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The Formulation and Evaluation of High-Fat Pellet on Lipid Profiles and Body Mass Index of Male Wistar Rats

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Abstract

This study aims to explore the manufacturing of high-fat pellets for obesity induction diets in male Wistar rats and determine their effects on lipid profiles and body mass index. It was conducted using an experimental laboratory method with posttest with control group. The formulation and evaluation of the physico-chemical characteristics of the high-fat pellets (HFD) were conducted in September 2019. In this study, the 28 male Wistar white rats used were 2 months old with 150-200 g bodyweight. The rats were acclimatized for 7 days and divided into 2 groups, namely the P0 group which was fed with standard PARS CP594 confeed as many as 7 rats (P0) and the P1 group which was fed high-fat diet (HFD FII) as many as 21 rats, each at 30 g/head/day for 8 weeks.. The result showed that the mean fat content of Formula II pellets (HFD FII), 25.44% ± 0.16 was higher than Formula I pellet (HFD FI) (22.55% ± 0.16) and 3% standard feed. Furthermore, the mean of body weight and BMI of obesity induction rat groups (P1) were significantly higher than the standard rat group (P0) ($p < 0.05$). The feed consumption in the rat fed with HFD FII pellets was also higher than the standard group (P0), indicating that rats preferred the HFD FII pellets. The lipid profile of the obesity induction group showed higher total cholesterol, triglycerides, and LDL, while the HDL levels were significantly lower compared to the standard feed group (P0). Therefore, giving HFD FII pellets, which are a source of fat from butter, full cream milk powder, and eggs of purebred chickens for 8 weeks can make male Wistar rats obese and dyslipidemic.

Keywords: Physio-chemical characteristics of pellets, High-fat diet, Body mass index, Lipid profiles, Wistar rats

Introduction

Obesity and related metabolic diseases are currently a priority of the study area. The increase in its global prevalence at various ages has profound economic and health impacts. Several factors such as genetic, environmental, and diet plays an important role in developing obesity. Meanwhile, scientific evidence shows that increased fat intake is associated with weight gain, leading to the disease[(1)]. For example, metabolic syndrome, impaired lipid metabolism and represents a risk factor for type 2 diabetes mellitus (DM), cancer, hypertension, hypercholesterolemia, coronary heart disease, heart failure, stroke, and osteoarthritis[(2,3)].

Moreover, white rats *(Rattus norvegicus)* are widely used as experimental animals in medicine, pharmacy, medicinal plants, nutrition, and other fields of science to study the effects of drugs, toxicity, metabolism, embryology, and behavior [(4,5)]. The induction of obesity in experimental animals can be mediated through neuroendocrine, genetic, and diet manipulation. However, the high-fat diet (HFD) induction method is often used due to its closeness to the human model [(6)]. A high-fat (atherogenic) diet is a food formula with high saturated fat (total fat> 10%) and cholesterol content of 0.28 mg/calorie of energy[7]. In America and Europe, the atherogenic diet for animal models is made from the main ingredients of egg flour or pure crystalline cholesterol, but it is not easily obtained in Indonesia due to a relatively high price. Therefore, there is a need to make a breakthrough through the use of local ingredients. This is possibly achieved from atherogenic diets made with local raw materials at affordable prices, sourced from cholesterol in purebred chicken egg yolk, coconut oil, which is 86% high in saturated fatty acids, and beef tallow.

Pellets are a mass form of feed or ration material formed by pressing and compacted through a mold hole mechanically. The use of pellet-shaped feed has several advantages, such as ensuring the balance of feed nutrients, being more durable, not producing dust-like mash feed, and reducing the amount of feed wasted[(8)].

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Furthermore, its use is also more practical without any prior preparation, and available in ready-to-use packages. Currently, the manufacturing of pellets for atherogenic diets in Wistar rats generally uses lard, coconut oil, beef fat, goat fat, and egg yolk as a source of fat[9]. Meanwhile, the use of lard in the manufacturing of high-fat diet feed is generally as a suspension, where the process uses water as a solvent medium for easy rancidity. Rancid odors arise from contact between fats and oxygen (oxidation), water molecules (hydrolysis), or metals to form hydroperoxides. This further becomes susceptible to more oxidation and degradation of secondary reaction products such as aldehydes, ketones, acids, and alcohols [10, 11]. Compared to the regular diet, the rancid aroma of the atherogenic diet gave a lower intake but higher fat content, affecting changes in body weight and lipid profile of the rat[12]. Therefore, the preference of feeding on fresh feed with a fragrant aroma by livestock affected its palatabilit and acceptance[13].

