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Biological response and organ morphology of Wistar rats fed differently processed beef steaks

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Abstract

Meat is an animal flesh utilised as food which also serves as the cardinal protein source vide its component elements and amino acids. Despite this, meat wholesomeness has recently been mutilated for its perceived association with polycyclic aromatic hydrocarbons (PAH) in smoked products and heterocyclic aromatic amines (HAA) in cooked products. Hence, this study was aimed at assessing the biological effects, nutritional value and safety of the differently processed beef steaks. Weanling male Wistar rats (n=63) weighing 55±1.9g were allotted in a completely randomized design to six treatments with ten rats per treatment. The rats were assigned to individual metabolic cages and had free access to water and assigned experimental diets *ad-libitum* for 21 days. Feed intake of rats on RBS (57.66 g) was significantly higher ($p<0.05$) than those on BSBS (56.02 g), SBS (50.10 g) and DFBS (46.00 g). Biological value of 91.46 % in rats on DFBS was higher than 89.17 % for RBS, SBS (88.59 %) and BSBS (87.46 %). Moderate fat deposition and scanty infiltration were noticed in the liver rats on DFBS and SBS, respectively. There were observable vascular congestion and tubular necrosis in the kidney of rats on DFBS. Rats on DFBS had fat infiltration in epicardia layer and vascular congestion. Consumption of DFBS altered kidney structure, caused fat infiltration and congested valves of rats. Therefore, dietary DFBS could heighten the risk of liver, kidney and heart diseases.

Keywords: Polycyclic aromatic amines, Heterocyclic aromatic amines, Biological value, Histopathology

1. Introduction

Human epidemiologic and animal studies have shown that diet plays an important role in cancer development [10, 22]. It has been reported that one third of human cancers are related to foods [31] which could be linked with the processing method. Food preparation at high temperatures was reported to elicit the formation of dangerous chemicals, including PAHs and HAAs that could alter genetic make-up [14, 18]. Research [1,19] showed that roasting, smoking, grilling and barbecuing of foods induces PAHs and HAAs which are carcinogenic.

When cooking is done at high temperatures, PAHs production levels become elevated.

Decomposition of organic substances like carbohydrates and proteins could contribute, although the main concentrations of PAHs proved to come from breakdown of fat [21].

The PAHs production occurs when the food dissolved fat dropping are on the heat source, The PAHs then settles on top of the meat which increases smoke or meat fat are broken down as a result of food exposure to heat [26]. The partial burning of the fuel itself is the other likely way for the production of PAHs.

Partial burning of charcoal produces PAHs which are located on top of the foods, then adsorbed [34]. The PAHs production is known to be influenced by the time of cooking [16] food type [11], proximity of food from the flame source, level of the food fat [35] and the allowed fat drip on the flame source and the cooking temperature [7]. Among the numerous PAHs identified, Phenanthrene, Fluoranthene, Pyrene, Benzo [a] anthracene, Chrysene, Benzo [b] fluoranthene, Benzo [k]fluoranthene, Benzo [a] pyrene, Benzo [ghi] Perylene and Indenol [123-cd] pyrene were the most

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[17]. It showed that broiled beef contained PhIP, MeIQx and IQ while ground beef contained DiMeIQx and MeIQx [9, 23, 30].

The formation of HAAs occurred as a result of fish and meat muscle exposure to temperatures higher than 150 °C when cooking [25]. The HAAs are carcinogens and mutagens occurring as parts per billion concentrations in processed meat. The amino acids, sugars and creatinine formerly present in meat are key initiators responsible for production of HAA [27]. Many heterocyclic amines are tagged to be possible carcinogens [36]. It recorded IQ, MeIQ, MeIQx and PhIP as chemicals reasonably expected human carcinogens [36]. Research revealed that nutritional ingestion of HAAs via consumption of heat-processed meat heightened the danger of breast, colon and stomach cancers in humans [20]. It surmised that the common HAAs is PhIP that is frequently present by levels near 40 ng/g [29]. The author also noted that deep-frying primarily generates bits of MeIQx, 4, 8-DiMeIQx and PhIP. Concentrations of 0–3 ng/g MeIQx and 0–70 ng/ PhIP were reportedly discovered in fried chicken [14].

The PAHs and HAAs absorption of occurs in the gastrointestinal tract when consumed, where they are carried to most of the body viscera tissues [33]. Despite avalanche of reports linking processed or cooked red meat products to diseases onset and progressions, there is dearth of information on the effect of the differently processed beef on biological, nutritional and organ histopathology of the teeming consumers of processed beef, which was the objective of this study.

