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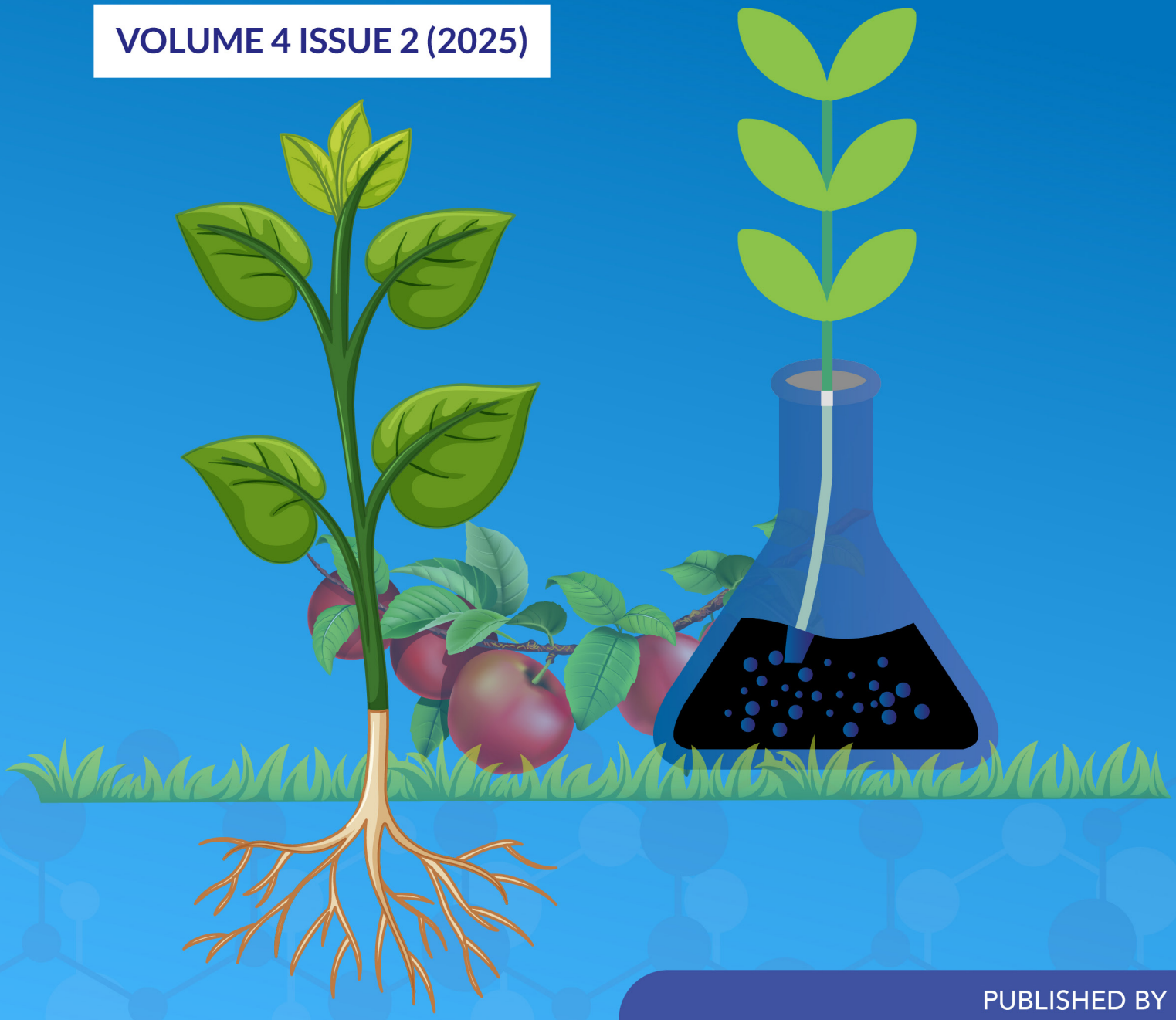

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Evaluation of the Nutritional Profile and Obesogenic Potential of a Formulated High-Fat Diet

Edith Reuben[1], Bruno Chukwuemeka Chinko[2*], Nimisoere Peace Batubo[1], Precious Whiskey Ikete[3], Fortune Somiari Amah-Tariah[1]

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ABSTRACT

High-fat diets (HFDs) are commonly used in nutritional research to model obesity and associated metabolic disorders. However, detailed profiling of their nutritional composition and physiological effects is essential for model validation. The present study developed standardized HFDs using locally available beef tallow as a cost-effective fat source and evaluated both the nutritional profile of these formulated diets and their efficacy in promoting obesity progression using male Wistar rat models. Fifteen (15) male Wistar rats were locally sourced and used for the study. They were grouped into three (3) of five (5) animals each. Group I served as the control and received standard rat chow (SRC) while groups II and IIIreceived SRC blended with 10% (HF-10) and 20% (HF-20) beef tallow respectively. The experiment lasted for ten (10) weeks during which all the animals were allowed access to feed and water *ad libitum*. Proximate analysis of the rat diets and faecal matter were determined using standard methods. Feed efficiency, nutrient digestibility, and morphometric parameters were determined using standard formula. Results from the study indicate that protein and fibre content of the rats' feed was reduced with increased fat supplementation with the HF-20 rat showing the highest energy (4144.1 Kcal/kg) and 64.8% increase in feed efficiency compared to the SRC diet. Also, HF diets increased body weight (35%), Lee's obesity index (18.5%), abdominal circumference (43.5%), absolute adipose tissue weight (776.43%) and adiposity index (529.2%) in a dose-dependent manner. The faecal proximate composition analysis showed a slight increase in ash and lipid excretion with an enhanced fat digestibility coefficient. Evidence from the present study shows that a high-fat diet containing 20% beef tallow (HF-20) effectively induced obesity and associated metabolic disturbances in male Wistar rats. The findings demonstrate a clear dose-response relationship between dietary fat content and the development of obesity-related phenotypes. These findings highlight the use of HFD in understanding the pathophysiology of obesity and metabolic disease, while also providing a practical animal obesity model for preclinical research.