The manufacturing of HFD pellets using lard also poses a particular problem for Muslim researchers due to its unavailability as other ingredients. Furthermore, high-fat feed for the obesity induction and atherogenicity for Wistar rats in the form of instant pellets is not yet available in the local Indonesian market, even though the need for its use in the fields of medicine, pharmacy, medicinal plants, nutrition, and others is relatively high. Therefore, this study used butter, chicken eggs, and full cream milk powder as sources of saturated fat to manufacture a high-fat (atherogenic) diet for male Wistar rats as experimental animals. The foods of animal origin such as fatty meat, cheese, butter, and milk cream, which contain saturated fatty acids and cholesterol, were used. Moreover, cheese raises less cholesterol at the same fat content as butter, whole milk is rich in saturated fat, cholesterol, and increases serum cholesterol[14-16].

Butterfat is generally recognized for its consistent increase in plasma cholesterol concentrations, especially in hypercholesterolemic subjects[17]. Previous study has shown an epidemiological link between butterfat consumption and cardiovascular mortality as the main cause of a decrease in the intake of full-cream milk fat[18]. Moreover, dairy products are rich in myristic and palmitic acids, which increase serum cholesterol[19]. The dominant effect of saturated fatty acids is increased total cholesterol and LDL cholesterol levels[20]. Several studies on human have shown that butter, which contains 66% of saturated fatty acids in hypercholesterolemic compared to other fat sources [21, 22]. The intake of this saturated fat had been associated with an increased risk of cardiovascular disease (CVD)[23], which is mediated primarily by high LDL cholesterol concentrations. In the United States, the primary dietary sources of saturated fatty acids are total fat dairy products and red meat.

An egg is a source of high-quality protein, which provides all essential amino acids for humans, especially in the white part. Furthermore, its yolk is a source of fat, containing 65.5% triglycerides, 28.3% phospholipids, and 5.2% cholesterol. Egg also contains many active lipid components such as phospholipids, choline, and carotenoids[24]. Monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) weighted 2.0 g and 0.7 g, respectively, and one medium egg contains 1.6 g saturated fatty acids. Moreover, egg consumption is positively associated with an increase in serum total cholesterol (TC), LDL-C, and a high incidence of coronary heart disease mortality[25,26]. Its intake and cholesterol were also related to all causes of higher CVD and cancer deaths [27], while increased mortality is influenced mainly by cholesterol intake. Therefore, it is advisable to limit cholesterol intake and replace whole eggs with egg whites/substitutes or other alternative protein sources.

This study aims to develop HFD pellets for dietary induction of obesity in experimental Wistar rats. The formulation was based on materials derived from halal fats, practical use, ready to use, relatively low prices, and easy to obtain in the local market such as butter, chicken eggs, and full cream milk.

Materials and Methods

This study was conducted using a laboratory experimental method with a post-test design a post-test design with control group. It obtained ethical approval from the Research Ethics Commission of the Hasanuddin University Faculty of Medicine, Makassar, number 1156 / UN4.6.4.5.31 / PP36 / 2019. Meanwhile, this study was carried out in 3 stages, namely (1) The formulation and manufacturing of high-fat pellets (HFD), with Physico-chemical characteristics investigations in September/ 2019 at the Animal Food Chemistry Laboratory, Department of Nutrition and Food, Faculty of Animal Husbandry, Hasanuddin University. (2) Maintenance of experimental animals and the induction of obesity by giving selected HFD pellets, Formula II HFD pellets (HFD FII) in male Wistar rats, for eight weeks from January to March 2020, at the Pharmacy Laboratory of the Faculty of Pharmacy, University of Muslim Indonesia (UMI) Makassar. Subsequently, anthropometric index measurement included body weight, length, and BMI was carried out every seven days, while pellet consumption was measured daily. (3) Lipid profile examinations such as total cholesterol, triglycerides, LDL, and HDL were carried out at the Laboratory of Molecular Biology and Immunology, Faculty of Medicine, Hasanuddin University Makassar, in May 2020.

High Fat Diet (HFD) Pellet Formulation

The HFD feed was made in pellets based on the study method with a modification[7]. The formulation consisted of two treatments, namely Formula I (HFD FI) and Formula II (HFD FII) as shown in Table 1.