2.Materials and Methods

2.1.Experimental Site

The feeding trial of the rats was carried out at the rat room, department of Animal Science, University of Ibadan, Ibadan located on latitude 7° 26'N and longitude 3° 54'E of the equator.

Beef loin, each weighing 10kg steaks were randomly assigned to four cooking techniques: roasting at 200°C, deep frying at 180 °C, microwaving at 600 watt and traditional smoking using *Tectona grandis* charcoal at 300 °C. The internal temperature of each steak was 75 °C using endorsed meat probe thermometer. Detail of the different processing of the steak has been documented [19]. The differently processed steak was used to formulate diets for the rats. Detail of dietary formulation for the rats is shown in Table 1. The dietary layout is as described below.

T1- Control diet (Standard Casein diet)

T2- Diet containing boiled-sundried beef steak meal (BSBS)

T3- Diet containing deep-fried beef steak meal (DFBS)

T4- Diet containing roasted beef steak meal (RBS)

T5-Diet containing smoked beef steak meal (SBS)

T6- Nitrogen free diet (NFD)

The rats were purchased from the rat section, Department of Biochemistry, University of Ibadan. Weanling male Wistar rats (n=63) weighing 55±1.9g were randomly allotted in a completely randomized design to six treatments with ten rats per treatment. Rats were individually housed in well ventilated stainless-steel metabolic cubicles (Multiple Compartment Rack Feature One Cage Model 1000, England) of 0.35 x 0.22 x 1.35 m. The cages were set at two layers of six cubicles in a 0.95 x 0.25 x 1.35 m; four cage sets were housed in a 41.25 m^2 room. The rats had free access to water and were fed *ad libitum* on experimental diets for 21 days.

Weekly record of feed consumption per rat was obtained by measuring the total amount feed consumed by each (offered feed weight minus the left over). The rats initial weights were recorded and were therefore weighed weekly through the twenty-one-day period. The total weight change was calculated by deducting the initial weight from the final weight as shown below:

$$\text{Weight change} = \frac{\text{Final weight} - \text{initial weight}}{21}$$

Table 1. Composition experimental feeds fed to weanling Wistar rats

Ingredients	Treatments					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Casein	11.11	-	-	-	-	-
Glucose Monohydrate	10.00	10.00	10.00	10.00	10.00	10.00
Corn Starch	65.69	58.65	58.65	58.65	58.65	76.80
Vegetable Oil	5.00	5.00	5.00	5.00	5.00	5.00
Non- nutritive Cellulose	5.00	5.00	5.00	5.00	5.00	5.00
Oyster Shell	0.50	0.50	0.50	0.50	0.50	0.50
Di-calcium Phosphate	1.50	1.50	1.50	1.50	1.50	1.50
Salt	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin Premix	1.00	1.00	1.00	1.00	1.00	1.00
DBS	-	-	18.15	-	-	-
BSBS	-	18.15	-	-	-	-
SBS	-	-	-	-	18.15	-
RBS	-	-	-	18.15	-	-
Total	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Nutrients ME (kcal/kg)	3686.77	3600.00	3600.02	3600.01	3600.00	3766.08
Crude Protein (%)	10.25	10.05	10.04	10.05	10.05	0.00

DBS = Deep fried beef steak, BSBS = Boiled-sundried beef steak, SBS =Smoked beef steak, RBS = Roasted beef steak. T1 – Standard rat diet (Positive Control, T2–Diet containing microwaved beef steak meal, T3 – Diet containing deep fried beef steak meal, T4 – Diet containing roasted beef steak meal, T5- Diet containing smoked beef steak meal, T6- Nitrogen free diet (Negative control)

The feed conversion ratio was calculated by dividing average daily diet ingested (kg) with average daily body weight gain (kg) as shown in equation below:

$$FCR = \frac{\text{Average daily feed consumption}}{\text{Average daily weight change}}$$

The total nitrogen in the diets and the faecal samples of rats were determined using the AOAC [5]. Each sample was analysed in triplicate. This was obtained through the estimation of nitrogen intake, faecal and urinary nitrogen (N) excretion. The biological value was calculated as shown below:

$$\text{Biological value} = \frac{NI - (F-F_o) - (U-U_o)}{NI - (F-F_o)} \times 100$$

Where:
NI = Nitrogen intake of rats on test diet
F = Faecal nitrogen of rats on test diet
F_o = Faecal nitrogen of rats on nitrogen free diet
U = Urinary nitrogen of rats on test diet
U_o = Urinary nitrogen of rats on nitrogen free diet

At the end of week three of trial, six rats from each treatment were selected and tagged. They were denied experimental diet for 12 hours and sacrificed by inactivation with carbon dioxide

The rats were carefully dissected. Liver, kidney and heart were harvested, weighed, fixed in 10 % formalin and processed for histopathological analyses as described [6].

