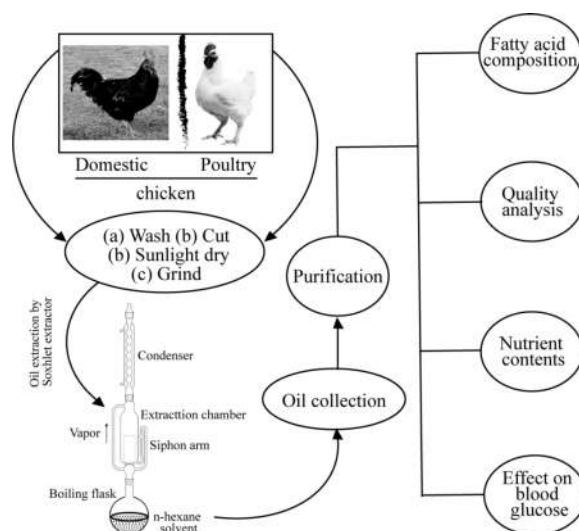


Fig. 1. Experimental procedure of oil extraction, purification and characterization, and in vivo analysis using poultry and domestic chicken

2.6 Experimental Animals

Female Wister strain rats weighing about 100~150 g and 21-28 days old were bought from Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong. All the animals were maintained at $(22\pm 2^\circ\text{C})$ temperature, humidity (45.75%), and 12 hours:12 hours day: night cycle, and were allowed free access to standard pellet diet and water ad libitum. The animals were divided into seven groups containing six rats in each group as mentioned below:

- NCD- Normal or non-diabetes rats
- DC- Control (Alloxan induced diabetic rats with no treatment)
- DG- Diabetic + glibenclamide
- DDO- Diabetic + domestic chicken oil (1% of the total diet)
- DPO- Diabetic + poultry chicken oil (1% of the total diet)
- DDF- Diabetic + domestic chicken flesh (1% of the total diet)
- DPF- Diabetic + poultry chicken flesh (1% of the total diet)

2.7 Diabetes Induction and Oil Treatment

Diabetes was induced by a single intraperitoneal injection of freshly prepared Alloxan Hydrate (55 mg/kg BW) in 0.9% saline solution. After three days of alloxan administration, serum glucose levels of each rat were determined. Rats with a fasting plasma glucose levels 280–350 mg/dL

were considered diabetic and included in the study. NDC and DC rats were fed with normal diet and plenty of water; DG rats were treated with glibenclamide (0.6 mg/kg body weight); DDO, DPO, DDF, and DPF rats were fed with domestic chicken oil (1% of the total diet), poultry chicken oil (1% of the total diet), domestic chicken flesh (1% of the total diet), and poultry chicken flesh (1% of the total diet), respectively. Blood was drawn every week throughout the three weeks from the tail vein (Fig. 3ab). The serum was separated by centrifugation at 4000 rpm for 15 min and estimated serum glucose level using a semi-autoanalyzer (Microlab 300).

2.8 Statistical Analysis

All the results were expressed as mean \pm SEM. The difference between the groups was calculated by using an independent t-test at 95% confident interval. $P < 0.05$ was considered as significant.

3. RESULTS

3.1 Characterization of Chicken Oil

The analyses show that the average amount of oil content in domestic and poultry chickens was 3.54% and 4.84%, respectively. Chemical characteristics of oils were investigated using various parameters, including iodine value, saponification value, acid value, peroxide value, and percentage free fatty acid. Iodine number

and unsaponifiable value were higher in poultry chicken (77.92 and 248.6) compared to domestic chicken (60.56 and 303.15). In addition, saponification value, acid value, peroxide value, and percentage FFA content in domestic chicken were higher compared to poultry chicken by 22%, 15%, 14%, and 15%, respectively (Table 1).