Table 1. High-fat pellet formulation for male Wistar strain white rat (Rattus novergicus)

Material	Formula I (HFD FI)		Formula II (HFD FII)	
	%	Amount (g)	%	Amount (g)
Confeed PARS CP594 (standard feed)	40	400	20	200
Wheat flour	20	200	20	200
Butter	20	200	30	300
Chicken eggs (whole)	10	100	20	200
Full cream powdered milk	10	100	10	100
Total	100	1000	100	1000

Furthermore, the composition of the ingredients in one kg of confeed PARS CP594 (standard feed) consisted of corn, bran, fish meal, soybean meal, coconut meal, broken wheat, peanut meal, leaf flour, and canola. The tools for making HFD pellets were a digital scale, electric oven, blender, pellet molding device (pelleter), water measuring device, 80 mesh filter, mixing bowl, spoon, airtight plastic packaging, and vacuum packaging device.

The preparation was ccarried out initially by weighting each ingredient according to the Formula's proportion (Table 1). The standard feed of Confeed PARS CP594 was mashed in a blender, filtered using an 80-mesh sieve, and mixed evenly with flour and full cream powdered milk (ingredient A). Subsequently, butter, eggs (yolks and whites) are evenly mixed and ingredient A was added into the mixture and mixed continually to form smooth dough, which was weighted and molded using a pelleter. It was baked in the oven at 160°C for 60 minutes, pellets were removed, allowed to cool, and weighted for yield calculation. The pellet was further packed with airtight plastic using a vacuum packaging tool and stored in an undamped place until its use for physical analysis and chemical composition of HFD pellets. The results of the high-fat pellet formulation with better chemical and physical characteristics were used as feed for obesity induction diets in male Wistar rats.

Chemical Characteristics of HFD pellets

The Duplo analysis of the chemical composition of HFD pellets carried out included moisture content (gravimetric method, SNI 01-2891-1992 item 5.1), ash content (AOAC 2005. 942.05), crude protein by Kjeldahl method (AOAC 2005.2000.11), crude fat in Soxhlet method (AOAC 2005.2003.06), carbohydrates (AOAC 2005), crude fiber (SNI 01-2891-1992 item 11), calcium (AAS method, AOAC 2005.968.08), and phosphorus (AAS method, AOAC 2005.965.17).

Physical characteristics

The physical characteristics of HFD FI and HFD FII pellets were measured using weight, length, and diameter parameters. The pellets were weighted using analytical scales, while the length and diameter were with a caliper. The measurement of yield was according to the Formula below:

$$\text{Yield (\%)} = \frac{\text{Pellet dry weight(gm)}}{\text{Pellet wet weight(gm)}} \times 100$$

Maintenance and induction of obesity in male Wistar rats

The 2 months old 28 male Wistar white rats, which were 150-200 g body weight, were from PT. Indoanilab Bogor (Laboratory Animal Development Facility, Faculty of Veterinary Medicine, IPB University, Bogor). The rats were acclimatized for 7 days, placed in individual cages, light/dark cycle for 12 hours, temperature 26-29 °C, humidity 60-70%, and given standard feed and drinking water ad libitum. Meanwhile, the cage was in form of a plastic box with a wire cover of 30 cm x 20 cm x 12 cm, covered with rice husks, and cleaned regularly two times a week to keep the cage dry and healthy.

After acclimatization, the body weight and length were estimated, and the remaining feed was measured daily, while the rats' body weight and length were measured every 7 days. The experimental animals were divided into 2 groups, namely the P0 group which was fed with standard PARS CP594 confeed as many as 7 rats (P0) and the P1 group which was fed high-fat diet (HFD FII) as many as 21 rats, each at 30 g/head/day for 8 weeks, while drinking water was given ad libitum. The amount of consumption was calculated from the amount of feed given minus the remaining feed. After eight weeks, the rats were examined for their level of obesity and were declared obese when the BMI value was ≥ 0.68 g/cm^2.

At the 8th week after treatment, rats were fasted for 12 hours and kept drinking before aseptic blood was drawn through the tails' lateral veins or ventral arteries. A total of 1 ml of blood was collected into an Eppendorf tube and left for 60 minutes. The blood serum appeared differently from the blood clot and was further separated by centrifugation 15 minutes, speed 3000 rpm. After

centrifugation, it was isolated with a single-use syringe and transferred to a new Eppendorf tube, labeled, wrapped in plastic, and stored in the freezer at –20 ºC upright until the serum was ready to be analyzed.