2.2. Statistical Analyses

Data were subjected to analysis of variance using SAS [28] package. Means were separated using Duncan's multiple range test option of the same software at $\alpha_{0.05.}$

3.Results

The biological values of differently cooked beef steaks in rats are shown in Table 2. The feed intake of rat fed RBS (57.66 g) was significantly higher ($p<0.05$) than those on BSBS (56.02 g), SBS (50.10 g), casein (49.60 g), DFBS (46.00 g) and NFD (24.13 g). Weight gain of rats on RBS (25.43 g) was significantly higher ($p<0.05$) than in those on BSBS (22.23 g), Casein (20.35 g) and SBS (19.57 g). However, rats on NFD had negative weight gain (-17.25 g).

Also, the final body weight of rats fed RBS (83.77 g) was higher than in those on BSBS (79.80 g), SBS (74.57 g), casein (73.95 g), DFBS (68.54 g) and NFD (36.76 g). However, the FCR of rats given DFBS diet (3.25) was significantly higher than rats fed SBS diet (2.56), BSBS diet (2.52) and casein diet (2.44) while NFD was -1.40. The biological value of casein diet (94.58 %) was higher than DFBS (91.46 %), RBS (89.17 %), SBS (88.59 %), BSBS (87.46 %) and NFD (-11.52 %).

3.1. Organ Morphology

The liver morphology of rats fed differently processed beef steaks are shown in Plate 1. The liver of rats offered casein diet had normal architecture. The central venules and portal tracts appeared normal and were not congested (see white arrows). Also, the sinusoids were normal without infiltration of inflammatory cells, the hepatocytes showed normal morphology (blue arrow). Also, the livers of rats fed BSBS and RBS diets were similar with those of rats given casein diet. However, the central venules of rats fed DFBS had moderate fat deposition (white arrow). The sinusoids appeared normal without infiltration of inflammatory cells. The hepatocytes showed normal morphology (blue arrow).

The central venules and portal tracts of rats offered SBS appeared normal and were not congested. The sinusoids and hepatocytes appeared normal. However, scanty infiltration of inflammatory cells was seen within the sinusoid (black arrow). Also, there was a focal area of moderate tumour (black arrow) in the liver of rats fed NFD. The central venules and portal tracts appeared normal and were not congested (white arrow). The sinusoids appeared normal without infiltration of inflammatory cells. Other parts of liver parenchyma showed normal hepatocytes.

The morphology of rats' kidney fed differently cooked beef steaks are shown in Plate 2. The renal cortex of rats fed standard casein diets showed normal glomeruli, mesangial cells and capsular spaces. The renal tubules (white arrow) and interstitial spaces appeared normal. There was mild vascular congestion noted (blue arrow). Also, the renal cortex of rats offered BSBS showed normal glomeruli. The mesangial cells, capsular spaces (white arrow) and interstitial spaces appeared normal. While some of the renal tubules lacked lumina spaces (blue arrow). However, there was a poor architecture with a mild to moderate vascular congestion noted (black arrow) in the kidney of rats fed DFBS. The renal cortex showed normal glomeruli, mesangial cells and capsular spaces (white arrow). The renal tubules and interstitial spaces appeared dilated and normal, respectively.