INTRODUCTION

Obesity is a chronic metabolic disorder characterized by an abnormal or excessive accumulation of body fat that adversely affects health. Recognized as a global epidemic by the World Health Organization (OECD/WHO, 2024; WHO, 2025). It is a leading risk factor for non-communicable diseases (NCDs) and represents a global public health crisis, with prevalence increasing across high, low, and middle-income countries (Azeez, 2022; Islam *et al.*, 2024). Obesity is a complex, metabolic, chronic, progressive, neurobehavioral disorder characterized by an increase in adiposity, which promotes adipose tissue dysfunction and abnormal deposition of fat mass resulting in adverse metabolic, biomechanical, and psychosocial health concerns (Brown *et al.*, 2024; Ikete & Chinko, 2022). It is associated with increased risks for several comorbidities, such as insulin resistance, type 2 diabetes, dyslipidaemia, hypertension, cardiovascular diseases, atherosclerotic cerebrovascular disease, chronic liver disease, gallbladder disease, colorectal cancer, osteoarthritis, psychosocial problems as well as a higher mortality rate (Zhang *et al.*, 2014). The development of obesity primarily arises from a chronic energy imbalance, characterized by sustained caloric intake exceeding energy expenditure. This imbalance is influenced by factors such as excessive energy consumption, physical inactivity, and genetic predisposition (Bays *et al.*, 2024; Billington *et al.*, 2000; Kopelman, 2000; Showalter *et al.*, 2018). While the aetiology of obesity is multifactorial, a prolonged positive energy balance remains a widely accepted mechanism underlying its pathogenesis. Notably, excessive energy intake has been identified as a key driver of obesity, with studies suggesting it may play a predominant (if not exclusive) role in weight gain (Bays *et al.*, 2024; Hu *et al.*, 2018).

Long-term management and prevention of obesity rely on lifestyle modifications, including diet and physical activity, while pharmacological and surgical interventions may be necessary in severe cases to achieve substantial weight loss (Encinosa *et al.*, 2005; Ikete & Chinko,

[1] Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Port Harcourt, Nigeria.
[2] Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Port Harcourt, Nigeria.
[3] Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Federal University Otuoke, Nigeria
* Corresponding author's e-mail: bruno.chinko@uniport.edu.ng

2022; Kushner, 2014). These interventions are enabled by research utilizing experimental models of obesity primarily rodents, to mimic human obesity and its metabolic complications (Hariri & Thibault, 2010). High-fat diets (HFDs) are well-established inducers of obesity in both humans and animal models reinforcing that obesity primarily arises from excessive energy consumption (Coelho *et al*., 2011; Folorunso *et al*., 2020), with a dose-dependent relationship between dietary fat content and increased body weight or adiposity (de Wit *et al*., 2011; Enos *et al*., 2013). The HFD used in experimental obesity models, typically comprises 40–60% of total caloric intake from fats with the type of fat, carbohydrate content, and protein sources significantly influencing metabolic outcomes (An *et al*., 2022; Wali *et al*., 2020). Lard and beef tallow, which are rich in saturated fatty acids (SFAs), are commonly used fat sources in high-fat diets (HFDs). Alternatively, plant-derived oils such as corn and safflower oil which are high in polyunsaturated fatty acids (PUFAs) are also employed in the experimental formulation of HFDs (Showalter *et al*., 2018; Wali *et al*., 2020). These diets exhibit higher energy density compared to standard rodent chow due to their elevated fat content, which contributes to their obesogenic properties (Sadie-Van Gijsen & Kotzé-Hörstmann, 2023).

The reliance on expensive commercial HFDs or nutritionally incomplete formulations presents major challenges for obesity research, particularly in resource-limited settings where diet-induced metabolic disorders are increasingly prevalent. Developing standardized yet affordable HFDs using regionally available fat sources could address both the nutritional inadequacies of current commercial formulations and the accessibility limitations of prepackaged diets. The present study is therefore aimed at formulating a standardising an HFD using locally and commonly available beef tallow and evaluating the nutritional composition of the HFD and monitoring its progression on induction of obesity using male Wistar rat models. This may lead to the development of evidence-based, standardized high-fat diets (HFDs) that are both cost-effective and nutritionally complete using locally available ingredients. This formulation could significantly improve the accessibility and reproducibility of formulated HFDs, particularly in low-income research settings, where the prohibitive expense of imported, commercial obesogenic diets often restrict experimental capacity.