3.2 Fatty Acid Compositions

GC analyses show that both the domestic and poultry chicken oils contain oleic acid (C18) as major fats. These oils contain the bulk of saturated fatty acids like palmitic acid (C16) and stearic acid (C18). Poultry chicken oil possesses monoenoic palmitoleic acid (C16), but domestic chicken possesses no palmitoleic acid. Both the oils contain a small quantity of arachidonic acid although domestic chicken contains a little higher

than poultry chicken. Besides, these oils contain a small quantity of myristic acid and behenic acid (Fig. 2).

3.3 Nutritional Analysis of Chicken Oils

The macronutrients of domestic and poultry chicken oils were mentioned in Table 2 and micronutrient contents were shown in Table 3. The results show the moisture and ash contents in domestic chicken were 73.89% and 2.25%, respectively, whereas in poultry chicken these contents were 75.22% and 2.4%, respectively. The total lipid content in domestic and poultry species was 2.82% and 4.78%, respectively. Besides, the total protein contents in domestic chicken and poultry chicken were 12.84% and 13.75%, respectively. Domestic chicken meat contains a considerable amount of glycogen

Table 1. Chemical characteristics of the domestic and poultry chicken oil

Parameters	Domestic chicken	Poultry chicken	Difference (%)
Oil content	3.54	4.84	26
Iodine value	60.56	77.92	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
Free fatty acid (%)	16.25	14.55	15

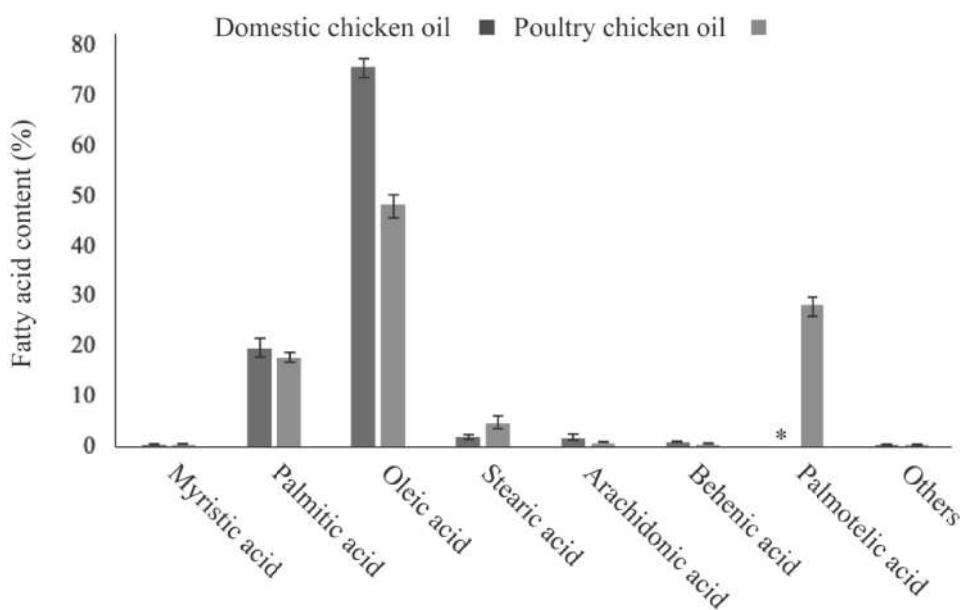


Fig. 2. Fatty acid composition in domestic and poultry chicken oil

(0.039%), free sugar (0.1%), and reducing sugar (0.002%). In poultry chicken these contents were 0.041%, 0.13% and 0.002%, respectively. It seems that all macronutrients, including ash, moisture, total protein, lipid, total carbohydrate, and total lipid were found to be higher in the poultry chicken. It was also noted that lipid content was about 40% higher in poultry chicken compared to domestic chicken.

