



BLOOD GLUCOSE LEVEL AND PANCREAS HISTOLOGICAL SECTION OF MALE RABBITS AFTER BEING FED COMMERCIAL FEED SUPPLEMENTED WITH COD LIVER OIL

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ABSTRACT

Fish oil contains eicosapentaenoic acid (E.P.A.) and docosahexaenoic acid (D.H.A.) which are beneficial for heart health, controlling blood glucose, reducing atherosclerosis, and lowering the risk of cancer. This study aims to determine the effect of commercial feed supplemented with cod liver oil on blood glucose levels and pancreatic histology of male rabbits. Twenty rabbits were divided into four groups, namely commercial feed supplemented with cod liver oil 3% (P1), 4.5% (P2), 6% (P3), and commercial feed without cod liver oil supplementation (control). Each group consisted of five rabbits, and the treatment was given for four weeks. The parameters observed were blood glucose levels in the 1st, 2nd, 3rd, 4th, and 5th weeks and the histology of the male rabbits' pancreas. Data were analyzed by Anova. The results showed that in the 1st, 2nd, and 3rd weeks there was no significant difference ($P > 0.05$). In contrast, in the 4th and 5th weeks, there was a significant difference ($P < 0.05$) between the control and treatment of cod liver oil supplementation on blood glucose levels in male rabbits, and the observational results on the histology of rabbits showed normal pancreatic histology. In conclusion, cod liver oil supplementation on commercial feed controlled blood glucose levels, and there was no disturbance in the histological structure of the pancreas of male rabbits.

INTRODUCTION

Blood glucose levels are controlled by hormones that work antagonistically, namely insulin and glucagon, which aim to maintain homeostasis in the body. Pancreatic beta cells secrete insulin which regulates blood glucose levels by helping to transport glucose from the blood to neighboring cells. Diabetes melitus (D.M.) occurs in conditions of insulin resistance, in which the pancreas produces little or no insulin. The cells can no longer absorb glucose from the blood, resulting in hyperglycemia. Hyperglycemia is responsible for most symptoms and complications of Diabetes mellitus (D.M.) (Alizadeh, 2020).

Each year, the number of people suffering from D.M. continues to increase globally. It is estimated that by 2040 it will grow up to 642 million people (International Diabetes Federation,

2013). D.M. can be prevented or delayed by keeping blood sugar levels within the normal range so that metabolism can be appropriately controlled. Blood sugar levels can be maintained by adopting a healthy lifestyle (Juwita & Fibrin, 2018). Risk prevention can be carried out in healthy individuals with the aim of keeping this group of people within the normal range to prevent possible risks (Ministry of Health, 2012).

Consumption of foods that contain excessive amounts of calories, high in saturated fats and sugars, low in fiber, and low in micronutrients are eating patterns that can lead to D.M. (Juwita & Fibrin, 2018). Fat is one of the components in the feed that is very important for life because it is the most significant energy contributor. Not all fats should be shunned, mainly unsaturated fatty acids (U.F.A.); however, foods that contain saturated fatty acids (S.F.A.) should be avoided. Fish oil is an example of a food source containing



polyunsaturated fatty acids (PUFAs) (Ermayanti *et al.*, 2018).

PUFAs are essential fatty acids needed by the body that cannot be synthesized by the body itself and, therefore, must be available in feed (Spector and Kim, 2015). PUFAs have broad physiological effects (Ristic-Medic *et al.*, 2013). PUFAs regulate various physiological processes, including the vascular and immune systems (Auger *et al.*, 2016), the brain, and the nervous system (Fenton *et al.*, 2013). PUFAs are essential components of the membrane's phospholipid bilayer (Samuelsson, 2012), which are precursors for synthesizing bioactive lipid mediators, prostaglandins, thromboxanes, leukotrienes, and lipoxins (Gurr *et al.*, 2016). PUFAs have an anti-inflammatory role (Vangaveti *et al.*, 2016) and are associated with gut health (Menni *et al.*, 2017), and can improve lipid profiles (Ermayanti *et al.*, 2018).

In addition, fish oil which is rich in PUFA content, namely omega-3 (n-3), especially eicosapentaenoic acid (D.H.A.) and docosahexaenoic acid (E.P.A.), is known to increase adiponectin hormone levels which are associated with insulin sensitivity (Adi *et al.*, 2016). Decreased adiponectin levels contribute to atherosclerosis, cardiovascular disease, insulin resistance, and inflammation; adiponectin is said to have antidiabetic, antiatherogenic, and anti-inflammatory effects (Sukumar *et al.*, 2012). The results also show that hilsa fish oil (*Tenuosella illisha*) is beneficial in treating D.M. because it has a hypolipidemic effect (Minarul *et al.*, 2014).