MATERIALS AND METHODS

Research Animals

Fifteen (15) male Wistar rats, 120-130g, were sourced from the animal house of the Department of Human Physiology, University of Port Harcourt, and used for the study. Males were selected to eliminate potential hormonal variations from the oestrous cycle that influence energy metabolism and to avoid inherent sex differences in body composition as females typically exhibit higher adiposity. The rats were maintained in well-ventilated wooden cages under controlled conditions (12:12 hr light-dark cycle; 28-31°C; 45-50% humidity) with ad libitum access to standard rat chow (SRC) and water. The floor of the cages was lined with sawdust and cleaned daily. The animals were allowed two (2) weeks of acclimatization before the commencement of the study.

Formulation of high-fat diet

The experimental high-fat diet was prepared following the previously described protocol from our centre (Alor & Chinko, 2022) with modifications to the preparation schedule. Fresh beef tallow was acquired from a local abattoir in the Aluu community, Rivers State under strict hygienic conditions immediately post-slaughter to ensure quality and minimize oxidation. The tallow was washed; impurities and bone particles were removed. It was then boiled and allowed to cool before the fat was removed and refrigerated until the time for use. The beef tallow was weighed using a digital weighing scale and melted before mixing with commercially standard rat chow. Two distinct high-fat diets (HFD) formulations were prepared: the first comprised 90% standard rat chow (SRC) supplemented with 10% beef tallow (HF-10), while the second consisted of 80% standard chow (SRC) blended with 20% beef tallow (HF-20). The obesogenic diets were prepared daily to avoid rancidity.

Experimental Design

The fifteen (15) male Wistar rats were randomly into three (3) groups of five (5). Group I served as the control and received SRC and water ad libitum while groups II and IIIreceived HF-10 and HF-20 respectively alongside water ad libitum. The experimental procedure lasted for ten (10) weeks.

Feed Analysis and Calorific Content

The proximate analysis of the SRC (Apple and Pears, Ogun State Nigeria Ltd.), HF-10 and HF-20 diets was conducted at the Department of Biochemistry, Faculty of Science, University of Port Harcourt. The calorific content was calculated by multiplying the weight of each macronutrient by its respective energy density per gram: 4 kcal/g for protein and carbohydrates and 9 kcal/g for fat (Food And Agriculture Organization of The United Nations, 2003). The total caloric value was determined by summing the individual calorie contributions while the percentage of calories from each macronutrient was derived by dividing its kilocalorie contribution by the total kilocalorie content and multiplying it by 100.

Proximate analysis

Crude Protein Determination

Crude protein was determined using the micro-Kjeldahl method, which measures nitrogen as the characteristic element in protein rather than the protein itself. A mixture of 15 g of potassium sulphate and 0.5 g of copper (II) sulphate was prepared in an 800 mL Kjeldahl flask. Approximately 2 g of SRC, HF-10 and HF-20

were weighed (Mettler Toledo AB 204) and transferred into the flask, followed by the addition of 25 mL of concentrated sulphuric acid. The flask was swirled to mix the contents and then inclined on a heating device in a fume cupboard. Heating continued until foaming ceased and the contents liquefied. Gentle boiling was maintained using the digestion catalyst, with occasional rotation of the flask, until the liquid turned clear and light blue. Digestion was continued for an additional 1.5 hours, ensuring a total digestion time of at least 2 hours. The flask was then cooled to about 40°C, and 50 mL of distilled water was cautiously added. After mixing, the solution was allowed to cool further. The digested solution was transferred into a 250 mL standard flask and rinsed several times to reach the mark. A 50 mL aliquot of the digest was transferred to a steam distillation apparatus. In a separate conical flask, 25 mL of 4% boric acid solution was prepared and positioned under the condenser, ensuring the outlet was submerged in the liquid. Then, 35 mL of 33% sodium hydroxide solution was added to the distillation flask, and steam distillation was carried out for 4 minutes after the first drop of distillate or until the distillate was no longer alkaline. The conical flask was then lowered so that the condenser outlet was above the liquid level, and distillation continued for an additional minute. Next, four drops of indicator solution (0.2 g methyl red and 0.1 g methylene blue in 100 mL ethanol) were added to the distillate, which was then titrated with 0.1 M hydrochloric acid until a grey endpoint was reached. The analysis was repeated with a second 50 mL portion of the digest. A complete blank determination was also performed regularly for calibration.

% Nitrogen content = ((ml of standard acid-ml of blank) × N of Acid × 1.4007)/(weight of sample in grams)

Nitrogen was finally converted to crude protein by multiplying by 6.25 and expressed in g/100g.

Determination of Moisture Content

Moisture content was determined by weighing the SRC, HF-10 and HF-20 in a porcelain crucible. The crucible with the sample was heated in an electric oven for about 6 hours at 105°C. It was then cooled in a desiccator and weighed again.

