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Oral Administration of a Hypercholesterol Feed Formula to Develop a Dyslipidemia Animal Model in Male Rats (Rattus norvegicus)

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Abstract. Dyslipidemia is a metabolic disorder characterized by elevated cholesterol levels, which can be induced through high-fat and high-cholesterol diets. Establishing an animal model of dyslipidemia is essential for studying its pathophysiology and for evaluating potential therapeutic agents. This study aimed to develop a dyslipidemia model in male white rats (Rattus norvegicus) through oral administration of a hypercholesterol feed formula. A true experimental design with a pretest–posttest control group was used, involving healthy male rats aged 2–3 months with an average weight of 150–200 g. The experimental group received a high-fat feed consisting of lard, duck egg yolk, and propylthiouracil administered orally for 14 days, while the control group received a standard diet. Total cholesterol levels and body weight were measured on day 1 and day 14. Statistical analysis using paired t-test showed a significant increase in body weight and total cholesterol levels in the dyslipidemia group ($p < 0.001$), whereas the control group only demonstrated slight, non-significant increases. These findings indicate that oral administration of hypercholesterol feed successfully induces dyslipidemia in rats. This model can be applied as a valid experimental platform for further research on dyslipidemia mechanisms and the evaluation of anti-hyperlipidemic agents..
Keywords: Dyslipidemia, Hypercholesterol feed formula, Animal model.

1 Introduction

Dyslipidemia is a metabolic disorder characterized by elevated total cholesterol, triglycerides, and low-density lipoprotein (LDL) levels, accompanied by decreased high-density lipoprotein (HDL) levels. This condition is a major risk factor for cardiovascular diseases such as atherosclerosis, coronary heart disease, and stroke, which remain the leading causes of death worldwide. Globally, approximately 24.1% of adults have hypercholesterolemia, 28.8% have hypertriglyceridemia, and 38.4% have low HDL levels [1].

Among hypertensive populations, dyslipidemia further worsens blood pressure control and increases the risk of cardiac and metabolic complications. A recent study conducted in Iran reported that the prevalence of dyslipidemia among patients with hypertension reached 86.4%**,** highlighting the strong comorbidity between lipid abnormalities and metabolic disorders [2].

Beyond human studies, animal models are essential to understand the pathophysiological mechanisms of dyslipidemia and to evaluate potential pharmacological or nutritional interventions. High-fat or high-cholesterol diet models administered orally in rats are widely used to simulate dyslipidemic conditions. Recent studies demonstrated that such dietary interventions can significantly increase TC, LDL-C, and TG levels, while decreasing HDL-C. Additionally, they induce histological alterations in the liver and sometimes in vascular tissues, resembling early stages of atherosclerosis or fatty liver disease [3].

However, a key research problem remains: despite numerous existing animal models, there is no consistent standard regarding diet composition (e.g., cholesterol and fat percentages, use of bile salts as enhancers), duration of feeding, and measurable outcome parameters. Some studies have indicated that lipid profile alterations require a certain induction period before becoming significant, and that the rat strain (e.g., Wistar vs. Sprague-Dawley) may influence the degree of response.

The importance of this study lies in several aspects: (1) **Standardization**: Developing a clearly defined and standardized hypercholesterol feed formula and administration protocol will

improve the reproducibility and validity of future studies comparing lipid-lowering interventions. (2) **Efficiency and Reproducibility**: Establishing a reliable model within a relatively short induction period (2 weeks) will facilitate faster development and screening of therapeutic or nutritional agents. (3) **Public Health Relevance**: With the rising global prevalence of dyslipidemia, particularly in Southeast Asia, animal models can serve as crucial translational tools for developing preventive and therapeutic strategies in humans [1].

The objectives of this study are to develop and validate a dyslipidemia animal model in male rats (*Rattus norvegicus*) through oral administration of a cholesterol- and fat-enriched feed formula designed to induce significant alterations in serum lipid profiles (TC) and to identify the optimal duration and diet composition required to produce a consistent and reproducible dyslipidemia model suitable for preclinical pharmacological and nutritional research.