Minerals are inorganic substances required for the organism in very small amounts for their growth and maintenance of functional activities. Food and vegetables are important sources of minerals for human beings and exist in food as organic and inorganic combinations. In our study, we show that the chickens contain many essential micronutrients including iron, calcium, zinc, and lead. These contents were higher in domestic chicken (32 µg/kg iron; 105.2 µg/kg calcium; 17.84 µg/kg zinc and 38.28 µg/kg lead) when compared to poultry chicken (27.0 µg/kg iron, 97.5 µg/kg calcium, 9.11 µg/kg zinc and 37.17 µg/kg lead). However, potassium and manganese were found to be higher in poultry chicken (127.8 µg/kg and 9.74 µg/kg, respectively) than domestic chicken (86.83 µg/kg and 6.83 µg/kg, respectively) (Table 3).

3.4 Chicken Oils in Lowering Blood Glucose Levels in Diabetic Rats

For this experiment Alloxan-induced diabetes rats were fed chicken oil and flesh of both domestic and poultry species for 21 days. After

the first week of treatment, chicken oil and flesh did not show significant effect on blood glucose levels. However, the treatment showed that oil and flesh significantly lower blood glucose levels at 21th day. The blood glucose levels were decreased by 12% and 10% by domestic and poultry chicken oil, respectively, which was comparable to glibenclamide administration (16%). On the other hand, the decrease rate was only 8% and 9% by domestic and poultry chicken flesh, respectively (Table 4).

4. DISCUSSION

Moisture plays a significant role in the cell since the protoplasm contains about 75% water that is required for the growth and metabolism of animal cells. It is also necessary for the absorption and transport of nutrients, and for the regulation of fluidity in living systems [31]. It serves as an essential part of life like other biomolecules such as carbohydrates, proteins, lipids, etc. Moisture is further necessary for most biological reactions that occur in animal tissues. Recently, Sorland et al. [32] carried out NMR analyses using six samples of pork meat and reported that it possesses at least 60% of moisture. Urh et al. [33] showed beef, pork, chicken, turkey possess 60~75% of water. Our analyses also show that both the domestic and poultry meats contain about 75% of water, which supports the recent findings. Ash serves as the source of most inorganic minerals. A study reported that hybrid meat contains 1.19% whereas polverara meats possess 1.31% of ash [34]. Edris et al. [35]

Table 2. The macronutrients content in chicken oils (gm %)

Parameters	Domestic chicken	Poultry chicken	Difference (%)
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

Table 3. The micronutrients content in domestic and poultry chicken (µg/kg)

Name of minerals	Domestic chicken	Poultry chicken	Difference (%)
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

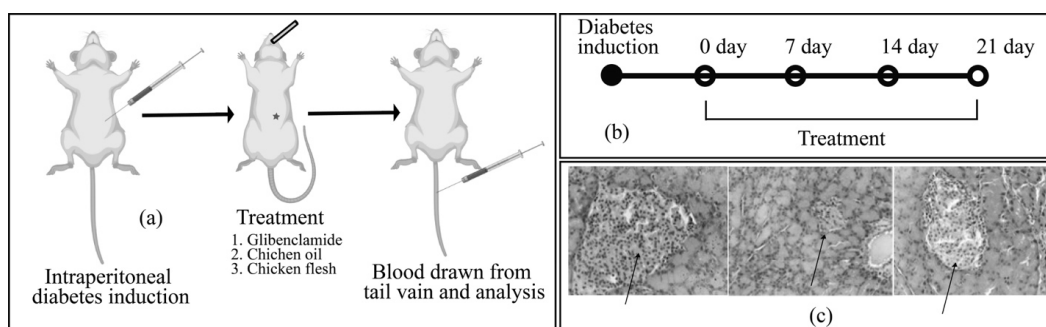


Fig. 3. In vivo animal model of diabetes treatment. (a) Mode of diabetes induction, oral treatment, and biochemical analysis; (b) roadmap of diabetes treatment; (c) mechanism of how diabetes treatment changes histological alteration of pancreatic β -cells to induce insulin secretion (adapted from reference [73])

Table 4. Effects of oil and flesh of domestic and poultry chicken on fasting blood glucose level of experimental rats