% Moisture content = (weight of moisture obtained (gm))/(weight of sample (gm)) × 100

Determination of Ash Content

The ash content was determined following the Association of Official Analytical Chemists (AOAC) method. Approximately 2 g of each sample (SRC, HF-10, and HF-20) was weighed in a pre-cleaned, pre-weighed crucible using an analytical balance (Mettler Toledo AB 204), and the initial weight was recorded. A muffle furnace was preheated and stabilized at 600°C. The weighed sample was evenly distributed in the crucible and carefully placed inside the furnace using tongs. The samples were ashed at the set temperature for 45 minutes to ensure complete combustion of organic matter. After ashing, the crucible was removed from the furnace and transferred to a desiccator to cool, preventing moisture absorption before final weighing. The ashed sample on the crucible was weighed, and the percentage of ash was determined using the formula:

% Ash content = Weight of Ash/Initial Weight of Sample ×100

Determination of Crude Fibre

The crude fibre content was determined through a sequential extraction protocol. Approximately 2 g of each sample (SRC, HF-10, and HF-20) was mixed with a sulphuric acid solution (1.25%) and maintained at boiling temperature for 30 minutes to solubilize non-fibrous components. The resulting acid-insoluble residue was isolated through vacuum filtration using a Whatman 4 filter paper and washing with warm (60°C) distilled water until a neutral pH was achieved. The resulting residue was then mixed with 1.25% sodium hydroxide solution at boiling temperature for 30 minutes to remove proteinaceous and hemicellulosic materials. The alkali-treated residue was similarly filtered and washed to neutrality. The purified fibrous residue was then quantitatively transferred to a pre-weighed crucible and dried to constant mass at 105±2°C in a forced-air oven (W1). To determine the inorganic contaminant fraction, the dried residue was then ashed in a muffle furnace at 550±25°C for 4 hours and recorded as (W2). The crude fibre content was calculated gravimetrically by subtracting the ash mass from the pre-ashed residue mass, with results expressed as a percentage of crude fibre relative to the original sample mass.

% Crude Fibre content = (W1-W2 (g))/(Initial Weight of Sample (g)) ×100

This analytical approach conforms to the standard principles of fibre quantification, effectively isolating the acid- and alkali-insoluble dietary fibre fraction while accounting for mineral contamination through the ashing correction.

Determination of Crude Lipid

About 3 g of the samples (SRC, HF-10 and HF-20) were weighed and poured onto a dry filter paper, which was then rolled carefully. The rolled filter paper was placed into an extraction thimble, and the fat was extracted using a Soxhlet apparatus with ethyl ether as the solvent.

After extraction, the fat-containing solvent was transferred into a pre-weighed flask, and the ether was evaporated. To ensure complete removal of residual solvent, the flask was placed in an air oven at 100°C for 30 minutes. Once dried, the flask was transferred to a desiccator to cool to room temperature. Finally, the flask was reweighed, and the total fat content was calculated.

% Fat content = (weight offat/lipid obtained (gm))/(weight of sample (gm)) × 100

Determination of Carbohydrate content

The carbohydrate content was calculated by difference, whereby the sum of the measured proximate components (moisture, crude protein, crude fat, ash, and crude fibre)

was subtracted from 100%. This approach assumes that the remaining fraction primarily consists of digestible carbohydrates, including sugars and starches.

%Carbohydrate content = 100 – [Moisture content(g/100g) + Protein content(g/100g) + Fat content(g/100g) + Ash content(g/100g) + Crude fibre content(g/100g)]

Abdominal and thoracic circumferences and of Lee Obesity Index

At the end of the experiment, morphometric parameters were recorded for all obese Wistar rats. The abdominal circumference (AC) and thoracic circumference (TC) were measured using a flexible measuring tape. Additionally, each animal's body length (nose-to-anus distance) and body weight were determined.

The Lee Obesity Index was subsequently calculated using the following formula:

Lee Obesity Index =($\sqrt[3]{}$weight(g))/(naso-anal lenght (cm))

The abdominal/thoracic ratio (AC/TC ratio) was calculated as follows:

AC/TC ratio =(Abdominal Circumference (cm))/(Thoracic Circumference (cm))

Determination of energy intake and feed efficiency

Determination of Energy Intake

Energy Intake (g) = Mean food consumed X dietary metabolizable energy (Kcal)

Determination of Feed Efficiency

Feed efficiency is the ability to convert ingested food into body mass (Zhang *et al*., 2023). The weight changes of the animals in the SRC, HFD-10 and HF-20 groups were summed up weekly to the end of the study. The weekly feed intakes were calculated and the feed efficiency was calculated as (Zhang *et al*., 2023).

Feed Efficiency= (weight gain (g))/(Feed intake (g))

Carcass Analysis and Relative Organ Weight

Carcass analysis of unshaven rats was carried out on the animals. After euthanasia by cervical dislocation, thoracotomy, and median laparotomy were performed on the animals to remove the organs as well as the adipose tissue from the retroperitoneal and epididymal compartments. The fat pads, which include the retroperitoneal fat attached to the posterior abdominal wall near the kidneys and inguinal fat, visceral fat (subcutaneous adipose tissue between the lower rib cage and thigh), epididymal fat located in the lower abdomen and connected to the epididymis will be dissected, isolated and weighed. The adiposity index was derived by the sum of epididymal, visceral, and retroperitoneal fat weights and the adiposity index is expressed as a percentage.

Adiposity Index = (Total body fat (g))/(Final body weight (g)) × 100

The liver, kidneys, heart and brain of the Wistar rats were harvested and weighed and are expressed as a percentage of the body weight.