2 Methods

2.1 Study Design

This study employed a *true experimental design* using a Pretest–Posttest Control Group Design. Male rats (*Rattus norvegicus*) were randomly assigned to two groups: a control group and a treatment (hypercholesterol-fed) group. The research aimed to induce dyslipidemia through oral administration of a high-cholesterol feed formula and to assess its effects on serum lipid profiles.

2.2 Study Location and Ethical Approval

The study was conducted at the Pharmacology Laboratory, Faculty of Medicine, Universitas Negeri Semarang (UNNES). All procedures involving animals were approved by the Ethical Committee for Animal Research, Faculty of Medicine, Universitas Negeri Semarang (Ethical Clearance No. 112/KEPK/FK/KLE/2025).

2.3 Study Location and Ethical Approval

The animal population consisted of healthy male white rats (*Rattus norvegicus*), aged 2–3 months and weighing 150–200 g. A total of 28 rats were used, divided equally into a control group and a treatment group (15 animals each), based on the Federer formula:
$(t–1)(n–1)\geq 15$
to ensure adequate sample size and statistical power.

Inclusion Criteria:

- Male *Rattus norvegicus* aged 2–3 months
- Body weight between 150–200 g
- Active, healthy, and showing normal feeding behavior
- Adapted to the environment for 7 days before treatment

Exclusion Criteria:

- Death during the study
- Signs of illness (loss of appetite, dull fur, inactivity)
- Anatomical abnormalities

2.4 Tools and Instruments

The equipment used in this study included: rat cages, oral gavage tubes (sonde), digital balance (*Ohaus*), measuring cylinders (*Herma*), beakers (*Herma*), glass containers, stirring rods, heating device, analytical balance, filter paper, graduated flasks (*Pyrex*), cholesterol test strips, *Easy Touch Multicheck Tester*, mortar and pestle, and test tubes (*Herma*).

2.5 Materials

The materials included:

- Standard feed (*BR2 Comfeed*, Indonesia)
- Duck egg yolks (local farm supply)
- Pork lard (Sigma-Aldrich, analytical grade)
- *Propylthiouracil* (PTU) tablets 100 mg (Kimia Farma, Indonesia)
- Aquadest (distilled water, sterile)
- 70% ethanol (for sterilization)

2.6 Preparation of Hypercholesterol Feed Formula

The hypercholesterol feed formula was prepared by mixing 150 mL of pork lard and 50 g of duck egg yolk in a 3:1 ratio (v/v). The mixture was homogenized and administered orally to the rats. In addition, Propylthiouracil (PTU) was prepared at a concentration of 1.25% (w/v) by dissolving 100 mg PTU in 8 mL distilled water, ensuring that each mL contained approximately 12.5 mg PTU[4,5].

2.7 Experimental Procedure

Adaptation Phase
All rats were acclimatized for 7 days under standard laboratory conditions (temperature 22–25°C, 12-hour light/dark cycle) with ad libitum access to food and water.
Induction of Dyslipidemia
Before treatment, baseline serum cholesterol levels were measured (Day 0). Rats in the treatment group were administered the hypercholesterol feed formula orally using an oral gavage at a dose of 4 mL/rat/day, divided into two equal doses (2 mL at 08:00 and 15:00 WIB) for 14 consecutive days. Additionally, each rat in the treatment group received 1 mL of PTU solution (1.25%) via oral gavage once daily at 08:00 WIB for 14 days to potentiate the lipid profile alteration. The control group received only standard feed and distilled water.
Monitoring Parameters
Body weight was recorded daily. Blood samples were collected from the tail vein on Day 0 (pretest) and Day 14 (posttest) after 12 hours of fasting. Serum total cholesterol levels were measured using the *Easy Touch Multicheck Tester* (Bioptik Technology, Taiwan)[6].
Data Collection and Analysis
The primary outcomes were changes in serum total cholesterol levels and body weight between pretest and posttest measurements. Data were analyzed using the Paired Sample t-test with SPSS. A *p-value* < 0.05 was considered statistically significant.

3 Results And Discussion

3.1 Changes in Body Weight

The mean body weight of male rats (*Rattus norvegicus*) significantly increased after 14 days of oral administration of the hypercholesterol feed formula. That is shown in table 1 and graph 1.