Table 2. Biological Value of Differently Cooked Beef Steaks in Rats

Parameters	Experimental Diets						SEM
	Casein	BSBS	DFBS	RBS	SBS	NFD	
IBW (g)	53.57^{f}	57.55^{b}	54.43^{d}	58.43^{a}	55.00^{c}	54.00^{e}	0.03
FBW (g)	73.95^{d}	79.80^{b}	68.54^{e}	83.77^{a}	74.57^{c}	36.76^{f}	0.02
BWG (g)	20.35^{c}	22.23^{b}	14.14^{e}	25.43^{a}	19.57^{d}	-17.25^{f}	0.02
FI (g)	49.60^{d}	56.02^{b}	46.00^{e}	57.66^{a}	50.10^{c}	24.13^{f}	0.00
FCR	2.44^{d}	2.52^{c}	3.25^{a}	2.27^{e}	2.56^{b}	-1.40^{f}	0.00
BV (%)	94.58^{a}	87.46^{e}	91.46^{b}	89.17^{c}	88.59^{d}	-11.52^{f}	0.00

^{a,b,c,d} Means with similar superscripts along the row are not significantly different (P>0.05);
SEM= Standard error of mean; DFBS (Deep-fried beef steak), SBS (Smoked beef steak), BSBS (Boiled-sundried beef steak), RBS (Roasted beef steak), NFD= Nitrogen free diet; IBW= Initial body weight; FBW=Final body weight; BWG=Body weight gain; FI=Feed intake; FCR=Feed conversion ratio; BV=Biological value