Relative organ weight = (Weight of organ (g))/(Final body weight (g)) × 100

Fecal matter proximate analysis and apparent nutrient digestibility coefficients

Faecal output was recorded three times per week throughout the experimental period. Upon completion of the experiment, faeces collected from individual rats were stored in pre-labelled plastic containers and subsequently pooled by the experimental group. The pooled faecal samples were oven-dried, homogenized, and ground to a fine powder for proximate analysis (crude fibre, crude protein and ash content). These prepared samples were also used to determine apparent nutrient digestibility coefficients by previously described methods (Ingweye, 2015).

Apparent digestibility coefficient = (Nutrient in feed-Nutrient in faeces)/(Nutritient in feed) × 100

Ethical Consideration

The animals were cared for and handled in full compliance with the most stringent ethical guidelines established for the use of animals in scientific research. The study protocol was approved by the Research Ethics Committee of the University of Port Harcourt with approval number UPH/CEREMAD/REC/MM107/054.

Statistical Analysis

The statistical analysis was performed using IBM Statistical Product and Service Solutions (SPSS)version 26. The one-way ANOVA was used to determine the difference among the groups followed by Fisher's Least significant difference. A p-value of less than 0.05 was considered statistically significant ($p<0.05$).

RESULTS AND DISCUSSIONS

Table 1 shows the proximate composition of the three rat diets; the standard rat chow (SRC), SRC supplemented with 10% beef tallow (HF-10), and SRC supplemented with 20% beef tallow. Our data show that the protein content decreased with increasing beef tallow inclusion (SRC: 19.38 g/100g; HF-10: 17.64 g/, HF-20: 14.22 g/100g). The ash content was highest in SRC (6.75 g/100g) and declined with tallow supplementation

Table 1: Feed Proximate Analysis

Nutrients (g/100g)	SRC	HF-10	HF-20
Protein	19.38	17.64	14.22
Ash	6.75	6.54	5.61
Carbohydrate	50.24	45.72	41.69
Fibre	4.22	3.84	3.5
Moisture	15.04	14.23	13.77
Lipids	4.37	12.03	21.21

SRC= standard rat chow
HF-10= SRC+ 10% beef tallow
HF-20= SRC+ 20% beef tallow

(6.54 g/100g and 5.61 g/100g for HF-10 and HF-20 respectively. Carbohydrates followed a similar trend of decreasing with increasing tallow supplementation (SRC: 50.24 g/100g; HF-10: 45.72 g/100g; HF-20: 41.69 g/100g). Also, dietary fibre was highest in SRC (4.22 g/100g) and decreased with tallow addition: 3.84 g/100g and 3.50 g/100g for HF-10 and HF-20 respectively. Expectedly, lipid content increased proportionally with tallow inclusion (SRC: 4.37 g/100g; HF-10: 12.03 g/100g; HF-20-21.21 g/100g) while moisture content marginally reduced with tallow inclusion; SRC: 15.04 g/100g vs. 14.23 and 13.77 g/100g in HF-10 and HF-20 respectively.

Table 2: Feed calorific content, percentage energy and efficiency

Calorific content and % energy	**SRC**	**HF-10**	**HF-20**
Protein calorific content (4 Kcal)	77.52	70.56	56.88
Protein energy percentage (%)	21.4	19.5	13.7
Carbohydrate (4 Kcal)	200	182.86	166.76
Carbohydrate energy percentage (%)	55.40	50.50	40.00
Fat calorific content (9 Kcal)	39.33	108.18	190.89
Fat energy percentage (%)	10.8	29.9	46.1%
Total calorific content	361.85	361.62	414.41
Total calorific content in Kcal/kg	3618.5	3616.2	4144.1
Feed Efficiency	37.63	43.82	62.00

SRC= standard rat chow
HF-10= SRC+ 10% beef tallow
HF-20= SRC+ 20% beef tallow

Table 2 presents the calorific content and percentage energy contribution of macronutrients (protein, carbohydrates, and fat) across three feed types: SRC, HF-10, and HF-20. Total calorific content per kilogram ranged from 3616.2 Kcal/kg (SRC) to 4144.1 Kcal/kg (HF-20), with HF-20 exhibiting the highest energy density. Protein contributed 21.4%, 19.5%, and 13.7% of total energy in SRC, HF-10, and HF-20, respectively, showing a progressive decline. Carbohydrates were the primary energy source in SRC (55.4%) but decreased to 40% in HF-20. Conversely, fat energy contribution increased from 10.8% (SRC) to 46.1% (HF-20), reflecting a shift toward higher fat content in HF feeds. The HF-10 and HF-20 diets showed a 16.5% and 64.8% increase in feed efficiency compared to the SRC diet

Table 3 compares the impact of the various feed

Table 3: Effect of feed formulations on morphometric parameters of male Wistar rats.