Lipid profile examination (Total Cholesterol, Triglycerides, LDL, and HDL)

The total cholesterol levels were measured by the ELISA sandwich method using the Rat Total Cholesterol (TC) ELISA Kit Cat. No: MBS2600008. Subsequently, HDL and LDL levels were examined by a homogeneous enzymatic (DiaSys) photometric test of Cholesterol Oxidase, Phenol Amino Phenazon (CHOD-PAP). Triglyceride levels were measured by the enzymatic colorimetric test method for Glycerol-3-Phosphatase Oxidase-Paminophenazone (GPO-PAP) using the system diagnostic kit (DiaSys) based on the kit procedure.

Statistical analysis

Processing and data analysis were carried out using the IBM SPSS program, while the data were presented as means ± standard deviations. Chemical and physical characteristics data of HFD FI and HFD FII pellets were analyzed descriptively. Furthermore, the anthropometric data such as BW, BL, BMI, average feed intake, lipid profile such as total cholesterol, triglycerides, LDL, and HDL were tested for normality by the Shapiro-Wilk test and data variance with the Homogeneity of Variance test. The parametric hypothesis test used was Independent T Test at 95% confidence level (α = 0.05).

Results and Discussion

Chemical characteristics of HFD pellets

According to Table 2, the 25.44% ± 0.16 average fat content of HFD formula II (HFD FII) pellets was higher than HFD formula I (HFD FI) pellets of about 22.55% ± 0.16 and 3% standard feed. The difference in fat content was due to the higher percentage of butter and eggs in the HFD FII formula, 30%, and 20%, compared to 20% and 10% in HFD FI. Furthermore, the protein, carbohydrate, fiber, calcium, ash, and phosphorus content of HFD FII pellets was lower than HFD FI pellets. Meanwhile, the standard dietary fat was from different meals such as fish, soybean, coconut, and peanut, and vegetable.

The moisture content of HFD FII pellets (7.87% ± 0.09) was lower than that of HFD FI pellets (8.47% ± 0.03). Moreover, the water content in an ingredient greatly affects the quality of feed ingredients. When the water content of a material does not meet the requirements, there will be physical and chemical changes identified by the growth of microorganisms which makes them unfit for consumption[29]. In addition, the reduction in water content also decreases the ration weight, which makes packaging easier[(8)]. Based on the physicochemical analysis of high-fat pellet formulations, HFD FII pellets had higher fat content and yield of 25.44% ± 0.16; 81.01% and lower moisture content of 7.87% ± 0.09 compared to HFD FI pellets. Therefore, HFD FII pellets were selected as obesity induction feed for male Wistar rats to obtain animal models of obesity and dyslipidemia

Physical characteristics of HFD Pellet

$$\text{Yield of HFD FI} = \frac{800.49}{1000} \text{ x } 100\% = 80.05\%$$

$$\text{Yield of HFD FII} = \frac{810.08}{1000} \text{ x } 100\% = 81.01\%$$

Table 3 shows the initial weight of each HFD FI, and the HFD FII formula was 1000 g. After drying, HFD FI and HFD II pellets had a weight reduction of 190.51 g and 189.20 g, respectively. However, the diameter and length of each pellet formulation did not change, while the yield on HFD FI was 80.05% and HFD FII 81.01%.

Table 2. Chemical composition of HFD FII pellets and standard feed

Nutrients (%)	Pellet HFD FI (%)	Pellet HFD FII (%)	Standard feed CP594* (%)
Fat	22.55 ± 0.16	25.44 ± 0.16	3
Protein	19.57 ± 0.35	19.37 ± 0.01	17.5 – 19.5
Carbohydrate	45.48 ± 0.26	44.51 ± 0.21	48.7
Fiber	6.34 ± 0.64	5.31 ± 0.49	8
Ash	6.08 ± 0.12	5.39 ± 0.09	7
Water	8.47 ± 0.03	7.87 ± 0.09	13
Calcium	0.85 ± 0.00	0.82 ± 0.01	0.9
Phosphor	0.17 ± 0.01	0.15 ± 0.01	0.9

Source: * PT. Pokphand (feed producer)