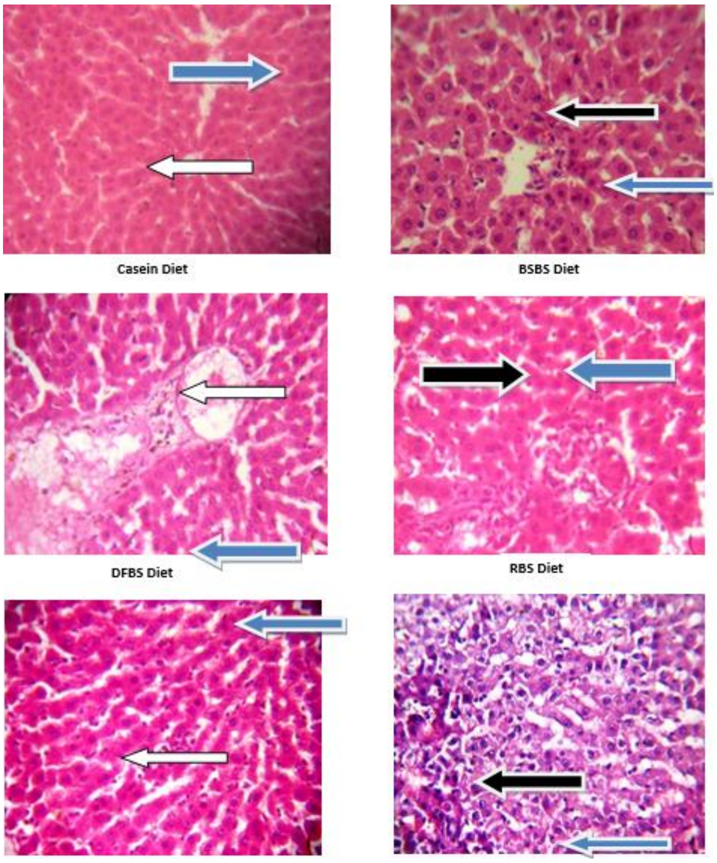

Plate 1: Histopathology of Rats Liver Fed Differently Processed Beef Steaks

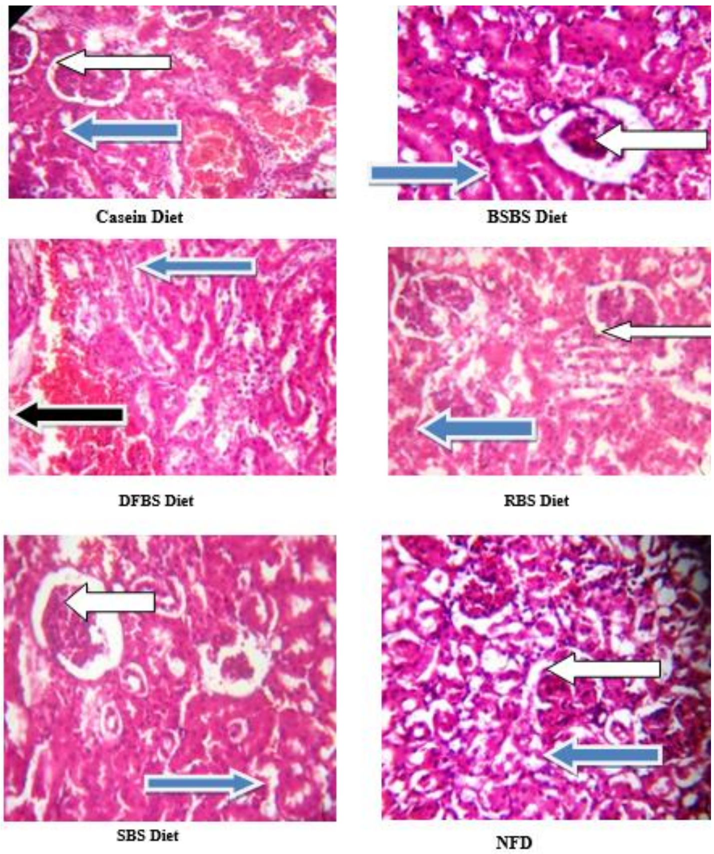

Plate 2: Histopathology of Rats Kidney Fed Differently Processed Beef Steaks

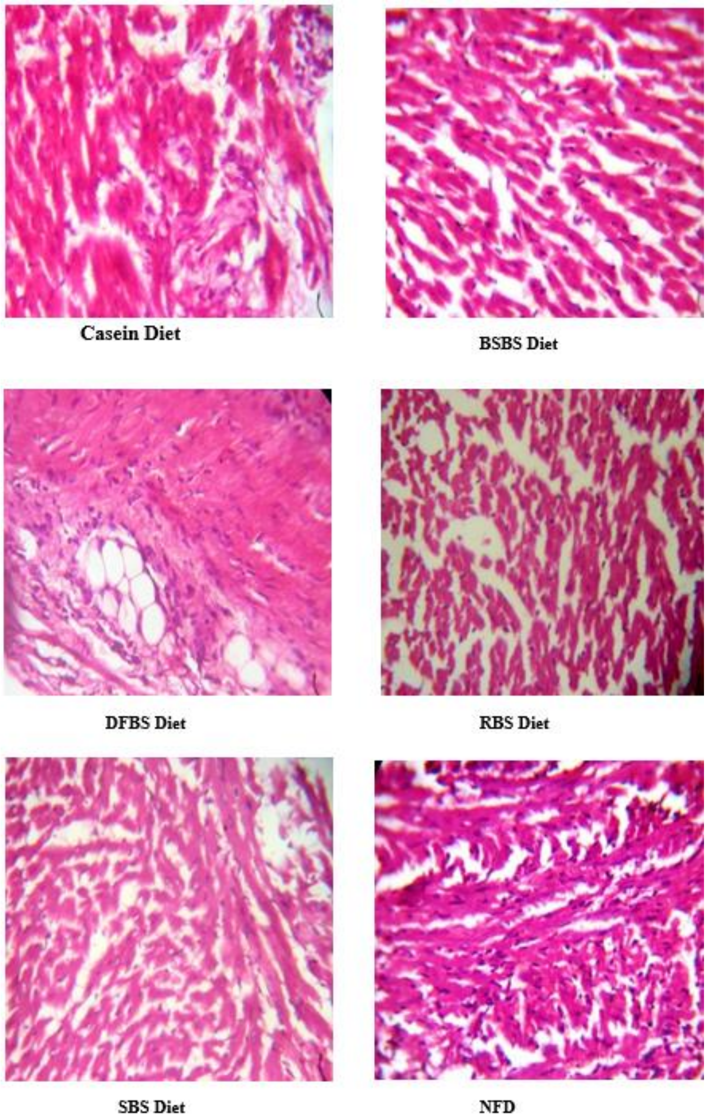

Plate 3: Histopathology of Rats Heart Fed Differently Processed Beef Steaks

The renal cortex of rats fed RBS showed glomeruli with normal mesangial cells and capsular spaces (white arrow). The renal tubules (blue arrow) and interstitial spaces appeared normal. Also, the renal cortex of rat offered SBS showed normal glomeruli with normal mesangial cells and capsular spaces (white arrow). The renal tubules (blue arrow) and interstitial spaces appeared normal. However, there was a poor architecture in those of rats on NFD diets. The renal cortex showed some abnormal glomeruli with sclerosis (black arrow). The renal tubules showed severe tubular necrosis and epithelial degeneration (white arrow).

The Photomicrograph of heart of rat fed differently cooked beef steaks are shown in Plate 3. The heart of rats fed standard casein diets showed normal epicardia (blue arrow) and myocardial layers (white arrow). The valve was congested and only visible at x40. Also, the heart of rats offered BSBS diet showed normal epicardia (black arrow) and myocardial layers (white arrow). There was neither haemorrhage nor any pathological lesion seen. However, the heart of rats fed DFBS diet, showed congested valves (blue arrow) and epicardia infiltrated by fat (black arrow). But, the epicardia (black arrow) and myocardial (white arrow) layers of rats fed RBS diet appeared normal.