Parameters	**SRC**	**HF-10**	**HF-20**
Body weight (g)	242.12± 2.92	272.16±0.02[a]	335± 1.12[a,b]
Lee's Obesity Index	0.32±0.01	0.33±0.02	0.38±0.01[a,b]
Abdominal circumference (AC)	15.70+0.35	15.52±0.97	22.48±0.93[a,b]
Thoracic circumference (TC)	15.40±0.24	16.40±0.39	16.44±0.70
AC/TC ratio	1.02±0.04	0.95±.06	0.95±.06
Absolute adipose tissue weight (g)	1.57±0.26	2.22±0.55[a]	13.76±1.43[a,b]
Adiposity Index (%)	0.65±0.11	0.82±0.20[a]	4.09±0.34[a,b]

SRC = standard rat chow; HF-10= SRC+ 10% beef tallow; HF-20= SRC+ 20% beef tallow
All values are expressed as Mean ± standard error of the mean
[a]p < 0.05, significant change when compared with the control,
[b]p < 0.05, significant change when compared with the HF-10 group

formulations (SRC (control), HF-10 (10% fat), and HF-20 (20% fat) on key morphometric parameters in male Wistar rats. The results indicate that the body weight of animals increased significantly from 242± 2.92g as seen in the SRC diet group to 272±0.02g and 335± 1.12g in the HF-10 and HF-20 diets ($p<0.05$). Following this, Lee's obesity index increased significantly from 0.32 in the SRC group to 0.38 in the HF-20 group ($p<0.05$), indicating a higher degree of obesity. Similarly, abdominal circumference (AC) also significantly increased in the HF-20 group (22.48 cm) compared to the SRC (15.70 cm) and HF-10 (15.52 cm) groups ($p<0.05$). However, the thoracic circumference (TC) only slightly increased among the high-fat diet groups compared to the SRC. Hence, the ratio of abdominal to thoracic circumference (AC/TC) remained consistent across all groups. Absolute adipose tissue weight and adiposity index showed a dose-dependent increase with dietary fat content. While the HF-10 group showed moderate increases in fat mass (2.22 g) and adiposity (0.82%), the HF-20 group showed a marked elevation (13.76 g and 4.09%, respectively).

Table 4 shows the impact of dietary formulation SRC,

Table 4: Effect of feed formulations on relative organ weights of male Wistar rats

Relative organ weights (g)	SRC	HF-10	HF-20
Left kidney	0.28±0.02	0.40±0.03	0.41±0.07
Right kidney	0.28±0.01	0.35±0.01[a]	0.42±0.02[a, b]
Liver	0.27±.014	2.95±0.26[a]	3.40±0.19[a]
Spleen	0.27±0.01	0.28±0.02	0.21±0.02[b]
Heart	0.32± 0.02	0.36±0.01	0.54±0.03[a, b]
Pancreas	0.36±0.03	0.37±0.04	0.54±0.03[a, b]
Left testis	0.54±0.03	0.54±0.01	0.45±0.01[a, b]
Right testis	0.57±0.04	0.54±0.01	0.47±0.02[a]

SRC = standard rat chow; HF-10= SRC+ 10% beef tallow; HF-20= SRC+ 20% beef tallow
All values are expressed as Mean ± standard error of the mean
[a]p < 0.05, significant change when compared with the control,
[b]p < 0.05, significant change when compared with the HF-10 group

HF-10 and HF-20 on the relative organ weights of male Wistar rats. The results revealed significant alterations in multiple organs, demonstrating a dose-dependent relationship with dietary fat content. Both kidneys exhibited increased weights in high-fat groups compared to SRC, with the right weighing heavier in the HF-10 (0.35 ± 0.01 g) and HF-20 (0.42 ± 0.02 g) compared to the control, $p < 0.05$). Similarly, the liver underwent apparent hypertrophy in fat-fat groups as shown as their relative weight increased from 0.27 ± 0.01 g (SRC) to 2.95 ± 0.26 g (HF-10) and 3.40 ± 0.19 g (HF-20) ($p < 0.05$). However, the spleen showed a reduced weight in the HF-20 group (0.21 ± 0.02 g) compared to SRC (0.27 ± 0.01 g) and HF-10 (0.28 ± 0.02 g) ($p < 0.05$). The heart and the pancreas increased significantly in HF-20 (heart: 0.54 ± 0.03 g; pancreas: 0.54 ± 0.03 g) vs SRC (heart: 0.32 ± 0.02 g; pancreas: 0.36 ± 0.03 g) and HF-10 ($p < 0.05$) while testicular weights declined in the HF-20 group (left: 0.45 ± 0.01 g; right: 0.47 ± 0.02 g) relative to SRC (left: 0.54 ± 0.03 g; right: 0.57 ± 0.04 g) ($p < 0.05$).

The faecal proximate composition analysis revealed distinct patterns in nutrient excretion across the three dietary groups (SRC, HF-10, and HF-20) as shown in Table 5. Crude protein content showed minimal variation between diets (19.7-20.5%), suggesting consistent protein metabolism regardless of dietary fat content. There was a slight increase in ash excretion in the high-fat groups (21.4% in HF-10, 20.4% in HF-20) compared to the control (18.9%). Carbohydrate and fibre fractions demonstrated remarkable stability across all diets, with values around 28-29% for carbohydrates and 14-15% for fibre. Moisture content showed a modest reduction in high-fat groups (12.6-13.0%) compared to the control (13.3%).