Table 3. Physical characteristics of HFD FI and HFD FII pellets

Formula	Wet			Drying		
	Weight (g)	Length (cm)	Diameter (cm)	Weight (g)	Length (cm)	Diameter (cm)
HFD FI	1000	1	0.5	800.48	1	0.5
	1000	1	0.5	800.50	1	0.5
Mean	1000	1	0.5	800.49	1	0.5
HFD FII	1000	1	0.5	810.10	1	0.5
	1000	1	0.5	810.06	1	0.5
Mean	1000	1	0.5	810.08	1	0.5

Maintenance and induction of obesity in male Wistar rats

After 8 weeks of obesity induction, the results of the independent T-test on body weight and BMI of Wistar rats obtained a p-value <0.05. This showed that there is a significant difference between body weight and BMI of the rat group fed with HFD FII pellets (P1) and the standard feed group (P0). The mean body weight and BMI of 21 rats in the obesity-induced group were significantly higher than those in the standard diet (P0).

Table 4. Anthropometric parameters and intake of male Wistar rats receiving standard feed and HFD FII pellets

Parameter	Groups	Mean ± SD	*p value*
Weight (gr)	P0	198.0 ± 2,82	0.000*
	P1	271.9 ± 4,52	
Length (cm)	P0	18.3 ± 0,55	0.336
	P1	18.0 ± 0,37	
BMI (gcm^{-2})	P0	0.49 ± 0,03	0.000*
	P1	0.71 ± 0,03	
Feed intake (g)	P0	21.8 ± 0,42	0.000*
	P1	23.7 ± 0,76	

*** Independent T Test; *significantly different (p<0.05).**

In Table 4, based on the results of Independent T Test, it is known that there are significant differences in mean body weight, BMI and feed intake, while body length does not differ significantly between groups P0 and P1.

Lipid profiles of Wistar rats

Lipid profile examination was carried out after 8 weeks of treatment. The obesity induction rat group (P1) was obese (IMT > 0.68 $g.cm^{-2}$), while the average body mass index for the standard feed group was within the normal range (IMT= 0.48 $g.cm^{-2}$). At the beginning of this study, the rats and the obesity induction were 8 weeks old; therefore, the lipid profile was examined when the Wistar rats were 16 weeks old. Reference values for the lipid profiles of 16-week-old male albino rats are as follows: serum total cholesterol 109.72 ± 3.67 (100.00–133.33) mg/dL, serum HDL cholesterol 45.46 ± 2.74 (36.36–54.55) mg/dL, serum triglycerides 92.67 ± 5.77 (72.00–130.00) mg/dL and LDL cholesterol 45.73 ± 3.54 (25.45–59.98) mg/dL. The results of the lipid profile of rats' measurements are shown in Table 5.

Table 5. Lipid profile of Wistar rats after the standard feed and high-fat pellets Formula II (HFD FII)

Parameter	Groups	Mean ± SD	*p value*
Cholesterol total (mg/dl)	P0	112, 96 ± 3,22	0,000*
	P1	178, 73 ± 6,73	
Triglyceride (mg/dl)	P0	96, 12 ± 5,41	0,000*
	P1	156,10 ± 26,77	
LDL (mg/dl)	P0	47,79 ± 2,64	0,000*
	P1	108,04 ± 5,45	
HDL (mg/dl)	P0	48,44 ± 2,47	0,000*
	P1	33,16 ± 1,78	

The results of the independent T test showed that the levels of total cholesterol, triglycerides, LDL in the P0 group were significantly lower and HDL levels were significantly higher than those in the P1 group (p<0.05) (Table 5). Meanwhile, food compositions that are commonly used to induce obesity are mostly rich in cholesterol/fats rather than carbohydrates and proteins, which help increase animal body weight and lipid levels[28].

Obesity status in the rat can be assessed using the parameters of Body Mass Index (BMI) and Lee's index[30]. Normal BMI in adult male Wistar rats ranged from 0.45 to 0.68 g/cm^2, therefore, rats were declared obese when the BMI was ≥ 0.68. Moreover, it is assumed that body fat and obesity in rats are better estimated in BMI than in Lee's index. Changes in BMI were associated with a profile of dyslipidemia and oxidative stress in rat serum and are used to predict the adverse consequences of obesity in rats. Such naturally available foods and their mechanisms maintain a healthy body weight, which reduces the morbidities and mortalities of obesity [31]. Maintaining a healthy weight is important for overall health, which helps prevent and control many of diseases [32].