Table 1. The mean body weight of male rats (*Rattus norvegicus*)

Group	Day 1 (g) ±SD	Day 14 (g) ±SD	Change (g)	p-value
Control (n=15)	203.71 ± 5.9	209.64 ± 6.3	+5.93	0.071 (ns)
Dyslipidemia (n=15)	203.78 ± 6.1	236.64 ± 7.2	+32.86	< 0.001

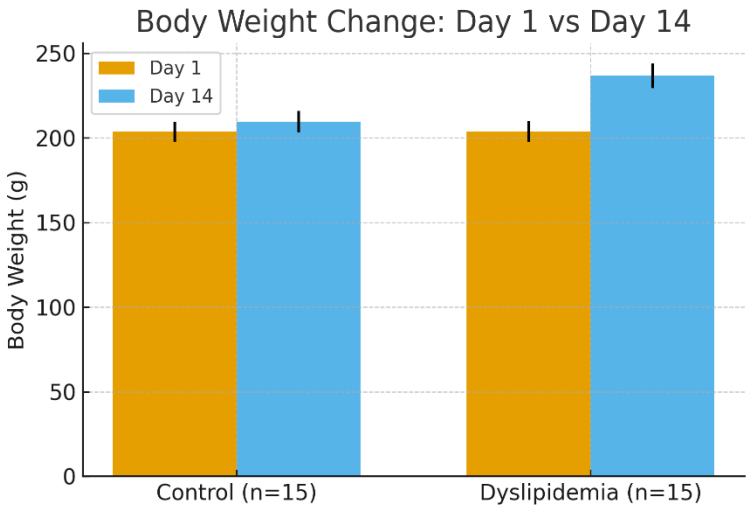


Fig. 1. Changes in Body Weight Day 1 vs Day 14

The significant increase in body weight in the treatment group indicates the metabolic impact of a cholesterol- and fat-enriched diet. Physiologically, such diets promote triglyceride storage in adipose tissues, leading to weight gain. The results showed that the administration of a high-fat/high-cholesterol diet combined with PTU for 14 days rapidly induced metabolic changes consistent with dyslipidemia, characterized by body weight gain and elevated total cholesterol levels.

HFD/HCD stimulates hepatic lipogenesis pathways through the activation of transcription factors such as SREBP-1c, along with increased expression of lipogenic enzymes (ACC**,** FAS). As a result, there is an elevation in fatty acid synthesis and triglyceride esterification within hepatocytes, which are subsequently exported as VLDL into the circulation, thereby increasing the lipid pool available for peripheral fat depots**.** The enhanced flow of VLDL and triglycerides (TG) promotes fat storage in adipose tissue and contributes to body weight gain [7].

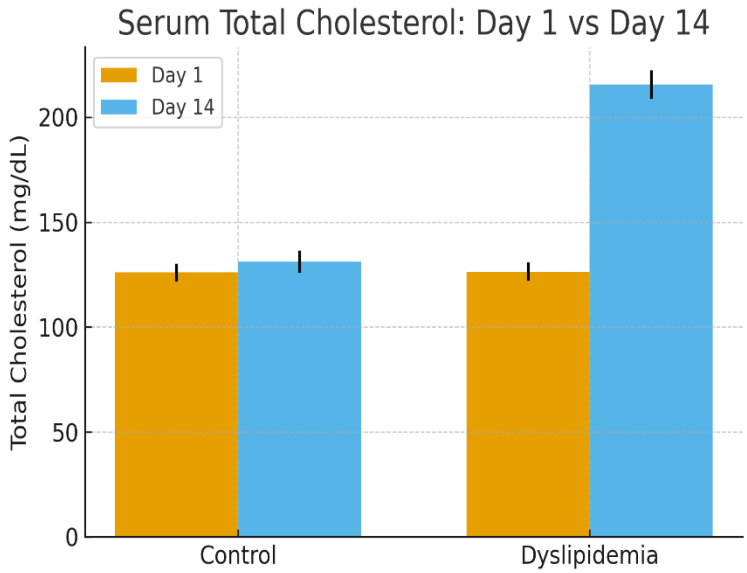
2. Serum Total Cholesterol

Serum total cholesterol was measured pre- and post-treatment using an *Easy Touch Multicheck Tester*. The study demonstrated that oral administration of a hypercholesterol feed formula for 14 days effectively induced experimental dyslipidemia in male rats, as evidenced by significant increases in serum total cholesterol and body weight. That is shown in table 2 and graph 2.

