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Nutritional profile of High Fat Simple Carbohydrate Diet used to induce metabolic syndrome in C57BL/6J mice

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ABSTRACT

Purpose: Many diet based studies on metabolic syndrome lack an in-depth study of feed nutrient profile post preparation. We analyzed the nutritional profile of formulated High Fat Simple Carbohydrate (HFSC) feed and studied its effect on target organs.
Methods: Control feed and HFSC feed were analyzed for macro and micronutrient profile and fed to one month male C57BL/6J mice for 5 months during which feed intake, energy intake and feed efficiency was monitored. The effects of feeds were preliminarily studied on kidney, liver, adrenal gland and pancreas by histological staining.
Results: The HFSC feed had significantly lower carbohydrate, moisture, crude fiber, ash content, mineral, vitamin content, higher ω-6/ω-3 fatty acid ratio as compared to control feed. The HFSC fed mice had higher food intake; energy intake; feed efficiency; lymphocyte infiltration in the liver; hypertrophy of the kidney, adrenal medulla and degenerated islets of pancreas as compared to the control mice after 5 months of feeding.
Conclusion: Food with a higher ω-6/ω-3 fatty acid ratio and simple carbohydrate leads to deterioration of structural integrity of vital organs.

1. Introduction

Lack of time, stress and increased per capita income has increased the consumption of nutritionally poor food. Availability of junk food rich in fat, carbohydrate or both in large portions and at an affordable cost has made it a daily occurrence [1,2]. Overconsumption of such food has led to an increasing epidemic of Obesity [3–5], Insulin resistance [6] and Dyslipidemia [7]. The International Diabetes Federation has defined clustering of lipid abnormalities and insulin resistance as Metabolic syndrome [8]. Its incidence has become increasingly prevalent in children and adolescents leading to a specialized definition for this age group [9]. Subjects born with a higher than average birth weight were found to have a greater risk of developing MetS in childhood and adolescence [10].

Therefore, knowledge of nutritional content of food consumed is of utmost importance. For example, Walker *et al.*, (2014) [11] reported fructose content in sugar sweetened drinks made with High Fructose Corn Syrup had higher fructose to glucose ratio than expected. Dietary habits play an important role in development of syndrome [12–15]. Few studies elaborating the relation of nutritional content of food and metabolic syndrome exist. We formulated a High Fat Simple Carbohydrate (HFSC) Diet mimicking fast foods according to Fraulob *et al.*, (2010) [16] with modifications and fed it to one month male C57BL/6J mice which led to the development of dyslipidemia and Type 2 diabetes inducing metabolic syndrome after five months [17]. In the present investigation, we have attempted to determine the nutritional content of the HFSC feed and its effect on some vital organs.

2. Materials and methods

2.1. Formulation of feed

The HFSC feed was formulated in reference to Fraulob *et al.*, (2010) [16] with modifications which included addition of sucrose

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(16% w/w) and reduction of cornstarch (10% w/w). All components for the formulation of the feed were purchased locally.

2.1.1. Control feed

Roasted Wheat flour (79% w/w), Amul Infant Spray (16% w/w) (nutritional details as per manufacturers details: Protein (22 g/100 g), Fat (18 g/100 g), Carbohydrate (50 g/100 g), Added sugar (18 g/100 g), Vitamin mix (2.24 g/100 g) and Mineral mix (1.41 g/100 g)) and pre-warmed Soyabean Oil (4.67% v/w) was mixed. Adequate amount of pre-boiled warm water was added to hold the mixture in the form of a pellet.

2.1.2. HFSC feed

Warmed lard (13% w/w), Melted Sucrose (10% w/w), Cornstarch (7% w/w) and Amul Infant Spray (70% w/w) was mixed and molded into a pellet as described above.

2.2. Food analysis

2.2.1. Calorific content

It was determined by multiplying the weight of each macronutrient with its respective energy density per gram (For Protein and Carbohydrate: 4 kcal/g and Fat 9 kcal/g [18]). The total calorific content was obtained by the sum of the individual calorie content. The percent energy (kilocalorie) obtained from each macronutrient was obtained by dividing the individual kilocalorie content and total kilocalorie content multiplied by 100.