The weight gain in the rats fed with HFD FII was significantly different from those given standard feed (P0). This showed that feeding on high fat for 8 weeks can significantly increase the body weight of the rat. This occurred due to the significant difference in feed intake between the standard feed group (P0) and the groups receiving HFD FII pellets (P1). In addition, there was a variation in the percentage of fat from the pellets where the fat content of HFD FII pellets was higher (25.44% ± 0.16) than the 3% standard feed. The source of fat from HFD FII pellets was from butter, chicken eggs, and full cream powdered milk, while in standard feed, the majority was vegetable fats such as soybean, coconut, and fish. However, feeding the standard 3% fat content did not have a significant increase in rat body weight and fat storage. Fat is the largest source of energy where each gram produces 9 kcal, while protein and carbohydrates only produced 4 kcal per gram. At the exact weight of feed ingredients, fat production was higher than carbohydrates and protein.

Previous studies showed that the consumption of a high-fat (HF) diet causes obesity and metabolic disorders in rodents that mimic metabolic syndrome in humans[33, 28, 34] .Although the weight gain in experimental animals can be assessed after 2 weeks, it is more noticeable after 4 weeks of HFD administration. In this study, prolonged feeding on a fat-rich diet-induced weight gain in the susceptible rat ranged from 10% to 20% compared to controls fed standard diets. This showed that obesity induction is most effective when the diet is started at a younger age and continues for several weeks. Providing a high-fat diet at early stages also increases the number of adipocyte cells. After an extended period, an enlargement of the adipocyte cell diameter due to the accumulation of fat associated with the risk of metabolic disorders was discovered[35].

The feed intake in the rat-given HFD FII pellets group was higher than the group fed with standard feed (P0), showing that the rat preferred the HFD FII pellets. This was because the high fat in HFD FII pellets gave a tasty and crunchy effect; therefore, it was preferred by rats. However, the more rancid smell of the atherogenic diet than the regular diet led to a lower intake of the rats' feed[12].

Obesity can cause lipid metabolism disorders characterized by abnormalities in the plasma of the lipid profile such as high total cholesterol and triglyceride levels, and low high-density lipoprotein (HDL) cholesterol levels. Since it is the key risk factor in the natural history of other chronic non-communicable diseases, its prevention strategies offer a cost-effective approach in avoiding other chronic non-communicable diseases[36]. Meanwhile, the administration of a high-fat diet causes an increase in the concentration of chylomicrons in plasma, while high triglyceride levels lead to increased LDL formation[37]. It was assumed that the high-fat diet causes triglyceride and LDL levels in the obesity-induced diet group to be higher than the standard feed group.

A study about intervention for 4 weeks was conducted in a group of healthy men and women in the general population, which consume 50 g per day of either these different dietary fats, namely extra virgin coconut, butter, or extra virgin olive oil. The results showed that the LDL-C concentration increased significantly in the buttered group than coconut and olive oil. Furthermore, cholesterol synthesis was lower during a diet rich in coconut fat and oil, which was safer than a diet rich in butter. This occurred due to lower production levels of lipoproteins containing apo-B.

In the obesity induction group, the HDL levels were lower than in the standard feed group (P0). A significant reduction in HDL cholesterol levels in Wistar rats after a high-fat diet showed that a high-fat diet affected serum cholesterol levels. Generally, an increase in HDL concentration is known as a protective cardio-protein because the lipoprotein functions to absorb excess cholesterol to the liver for excretion. However, a low HDL concentration is a diagnostic marker for Metabolic Syndrome (MetS) and CVD. Recent studies have shown that metabolic syndrome is associated with an increase in the liver enzymes such as ALT, AST, GGT, and albumin[38]. Similarly, epidemiological and clinical studies showed the inverse correlation between serum high-density lipoprotein cholesterol (HDL-C) concentration and the risk of atherosclerotic diseases[39]. Also, a strong inverse relationship exists between HDL and CVD concentrations at the epidemiologic level, which makes it a significant marker for assessing cardiometabolic health [40].

The limitation of this study is that the total cholesterol and trans fatty acid content of the high-fat pellets produced were not examined. Also, the texture of the pellets was still rather fragile and easily crushed, which could affect the amount of feed intake in the experimental animals.

Conclusion

The results recommended the use of high-fat formula II (HFD FII) pellets for 8 weeks to make male Wistar rats a model of obesity and dyslipidemia. The intervention of HFD FII pellets made from butter, eggs, full cream milk as a source of fat for eight weeks in male Wistar rats can significantly increase the body weight, body mass index (BMI), total cholesterol, triglycerides, LDL, while the HDL levels were lower compared to the group that received a standard feed.

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