The fish oil diet is a good and healthy diet because it contains fiber, high protein, and unsaturated fats (D.H.A. and E.P.A.). Several research results show the benefits of fish oil in the treatment and prevention of certain diseases, such as atherosclerosis and cardiovascular disease (Oppedisano *et al.*, 2020). Although fish oil is considered capable of effective disease prevention, concern over the possibility of increased lipid peroxidation caused by a diet rich in PUFAs remains a problem. In general, fish oil rich in PUFA is easily oxidized because the incorporation of PUFA in cell membranes makes cells more susceptible to oxidation (Osterud & Elvevoll, 2011). PUFA can cause lipid peroxide because it can react with free radicals, and free radicals can damage normal tissue (Ayala *et al.*, 2014). In addition, Djosee *et al.* (2011) stated that there was an increased risk of type 2 diabetes (T2D) in women aged ≥ 45 years who were given high doses of n-3 fatty acids (≥ 0.20 g n-3/day).

This study aims to determine blood glucose levels and pancreatic histological features of male rabbits after being given commercial feed supplemented with cod liver oil with different groups, namely 0.3%, 4.5%, and 6%. The research results are expected to add references and be helpful in developing science.

MATERIALS AND METHODS

Test animal

Experimental animals were taken from Riang Gede Tabanan, and each was kept in iron cages measuring 45x30x40 cm. A total of 20 local male rabbits were used, aged four months old, and weighing between 1450-1500 g. Rabbits as experimental animals in this study have been approved by the Animal Ethics Committees of the Faculty of Veterinary Medicine, Udayana University Denpasar, with an animal ethical approval certificate Number: B/154/UN14.2.9/PT.01.04/2021.

Feed

The feed pellets tested were commercial feed supplemented with cod liver oil (CLO). Commercial feed pellets produced by P.T. Japfa Comfeed Indonesia. The CLO used is a commercial CLO emulsion produced by P.T. Merck Tbk, Jakarta. Every 10 mL of CLO contains 4000 IU of vitamin A and 200 mg of CLO. Feed is as much as 120 g/day (the average feed requirement for adult rabbits).

Experimental design

The experimental design used was a complete randomized design (C.R.D.). Twenty local male rabbits were randomized into four treatments, including control, each with five replications. Each treatment tested was P0: commercial feed without CLO supplementation as a control, commercial feed supplemented with 3% CLO (P1), commercial feed supplemented with cod liver oil 4.5% (P2), and commercial feed supplemented with cod liver oil 6 % (P3). The feeding treatment lasted for one month, starting when the rabbit was four months old and ending when the rabbit was five months old.

Blood glucose

Blood glucose levels measured were fasting blood sugar levels using the auto check method. Blood is taken from the rabbit's ear using a needle (lancet). The blood that comes out is then attached

to a glucose strip. Blood glucose levels will be measured and appear on the auto-check screen after 5 seconds, expressed in mg/dl. Glucose measurements were carried out five times, on the 1st (before treatment), 2nd, 3rd, 4th, and 5th weeks.

Preparation of pancreatic histology preparations

The day after the treatment ended, the rabbits were sacrificed and dissected to make pancreatic histology preparations. The preparation of pancreatic histology was carried out using the paraffin method, 10% N.B.F. fixative, 5 mm thick incision, and H-E staining (Alturkistani *et al.*, 2016). Histological observations were counted in 5 fields of view using an optilab XSZ-107BN microscope camera with a magnification of 400x.

Data analysis

Statistical analysis was done using SPSS for Windows version 22. Normality test was done using the Kolmogorof-Smirnof test, and then the data were analyzed by Way ANOVA and DMRT for further analysis.

and P3. It shows that on weeks 1st, 2nd, and 3rd, the treatment of cod liver oil supplementation does not affect rabbit blood glucose levels. On the 4th and 5th weeks the P2 treatment was able to produce a decrease in rabbit blood glucose levels, while in the P3 treatment, there was no longer a decrease in blood glucose levels. Thus, the P2 (4.5%) treatment was able to affect the rabbits' blood glucose levels, but if the cod liver oil supplementation level was increased to 6% (P3), it did not affect on rabbit blood glucose.