Treatment Group	7 th day	14 th day	21 th day
NDC	5.7±0.4	5.6±0.46	5.5±0.57
DC	17.7±1.5	21.2±0.96	25.0±1.4
DG	16.0±1.12	13.7±0.82	10.9±0.81*
DDO	15.6±1.09	14.5±1.29	11.9±0.7*
DPO	16.11±0.71	14.13±0.77	12.8±0.56*
DDF	16.4±0.46	15.1±0.45	13.1±0.46
DPF	16.7±0.6	14.8±0.7	13.9 ±0.51

* Indicate significance change compared with the normal control group ($P < 0.05$). The results are expressed as \pm SEM

has demonstrated that the breast, thigh, and drumsticks portion of chicken possess 2.37%, 2.52%, and 3.56% of ash, respectively. Our analyses also show that domestic and poultry meats contain 2.25% and 2.406% of ash, respectively (Table 2).

Our findings demonstrate that poultry oil possesses a higher iodine value (78) compared to domestic chicken oil (61), which indicates the presence of large number of long-chain unsaturated fatty acids because a greater number of double bonds give more sites for oxidation [36]. Gheisar [36] has also reported that chicken oil possesses a higher iodine number (80) than cattle (52) and camel (40). Feddern et al. [37] have found that chicken contains (73-85) higher iodine value than beef (35-48) and pig (55-68). Our results support these findings. Poultry meat contains lower saponification number (249) than domestic chicken oil (303). Saponification value is a quality indicator of the fatty acid chain, low saponification number indicates the presence of long chain fatty acids [38]. Feddern et al. [37]

have reported the saponification number of chicken skin and chicken meat oil was 216 and 190-196, respectively, which closely match with our finding.

Acid value, peroxide value, and percentage of free fatty were higher for domestic chicken than poultry chicken by 15%, 14%, and 15%, respectively (Table 1). Lipids readily undergo oxidation to form peroxides. Peroxides are intermediates of several reaction products that may react to generate odorous aldehydes and ketones, an indicator of rancidity. In long-chain unsaturated fats, the number of peroxide intermediates remains low. In this case, although the peroxides primarily yielded from unsaturated fats, which are highly unsaturated as well as unstable, and therefore, quickly react to generate secondary oxidation products. Low peroxide values might also be received for any highly rancid products; however, the peroxides yielded in the initial step have all subsequently undergone oxidation reactions [39]. On the other hand, FFA content is an estimation of the extent to which rancidity has occurred in a sample. It

can be overvalued if other acid constituents, including amino acids in meat, exist in the system. FFA content is employed largely as an indicator of the food condition as well as the edibility of pure oils and fats isolated from food products, such as meat [36,40]. The unsaponifiable matter is a fraction of fat or oil that remains insoluble after saponification of the sample by alkali. The unsaponifiable matter of 0.5-2.0% in an oil represents the presence of a mixture of several lipid classes viz, sterols, tocopherols, hydrocarbon, higher aliphatic fatty acids, and terpenoids alcohols. This assay shows that poultry meat possesses three times higher unsaponifiable matter than domestic chicken (Table 1) [38]. Our result is also supported by several research reports [37,41,42].

Essential fatty acids including omega-3 fatty acid (alpha-linolenic acid) and omega-6 fatty acid (linolenic acid) are well-known owing to their strong health function in humans [43]. Eicosanoids and cannabinoids, derivatives of essential fatty acid, affect inflammation, mood, and behavior, and cellular signaling [44,45]. They also regulate transcription factors that are associated with proinflammatory cytokines formation including NK-kB [46]. Besides, essential fatty acids perform crucial roles in the life and death of heart tissues, development of several endocannabinoids [47–50]. Lipid acts as components of the cell membrane, sources of energy as well as insoluble material in the cells [51,52]. Dietary fat assists to absorb a variety of lipoproteins and soluble vitamins that act as essential cellular compositions [53]. The protein serves as essential constituents of nuclear as well as cytoplasmic structures and also acts as the complement of many enzymes necessary for metabolism during growth and development [54–56]. Carbohydrates are essential biomolecules that play vital functions in many physiological activities in all forms of life by providing essential energy. Besides, the animal body reserve carbohydrate-based nutrients in the form of glycogen [57,58]. The poultry chickens possess a higher quantity of carbohydrates including glycogen and total sugar, various macronutrients, such as essential fatty acids, lipid, total protein, carbohydrate, reducing sugar, glycogen, etc, which is supported by several studies [1,59–61].