Table 6 shows the apparent nutrient digestibility coefficients in macronutrient utilization across the experimental diets. Fat digestibility exhibited a pronounced dose-dependent increase, rising from 42.1% in the SRC group to 73.3% in HF-10 and further to 84.7% in HF-20, demonstrating enhanced lipid absorption efficiency with higher dietary fat content.

Protein digestibility showed negative digestibility coefficients in both SRC (-5.7%) and HF-10 (-7.7%) groups while the HF-20 diet showed a remarkable reversal to positive protein digestibility (39.7%). Carbohydrate digestibility displayed an inverse relationship with dietary fat content, gradually declining from 41.4% in SRC to 38.0% in HF-10 and 31.6% in HF-20.

Table 5: The faecal proximate composition analysis

Nutrients (%)	SRC	HF-10	HF-20
Crude Protein	20.49	19.7	19.86
Ash	18.93	21.36	20.44
Carbohydrate	29.42	28.35	28.53
Fibre	15.35	14.79	14.89
Moisture	13.28	12.6	13.03
Crude Fat	2.53	3.2	3.25

SRC= standard rat chow
HF-10= SRC+ 10% beef tallow
HF-20= SRC+ 20% beef tallow

Table 6: The apparent nutrient digestibility coefficients of various feed formulations in male Wistar rats.

Nutrient Digestibility	SRC	HF-10	HF-20
% Fat digestibility	42.1	73.34	84.67
% Protein	-5.73	-7.70	39.66
% Carbohydrate	41.44	37.99	31.57

SRC= standard rat chow
HF-10= SRC+ 10% beef tallow
HF-20= SRC+ 20% beef tallow

Discussion

Obesity and its associated metabolic disorders have become major public health concerns worldwide, with high-fat diets (HFDs) widely implicated in their development (Duan *et al.*, 2018; Tang *et al.*, 2024). High-

fat diets (HFDs) are employed in experimental settings to induce obesity, with formulations typically deriving 40–60% of total caloric intake from lipids (An *et al*., 2022; Wali *et al*., 2020). This approach reinforces the well-established paradigm that obesity development is fundamentally driven by chronic positive energy balance. To address the dual challenges of nutritional inconsistencies in non-commercial high-fat diet formulations and limited accessibility of prepackaged diets, the study developed standardized HFDs using regionally available beef tallows as a cost-effective fat source. The present investigation systematically evaluated both the nutritional profile of these formulated diets and their efficacy in promoting obesity progression using male Wistar rat models.

Proximate analysis of the diets in the present study revealed that increasing beef tallow supplementation from 10 to 20% reduced the protein content from 19.38 to 14.22 g/100g, carbohydrates from 50.24 to 41.69 g/100g and fibre from 4.22 to 3.50 g/100g) while increasing the lipid content from 4.37 to 21.21 g/100g (Table 1). The reduction in carbohydrate content is consistent with findings from a previous study that reported that HFDs typically displace carbohydrates to elevate energy density (Hariri & Thibault, 2010). The HF-20 diet exhibited the highest energy content (4144.1 kcal/kg), with fat contributing 46.1% of total energy, which is consistent with diet-induced obesity models (Hariri & Thibault, 2010; Winzell & Ahrén, 2004). The dramatic shift in the energy contribution from fat is a key feature of an obesogenic diet, which promotes the accumulation of fat stores while potentially impairing glucose and lipid metabolism (Grabner *et al*., 2021; Richard *et al*., 2020).

However, the decline in protein-derived energy from 21.4% in SRC to 13.7% in the HF-20 diet contrasts with some commercial HFDs where protein levels are often stabilized to prevent malnutrition (Levin *et al*., 1997). HFDs, particularly those in saturated fats suppress protein metabolism in favour of lipid utilization (Yiannakou *et al*., 2023). Feed efficiency measures the ability of the animal to convert ingested food into body mass (Benoit & Mottet, 2023). The 64.8% higher feed efficiency in HF-20 mirrors predictive models linking energy density to calorie intake efficiency in rats (Beheshti *et al*., 2018) and suggests that high-fat diets support the promote obesity and lipogenesis and promote fat storage, due to the higher caloric density provided by the fat by lipogenesis and, even when food intake is not significantly increased (Jahan *et al*., 2024; Strable & Ntambi, 2010; Zhang *et al*., 2023).

The present study demonstrated significant dose-dependent increases in body weight and adiposity markers in male Wistar rats fed beef tallow-supplemented high-fat diets (HFDs).