Figure 2 shows the pancreatic histology of male rabbits after being fed commercial feed supplemented with CLO. At P0 (control) and treatment P1 (3%), P2 (4.5%), and P3 (6%), the histology of the rabbit's pancreas was normal. At P0, P1, P2 and P3 showed an orderly arrangement of cells that spread across the islets of Langerhans, and there was no empty space in the middle of the islets of Langerhans. Histology of the pancreas consists of the islets of Langerhans as endocrine glands, and between the islets of Langerhans, there are acini cells as exocrine glands. Each islet of Langerhans consists of many cells.

RESULTS

Observation results of male rabbit blood glucose levels for four weeks can be seen in Figure 1. Figure 1 shows the results of measuring blood glucose levels in local male rabbits for four weeks of observation. Based on the words listed in Figure 1, it appears that on weeks 1st, 2nd, and 3rd between control (P0) and cod liver oil supplementation (P1, P2, and P3), the results were not significantly different ($P > 0.05$) on the mean blood glucose levels of male rabbits. On the 4th and 5th weeks, there was no significant difference between P0 and P1 ($P > 0.05$), but there was a significant difference ($P < 0.05$) between P0 and P2

DISCUSSION

Research on n-3 PUFAs for the treatment and prevention of disease has increased in recent years. The benefits of ω -3 PUFA in patients with various health conditions and diseases, such as cardiovascular disease, diabetes, cancer, depression rheumatoid arthritis, Alzheimer's disease, dementia, brain development, and maternal and child health, have been studied. (Shahidi & Ambigaipalan, 2018). In addition, there is some controversy about specific side effects of fish oil on human health, including bleeding and giving fish oil can increase blood glucose.

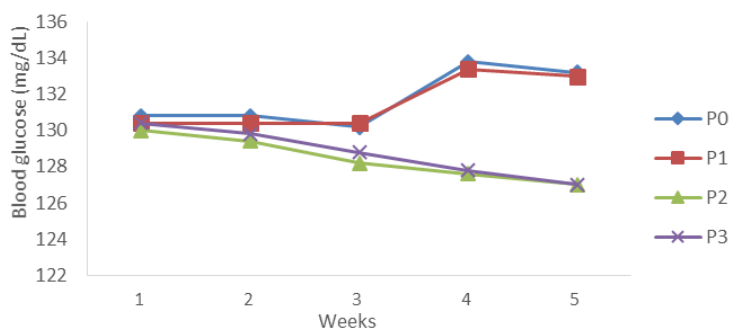


Figure 1. Rabbit blood glucose levels; P0 (control): without CLO supplementation, P1: 3% CLO supplementation, P2: 4.5% CLO supplementation, P3: 6% CLO supplementation.