Minerals are very essential inorganic constituents needed by the cellular organism for their growth as well as regulation of functional activities [62]. Some minerals including iron may form a

complex with the organic molecule to generate hemoglobin, others are necessary for bone regeneration and teeth formation. Some inorganic nutrients are components of diverse regulatory compounds, including vitamins, hormones, and enzymes [62]. For example, succinate dehydrogenase and lipase require calcium for their functions. Calcium is also important for accurate functioning of muscles and nerve, formation of fingernails and hair, blood clotting, and maintains healthy body fluids and membranes, and sometimes, calcium may help to prevent cardiovascular disease by lowering blood pressure [63]. Iron is needed for many enzymatic functions including ferredoxin catalase, aldehyde oxidase, indophenol oxidase, etc. Some minerals also exist in both intra- and extra-cellular spaces [62]. Zinc is an essential component to boost immune response in the body, and also involves numerous enzyme systems as well as a component of insulin [64–66]. Potassium assists to control extracellular body fluid, regulate blood pressure, transmit nerve impulses and help the heart and muscles to contract [67,68]. Manganese is an essential constituent of diverse enzymes and is required for metabolism, tendon formation, and growth of bones [69]. Food and vegie are essential sources of various minerals and present in food as inorganic and organic salt. In our study, we show that chicken possesses significant quantities of mineral, which have been supported by several other findings [70,71].

Alloxan has been being applied for inducing diabetes in vivo models for a long time. The intraperitoneal injection partly damages pancreatic β -cells, which affects insulin production leading to the development of diabetes mellites (Fig. 3C, middle) [72]. Our result shows that both poultry chicken and domestic chicken oil lower the blood glucose levels in alloxan-induced diabetes to near the control levels. The blood glucose levels were decreased by 12% and 10% for domestic and poultry chicken oil administered subjects whereas 8% and 9% for domestic and poultry chicken flesh administered subjects, which is close to glibenclamide administered subject (16%). The result indicates that the treatment with domestic and poultry chicken oil significantly reduced blood glucose levels through similar mechanisms of glibenclamide. However, the flesh could not significantly reduce sugar levels. We could not identify the exact reason(s) for this phenomenon, we are assuming the presence of

lower quantities of hypoglycemic constituents in flesh portion of the chickens. Further studies are essential to resolve this interesting problem. Another limitation of our experiment is that we could not find out the exact mode of action of chicken oils on lowering blood glucose levels. Some authors have proposed that diabetes condition might be improved either through the regeneration of affected pancreatic β -cells by antidiabetic compounds (Fig. 3, right) [73], or by release of bound insulin from pancreatic cells by blocking ATP-sensitive- K^+ (potassium ion) channels [74]. Similar mechanism(s) on blood glucose reduction might be exerted by chicken oils. However extensive studies are necessary to trace the exact mechanism(s) of actions of chicken oils on diabetes.

5. CONCLUSION

In conclusion, chickens are the sources of several nutrients, including total sugar, glycogen, protein, essential fatty acids, and minerals. Besides, it prevents diabetes in alloxan-induced diabetes (Wister) rats. Considering the health benefit as well as taste, it should be considered as good food for our healthy life.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This research work was approved by the Institutional Animal, Medical Ethics, Bio-Safety and Bio-Security Committee (IAMEBBC) for Experimentations on Animal, Human, Microbes, and Living Natural Sources, memo no. 118/320-IAMEBBC/IBSc on 19/03/2019. Institute of Biological Sciences, University of Rajshahi, Bangladesh.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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