There was neither haemorrhage nor any pathological lesion seen. Also, the heart of rats fed SBS diet showed normal epicardia (black arrow) and myocardial layers (white arrow). There was neither haemorrhage nor any pathological lesion seen.

However, the heart of rats fed NFD showed moderate infiltrated epicardia layer (black arrow) and myocardial layer (white arrow) appeared normal.

4. Discussion

Observations revealed that the rats fed RBS diet had higher feed intake than those on other treatments which showed that RBS was relatively more acceptable compared with other diets. Perhaps, roasting improved the preference of steaks by the rats. Among the beef steaks based diets, DFBS had the least feed intake, which shows that deep frying reduced the steak preference. Also, the feed intake of rat given RBS, BSBS and SBS were significantly higher than for the casein diet while NFD was lowest. The least preference of the NFD feed could be expectedly linked to the absence of protein in the diet.

The weight gain of rat fed RBS was the highest which could be attributed to higher feed intake as a result of high protein content of the steak. This was followed by microwaved beef steak, casein diet, then SBS but NFD had a negative weight gain which showed that the rats were depreciating and this could be linked with the protein deficiency in the diet. The weight change of rat reported by [8] was higher than the values recorded in this study. However, [3] recorded relatively lower weight changes for Wistar rats.

There was a poor feed utilisation of rats fed DFBS as the feed conversion ratio was significantly higher. However, rats given RBS had the lowest FCR and this indicates that there has been more effective feed utilisation by the rats.

The biological value of casein diet was higher than the other treatments, indicating that the protein quality of casein diet was higher than in other treatments. Among the steak diets, DFBS diet had the highest BV and this could be due to the improper utilisation of the amino acids as a result of maillard reaction during cooking. This is an indication that deep frying has a significant influence on the consumption of steaks thereby increasing the BV of the steaks. However, the BV of NFD was negative and this is an indication that the diet was of poor protein quality. [15, 4] both recorded a range of 85 to 96 % BV for rats fed solvent extracted neem seed cake and 93.24 to 100.98% BV for male Wistar rats fed processed Atlantic horse mackerel, respectively which were similar to the values obtained in this study.

It was observed that the liver of rats fed BSBS and RBS diets were similar with those given casein diet. This shows that boiling-sun drying and roasting did not have any negative effect on the liver of experimental rats. However, the liver of rats on DFBS diet showed moderate fat deposit in the central venules. This is an indication that deep frying could cause fat deposition in the liver which is not a good sign of health. The scanty infiltration of inflammatory cells seen within the sinusoid of rat offered SBS shows that smoking of beef could lead to cell membrane leakage. Therefore, continuous consumption of DFBS and SBS may lead to liver failure due to fat deposition and cell membrane leakage. Also, the focal area of moderate tumour found in rat fed NFD could be attributed to gross dietary protein deficiency. The results above corroborated the report of [2, 15].

The renal cortex and tubules of rats fed BSBS, RBS and SBS diets were normal, it showed that microwaving, roasting and smoking did not affect the morphology of the kidney thus, lending credence to the safety of the consumption of boiled-sundried, roast and smoked beef steaks. However, the mild to moderate vascular congestion noted in rats offered DFBS is an indication that the consumption of deep fried beef steaks is capable of altering the structure of kidney. The renal cortex sclerosis, severe tubular necrosis and epithelial degeneration found in the kidney of rats given NFD diets showed the importance of dietary protein. These results were juxtaposed with those of [12, 15].

The normal epicardia and myocardial layer of heart found in rats fed BSBS, RBS and SBS, appeared normal and this shows that these three cooking methods (boiling-sun drying, roasting and smoking) did not influence the morphology of the heart. Although, fat infiltration and congested valves were noted in the epicardia layer of rats offered DFBS. It was evident from the morphology that deep frying increased fat deposition in the delicate organs such as the heart which on accumulation could lead to cardiovascular diseases or heart failure.

5. Conclusion

Dietary DFBS has tendency of heightening the danger of heart, kidney and liver ailments as the consumption triggered congested valves, fat infiltration and changed kidney structure. As a result, the intake of DFBS should be greatly reduced as the consumption risk is high.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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