The progressive increase in body weight from 242 ± 2.92 g (SRC) to 335 ± 1.12 g (HF-20) ($p<0.05$) corresponds with previous reports of HFD-induced weight gain in rodent models (Levin *et al*., 1997), however, the magnitude of increase (38% in HF-20 vs SRC) exceeds the 20-30% typically reported for 60% fat diets (Buettner *et al*., 2007) but falls with the 35% weight increase for obesity classification reported in our facility (Alor & Chinko, 2022). This suggests that have potent obesogenic effects. The elevated Lee's index (0.38 in HF-20) confirms the development of true obesity, not merely increased lean mass, matching the standard criteria (Hariri & Thibault, 2010). The larger increase in abdominal circumference (43% in HF-20 vs SRC) when compared to the change in thoracic circumference (6.7% in HF-20 vs SRC) indicates preferential visceral fat deposition. This aligns with clinical observations of classic abdominal obesity in metabolic syndrome (Choi *et al*., 2015; Després & Lemieux, 2006). The stable AC/TC ratio across groups suggests this regional fat partitioning occurred proportionally at all diet levels. Visceral obesity, as indicated by an elevated AC/TC ratio, is a well-known risk factor for metabolic diseases, including insulin resistance, fatty liver disease, and cardiovascular conditions (Agboola *et al*., 2023). This highlights the detrimental effects of high-fat diets on fat distribution and underscores the importance of abdominal fat as a marker for obesity-related metabolic diseases (Després, 2012; Ritchie & Connell, 2007). The substantial increase (8.8 fold) in absolute adipose tissue weight in high-fat diets (13.76 g in HF-20 vs 1.57 g in SRC) exceeds the 5-6-fold changes previously reported with 45-60% fat diets (Softic *et al*., 2017). The adiposity index (4.09% in HF-20 vs 0.65 in SRC) reached levels comparable to genetically obese Zucker rats (She *et al*., 2013; Sisk *et al*., 2001). This suggests that beef tallow promotes particularly efficient fat storage due to increased caloric intake as observed in this study (Table 1). This contrasts with plant-oil-based HFD showing lower adiposity indices (2.5-3.5%) at similar fat percentages (Cintra *et al*., 2012). Organ weight alterations were particularly striking with an increase observed in the weight of the liver and the kidney and a reduction in the weight of the spleen and testes among the HF-10 and HF-20 diet groups compared to the control SRC group (Table 4). The apparent hepatic hypertrophy (from 0.27 g in SRC to 3.40 g in HF-20) aligns with studies showing lipid accumulation in HFD-fed rats (Softic *et al*., 2017), while renal enlargement may reflect early metabolic stress (Declèves *et al*., 2011). The apparent spleen atrophy in HF-20 (0.21 g vs. 0.27 g in SRC) aligns with a previous study that observed a reduction in spleen weight in mice chronically fed with HFD (Strandberg *et al*., 2009), however, this conflicts with reports of HFD-induced splenomegaly in obese female rats fed with HFD (Altunkaynak *et al*., 2007) suggesting fat-source-specific immune modulation. Testicular weight reduction (0.54 g in SRC to 0.45 g in HF-20) supports evidence of HFD-induced reproductive dysfunction (Aly & Polotsky, 2017; Bakos *et al*., 2011).

Faecal proximate analysis revealed stable protein (19.7–20.5%), carbohydrate (28–29%) and fibre (14–15%) and water (13.28% in SRC vs 12.6 and 13.03% in HF groups) excretions across diets. There was a marginal increase in

ash ((18.9 - 21.4%) and fat (2.53 to 3.25%) in HF groups compared to the control. The rise in fat excretion was lower than expected given the dietary fat load, suggesting enhanced lipid absorption efficiency. The observed faecal fat excretion reflects a saturation of intestinal absorption. These findings demonstrate that while high dietary fat intake led to modest increases in faecal fat excretion, the majority of absorbed lipids are efficiently partitioned into adipose tissue storage leading to disproportionate gains in adiposity despite marginal lipid excretion. The preferential visceral/abdominal fat accumulation suggests adipose tissue acts as a primary lipid dump, particularly for saturated fats. These observations align with the "spillover" hypothesis of lipid metabolism, where adipose tissue buffers excess lipid influx, explaining how even marginally reduced absorption efficiency can coexist with significant obesity development (Ravussin & Smith, 2002; Sakers *et al.*, 2022).

A progressive increase in fat digestibility was observed with rising fat content, which can be attributed to increased bile acid secretion stimulated by fat leading to an enhanced activity of pancreatic lipase and improved micelle formation in the intestine, which facilitate efficient fat emulsification and absorption in high-fat diets (Wang *et al.*, 2025). The apparent negative protein digestibility, especially in the 20% HFD group may indicate inefficient nitrogen utilization, possibly due to metabolic disturbances induced by chronic high-fat intake such as low-grade inflammation, gut microbiota dysbiosis, and increased oxidative stress, which may impair amino acid absorption and increase protein turnover (Wang *et al.*, 2025). Additionally, high-fat diets have been shown to downregulate intestinal amino acid transporters, further reducing protein digestibility (Hijo *et al.*, 2019). The decline in carbohydrate digestibility as seen in a study may be due to the metabolic shift from glucose oxidation to greater reliance on lipid metabolism (Ludwig *et al.*, 2021). This metabolic adaptation is well-documented in high-fat experimental models, where insulin resistance and reduced expression of carbohydrate transporters (e.g., SGLT1, GLUT2) impair glucose uptake and utilization (Hijo *et al.*, 2019; Ludwig *et al.*, 2021).

CONCLUSION

Evidence from the present study shows that a high-fat diet containing 20% beef tallow (HF-20) effectively induced obesity and associated metabolic disturbances in male Wistar rats. The findings demonstrate a clear dose-response relationship between dietary fat content and the development of obesity-related phenotypes. The HF-20 increased body weight, feed efficiency, nutrient digestibility and visceral fat accumulation. These findings highlight the use of HFD in understanding the pathophysiology of obesity and metabolic disease, while also providing a practical animal obesity model for preclinical research.

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