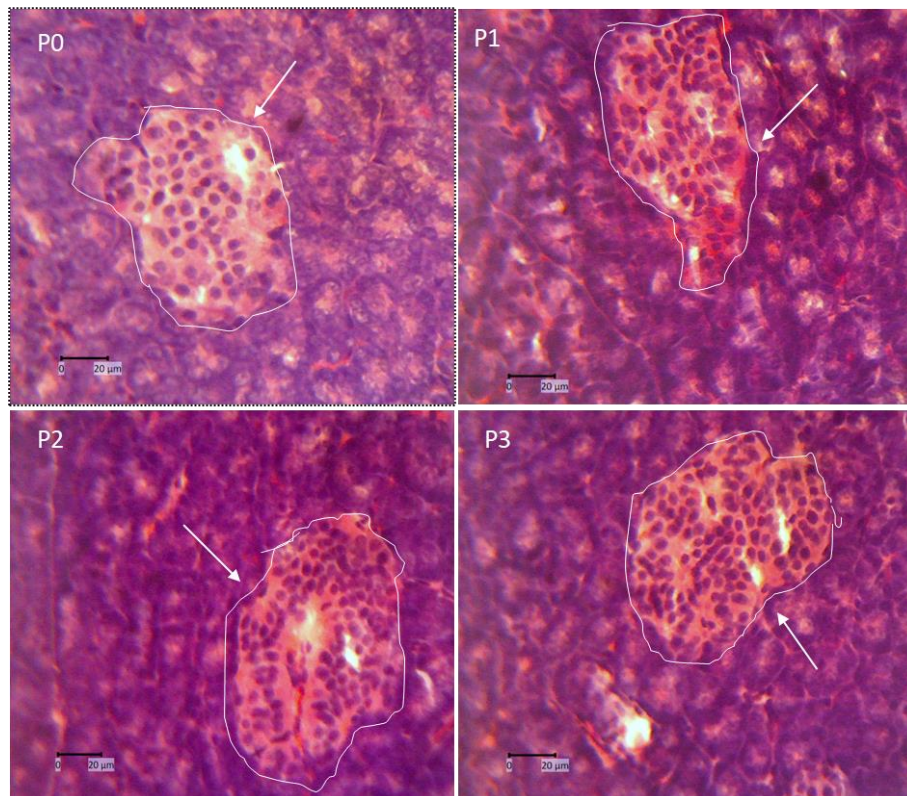


Figure 2. Histology of the male rabbit pancreas (400x, HE); P0 (control): without CLO supplementation, P1: 3% CLO supplementation, P2: 4.5% CLO supplementation, P3: 6% CLO supplementation 6%, islets of Langerhans (arrow)

In this study, based on measurements of blood glucose levels in rabbits supplemented with cod liver oil on commercial feed, the results were 130.0 – 133.8 mg/dL (Figure 1). These results represent the normal range of blood glucose levels in rabbits. According to Smith (2013), normal blood glucose levels in rabbits range from 75.6-140.4 mg/dL, although there are slight differences between those published. Male local rabbits have an average blood glucose content of 178.50 mg/dL (Nuriyasa, 2012), 75.6-149.4 mg/dL (Common, 2016), 50-135 mg/dL (Barus & Rahmi, 2021). Blood glucose levels up to 15mmol/L (270mg/dL) can cause hyperglycemia in rabbits (Harcout-Brown & Harcourt-Brown, 2012). This shows that giving fish oil with different levels can control blood glucose levels in rabbits so that it can be used for the treatment and prevention of diabetes.

Fish oil supplements, rich in n-3 PUFAs, are known to increase levels of the hormone adiponectin, which is linked to insulin sensitivity. Adiponectin is one of the specific proteins secreted by adipose tissue and can be detected in circulation. Adiponectin has a protective effect as an antidiabetic, antiatherogenic, and anti-inflammatory (Sukumar *et al.*, 2012). Clinical and

experimental studies have shown that decreased adiponectin levels contribute to obesity-related diseases, type 2 diabetes, dyslipidemia, and insulin resistance (Hariawan, 2010). Insulin resistance causes postprandial hyperglycemia, hyperinsulinemia, and pancreatic β -cell dysfunction (Lalia & Lanza 2016).

Adiponectin has many biological functions, including regulating metabolism (glucose and fatty acids) and functioning in several cellular processes (proliferation, inflammation, and oxidative stress) (Polito *et al.*, 2020). Adiponectin is insulin-sensitizing, antiatherogenic, and anti-inflammatory, which plays a role in preventing the progression of D.M. disease (Park & Sweeney, 2012). Omega-3 PUFAs contained in fish oil could control blood glucose levels and increase insulin production in Langerhans beta cells (Minarul *et al.*, 2014), and the benefits of intake of n-3 PUFAs in the prevention and treatment of type 1 diabetes (Purdel *et al.*, 2021).

Adiponectin can prevent insulin resistance by increasing glucose utilization by muscle cells and improving insulin sensitivity by reducing hepatic glucose production (gluconeogenesis), stimulating glucose and fatty acid metabolism, and increasing glucose transporter-4 (GLUT-4)

translocation in muscle cells. Adiponectin is helpful in the maintenance of insulin sensitivity (Polito et al., 2020). In addition, anti-inflammatory adiponectin can suppress the expression of proinflammatory cytokines, namely tumor necrosis factor- α (TNF- α) and interleukin 6 (IL-6), which play a role in inducing insulin resistance (Chimen et al., 2015).

Hyperlipidemia is a significant risk factor for diabetes and cardiovascular disease (CVD). Polyunsaturated fatty acids, especially omega-3, can contribute to the management of dyslipidemia and the prevention of D.M. and CVD (Kantostathi et al., 2021). The results of research conducted by Minarul et al. (2014) showed that hilsa fish oil (*Tenualosa illisha*) is helpful in the treatment of D.M. because it has a hypolipidemic effect. Its hypolipidemic effect may represent a protective mechanism against the development of atherosclerosis usually associated with D.M. Takkunen et al. (2016) reported that administration of serum long-chain omega-3 fatty acids can improve insulin sensitivity in groups of people who have a high risk of D.M. Rocha et al. (2022) reported that the prevention and treatment of dyslipidemia could be done by improving nutrition, namely replacing saturated fatty acids in food with MUFA or PUFA.