2.3. Proximate analysis

2.3.1. Moisture content

5 g of feed was dried in pre-weighed crucible in an oven at 100 °C and cooled. The process of heating and cooling was repeated till a constant weight was obtained [19].

$$\text{Moisture content}\,(\%) = \frac{[\text{Initial weight(g)} - \text{Final weight(g)}]}{\text{Weight of sample(g)}} * 100.$$

2.3.2. Protein content

100 mg of sample, 1 g of digestion mixture (Copper sulphate: Selenium: Potassium sulphate in a ratio 1:1:20) and 20 ml of concentrated sulfuric acid was digested in a Kjeldahl flask and. Post dilution, 10 ml of the sample and 10 ml of 40% Sodium hydroxide was distilled. The liberated ammonia was collected into a receiver containing 25 ml of 4% Boric acid and methylene blue. This was back titrated using 0.02 N Hydrochloric acid. A reagent blank was similarly treated. The protein content was determined by using the following formula [19].

$$\textbf{Protein content}\left(\frac{\textbf{g}}{\textbf{100g}}\right) = 6.25 * \frac{\text{Acid required to neutralize sample(ml)} * \text{acid required to neutralize blank (ml)} * \text{final volume}}{\text{weight of sample (g)} * \text{aliquot volume (ml)}}$$

2.3.3. Fat content

1 g of sample, methanol and chloroform (2:1) was vortexed for 20 min. To this, 1 ml of chloroform and 1.8 ml of distilled water was added. On centrifugation, organic layer was evaporated. The fat content was determined by subtracting difference in weights [20].

2.3.4. Fatty acid profile analysis

The fat obtained by following the Bligh and Dyer method was further analyzed by Gas chromatography (GC) [21]. Fatty acids were transmethylated using 2 M methanolic sodium hydroxide followed by 2 M methanolic hydrochloric acid to obtain Fatty Acid methyl Esters (FAME). FAMEs were analyzed by GC (Shimadzu GC 2014; M/s Shimadzu, Kyoto, Japan) fitted with a flame ionization detector for identifying fatty acids. FAME dissolved in hexane was analyzed using GC with the following conditions: Column-Omegawax TM 320 fused silica capillary column (30 m × 0.32 mm × 0.25 μm), Split ratio - 1/10, Injection temperature used was - 250 °C, Detector (FID) temperature - 260 °C, Column temperature - 200 °C. The peaks were identified by comparing with authentic standards (Supelco® 37 Component FAME Mix, Bangalore, India). Peak areas above 1% of total were only considered for calculation of % composition of fatty acids.

2.3.5. Crude fibre

2 g of moisture and fat free samples and 200 ml of 0.255 N Sulfuric acid was boiled for 30 min and filtered. The residue was washed with hot water and boiled with 200 ml 0.313 N Sodium hydroxide for 30 min. The residue was washed with hot water followed by washing with alcohol and ether (crucible labelled as We). It was dried overnight at 80–100 °C in a crucible and weighed. The crucible was heated in a muffle furnace at 600 °C for 2–3 h, cooled and weighed again (crucible labelled as Wa). The difference in the weights (V-Wa) represented the weight of crude fibre [19].

$$\text{Crude fibre} = \frac{\{[100 - (\text{Moisture(g)} + \text{Fat(g)})] * (\text{We} - \text{Wa})\}}{\text{Weight of moisture and fat ree sample(g)}}$$

2.3.6. Ash content

5 g of feed was heated in a muffled furnace for 3–5 h at about 600 °C in a crucible. This was repeated till constant weight was obtained and the ash was almost white or grayish white in color [19].

2.3.7. Carbohydrate content

Carbohydrate content was determined as follows [19].

$$\begin{aligned}\textbf{Carbohydrate content} = 100 - [&\text{Moisture content(g/100g)} \\ &+ \text{Protein content((g/100g)} \\ &+ \text{Fat content(g/100g)} \\ &+ \text{Ash content(g/100g)} \\ &+ \text{Crude fibre content(g/100g)}]\end{aligned}$$

2.4. Mineral analysis

2.5 g of feed and 25 ml of concentrated Nitric acid was boiled for 45 min 10 ml of 70% Perchloric acid was added to the cooled solution and boiled till the solution became colorless. This was filtered and made up to 100 ml with deionized double distilled