Table 2. Serum total cholesterol

Group	Day 1 (mg/dL) ±SD	Day 14 (mg/dL) ±SD	Δ (Change)	p-value
Control	126.07 ± 4.2	131.14 ± 5.3	+5.07	0.086 (ns)
Dyslipidemia	126.42 ± 4.5	215.64 ± 6.7	+89.22	< 0.001

The increase in total cholesterol in the data is due to several mechanisms, namely: Increased lipid and cholesterol intake: enhanced intestinal absorption and hepatic lipid influx. Excess dietary cholesterol enters hepatocytes and disrupts hepatic lipid homeostasis, leading to increased triglyceride esterification and VLDL secretion, while simultaneously reducing LDL clearance capacity through inhibition or downregulation of the LDL receptor (LDL-R). This mechanism aligns with previously reported models of diet-induced dyslipidemia in rats and rodents [8]**.**



Graph 2. Serum total cholesterol Day 1 vs Day 14

The role of PTU (an antithyroid agent) as a contributing factor is significant: PTU suppresses thyroid hormone synthesis, which in turn reduces the basal metabolic rate and lipid oxidation, thereby slowing lipoprotein catabolism and promoting lipid accumulation in circulation and adipose tissues. The combination of HFD/HCD (high-fat or high-cholesterol diet) with PTU has been shown to accelerate and intensify the hyperlipidemic and steatotic phenotype in animal models, thus shortening the induction period compared to HFD alone**.** Similar and mechanistic findings in HFD+PTU models have been reported recently [9].

Dysregulation of hepatic metabolic genes: A high-fat/high-cholesterol diet alters the expression of key genes involved in lipid metabolism (e.g., SREBP-1c, ACC, FAS) and inflammatory pathways (NF-κB), contributing to intrahepatic triglyceride accumulation and oxidative stress, processes that have been well described in HFD/HCD animal models [10].

The induction time and magnitude of lipid changes obtained in this study (Δ TC ≈ +89 mg/dL within 14 days) indicate a rapid response; several HFD/HCD protocols without PTU usually require 4–12 weeks to achieve comparable lipid alterations, depending on the diet composition and animal strain. Model modifications that include additional agents such as PTU, cholic acid, or Triton have indeed been reported to accelerate the induction process [11]**.**

Recent comparative studies have shown that the addition of 1–2% cholesterol to the diet results in a significant increase in serum lipid levels after several weeks; the inclusion of supporting agents such as bile salts or PTU further accelerates the onset and exacerbates the steatotic/hyperlipidemic phenotype. Our findings are consistent with these reports; however, the 14-day duration used in our study is relatively short compared to many others thus, long-term confirmation is still required [8]**.**

Overall, this study confirms that the oral hypercholesterol-PTU combination model is a valid and efficient animal model for preclinical testing of lipid-lowering agents, both synthetic and natural. The model offers advantages in terms of short induction time (14 days)**,** reproducibility**,** and practical laboratory application**.**

4 Conclusion

This study demonstrated that oral administration of a high-fat and high-cholesterol feed formula combined with Propylthiouracil (PTU) for 14 days was able to rapidly and significantly induce

dyslipidemia in male rats (Rattus norvegicus). This was evidenced by a marked increase in total serum cholesterol levels of +89.22 mg/dL ($p < 0.001$).

The mechanism of dyslipidemia induction involves enhanced intestinal cholesterol absorption, downregulation of low-density lipoprotein receptor (LDL-R) expression, and reduced lipid metabolism due to thyroid suppression by PTU. The combined effects of a high-cholesterol diet and PTU resulted in a metabolic disturbance closely resembling human dyslipidemia within a relatively short period.

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