In this study, the organ observed was the pancreas because it is the most crucial organ in relation to blood glucose levels. The pancreatic islets of Langerhans function as endocrine glands that secrete insulin and glucagon hormones (Mescher, 2018). Insulin and glucagon work antagonistically to maintain blood glucose levels within the normal range. Insulin is an anabolic hormone because it inhibits gluconeogenesis and glycogenolysis while glucagon promotes gluconeogenesis and glycogenolysis. Insulin promotes the storage of nutrients as glycogen in the liver and muscles and as triacylglycerols in adipose tissue. In response to high blood glucose levels, insulin is secreted by pancreatic β cells. Impaired insulin secretion and sensitivity can lead to metabolic diseases, such as type 2 diabetes mellitus (T2D) (Roder et al., 2016).

Based on the observational results of the pancreatic histology of male rabbits fed commercial feed supplemented with cod liver oil, there was no change in the histology of the rabbit pancreas (Figure 2). The histology of the rabbit pancreas appeared normal, the cells appeared to be arranged in an orderly and compact manner, and there were no empty spaces in the islets of Langerhans. Zubaidah (2015) reported that the

islets of Langerhans are said to be expected if there is an orderly arrangement of endocrine cells that spread in the islets of Langerhans with uniform cell shapes, rounded shapes, and clear cell nuclei and no disturbed cells (edema, necrosis, degeneration).

Several researchers have reported changes in the histology of the islets of Langerhans in D.M. patients. These changes can occur both quantitatively and qualitatively. Quantitative changes include a reduction in the number or size of the islets of Langerhans, and qualitative changes include necrosis, degeneration, and amyloidosis in the cells of the islets of Langerhans. Many factors trigger the destruction of the beta cells of the islets of Langerhans, including infection by germs, genetic factors, nutrition, diabetogenic substances, and free radicals (oxidative stress) (Suarsana et al., 2010).

Free radicals can cause oxidative stress due to an imbalance between oxidants and antioxidants that have implications for various diseases, including heart failure, atherosclerosis, D.M., hypertension, stroke, and other chronic diseases (Ray et al., 2012; Kim et al., 2016). Fish oil can provide an antioxidant effect by increasing the expression of antioxidant enzymes. Based on previous research, Rocha et al. (2022) found that the administration of fish oil can reduce malondialdehyde (M.D.A.) levels and increase superoxide dismutase (S.O.D.) in rats. Ayala et al. (2014) stated SOD works by catalyzing the dismutation reaction of superoxide anion radicals into H_2O_2 .

Fish oil is an antioxidant because it can inhibit lipid peroxide, counteract free radicals and increase antioxidant enzymes. CLO exerts an antioxidant effect through increasing the expression of antioxidant enzymes, such as glutathione peroxidase (GSHPx) and catalase (CAT). Treatment with CLO can control blood glucose and lipid metabolism and improve the activity of endogenous antioxidant enzymes in various tissues in diabetic rats (Hunkar et al., 2002). As antioxidants, these enzymes inhibit the formation of free radicals, by breaking the chain reaction (polymerization), then turning them into more stable products. The enzymes GSH-Px and CAT work by converting H_2O_2 to H_2O and O_2 (Ayala et al., 2014). In addition, Hunkar et al. (2002) stated that vitamin A contained in CLO is a potent antioxidant that functions in the prevention of diabetes-induced damage so that it can reduce the risk of complications in diabetes.

Diabetes mellitus is a metabolic syndrome disease associated with dyslipidemia. Supplementation with n-3 PUFAs can be used as a complementary therapy for dyslipidemia because it has an antihyperlipidemic effect (Kantostathi *et al.*, 2021). Fish oil supplementation can reduce serum triglyceride concentrations by decreasing the occurrence of liver lipogenesis and suppressing the expression of sterol regulatory element-binding protein-1c (SREBP-1c) so that there is a decrease in the face of cholesterol, fatty acids, and enzymes that synthesize triglyceride formation (Lupei *et al.*, 2020). Based on previous research was done by Ermayanti *et al.* (2018), they stated that local rabbits supplemented with 4.5% CLO on commercial feed were able to reduce triglyceride and low-density lipoprotein (LDL) levels.

CONCLUSION

The cod liver oil supplementation on commercial feed was able to control blood glucose levels, and there is no disturbance in the pancreatic histology of male rabbits.

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