

# Striped catfish oil and turmeric extract reduces inflammation in metabolic syndrome rats

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## ABSTRACT

Metabolic syndrome is a growing global health problem. Long-term treatment for metabolic syndrome causes side effects. Therefore, the use of nutraceuticals could also be considered. This study analyzed the effect of the administration of striped catfish oil and turmeric extract on tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels and homeostasis model assessment of insulin resistance (HOMA-IR) in metabolic syndrome rats. Metabolic syndrome was induced in rats by administration of high fat fructose diet (HFFD) containing 3 g pork fat (15%), 2 g duck egg yolk (10%), 15 g standard diet (75%), and 2 ml fructose (1%). Thirty rats were randomized into five groups: C1 (normal control group), C2 (metabolic syndrome control group without treatment), P1 (striped catfish oil at 0.08 ml/200 g BW/day), P2 (turmeric extract at 5.04 mg/kg BW/day), P3 (combination of striped catfish oil at 0.08 ml/200 gBW/day and turmeric extract at 5.04 mg/kg BW/day). There was a significant decrease ( $p < 0.05$ ) in TNF- $\alpha$  levels and HOMA-IR in treatment groups (P1, P2, P3) compared to C2. The P3 group had the lowest TNF- $\alpha$  levels. Treatment groups had the same potential effect in reducing HOMA-IR. Striped catfish oil, turmeric extract, and their combination reduce inflammation and insulin resistance in metabolic syndrome rats.

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## 1. INTRODUCTION

Metabolic syndrome is a growing global health problem. Its prevalence ranges from 12.5% to 31.4%, depending on the diagnostic criteria [1]. The prevalence of metabolic syndrome in the multi-ethnic population of Indonesia from the Indonesian family life survey wave 4 (IFLS4) in 2019 was 21.66% [2]. Metabolic syndrome is important because it increases the risk of developing cerebrocardiovascular disease and type 2 diabetes mellitus [3].

Consumption of a high-calorie diet and physical inactivity are major contributors to the increase in visceral adipose tissue, which triggers most metabolic syndrome pathways [4]. The pro-inflammatory mediator tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) strongly correlates with insulin resistance in metabolic syndrome. TNF- $\alpha$  disrupts insulin signaling in adipocytes. This leads to decreased expression of the substrates insulin receptor-1 (IRS-1) and glucose transporter member-4 (GLUT-4) [5]. Insulin resistance is diagnosed by measuring the homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR is calculated from fasting blood glucose and fasting blood insulin levels [6].

Interventions for metabolic syndrome in individuals should be started early, without waiting for other components of metabolic syndrome to appear. This will reduce the incidence of complications of the disease [7]. As long-term medication for the management of metabolic syndrome can cause side effects, the use of nutraceuticals can be considered to support medication when therapeutic targets are not met [8]. One potential strategy is to supplement daily intake with bioactive compounds naturally found in foods that have anti-inflammatory, antioxidant, and endothelial-enhancing effects. These include long-chain omega-3 polyunsaturated fatty acids (LC) omega-3 polyunsaturated fatty acids (PUFA) from fish oil and polyphenolic compounds from turmeric extract [9].

Striped catfish (*Pangasius hypophthalmus*) is one of the freshwater fish that is easily farmed in Indonesia and has the potential to develop fish oil. It is therefore expected to provide an alternative to marine fish oil to protect and reduce the exploitation of marine ecosystems [10]. A recent study [11] has shown that striped catfish oil, obtained by the wet extraction method of rendering, contains 2.92% of *Eicosapentaenoic acid* (EPA) and 5.54% *Docosahexaenoic acid* (DHA) and has the potential to reduce inflammation in malnourished Wistar rats, as G-protein 120 (GPR 120) activated by omega-3 PUFA inhibits the production of inflammatory mediators by macrophage cells and inhibits cytokine expression.

Turmeric (*Curcuma longa* Linn.) rhizome is a member of the Zingiberaceae family with a variety of biological activities [12]. Recent studies [13] have shown that hot water extract of turmeric rhizome can reduce TNF- $\alpha$  levels and fasting serum glucose in subjects with overweight or prehypertension/mild hypertension. Polyphenolic compounds can scavenge free radicals and hypoglycaemic effects that can inhibit carbohydrate digestion by salivary and pancreatic  $\alpha$ -amylase and  $\alpha$ -glucosidase at the brush border of the small intestine [14].

The combination of omega-3 PUFAs and polyphenolic compounds has a reciprocal effect on bioavailability [15]. In this study, we also evaluated the synergistic and complementary effects of omega-3 PUFAs and polyphenols on inflammation and insulin resistance in Wistar rats, which have a good response to the metabolic syndrome [16]. Currently, no research evaluates the potential of omega-3 PUFA (EPA and DHA) of freshwater striped catfish oil, and the potential of polyphenolic ethanol extracts of turmeric rhizomes followed by freeze drying to improve metabolic syndrome. This study aimed to analyze the effect of the administration of striped catfish oil and turmeric extracts on TNF- $\alpha$  levels and HOMA-IR in metabolic syndrome rats.

## 2. METHOD

### 2.1. Design

This is a true experimental study with a randomized pre-post control group design. This study used 30 male Wistar rats aged 6-8 weeks weighing 150-200 g. The housing system used individual cages with an ambient temperature of 25-26°C, a 12-hour light cycle, and water ad libitum. Body weight was weighed weekly. The rats were acclimatized for seven days and then randomly divided into five groups (n=6 per group): group C1, administered with a standard regular diet Comfeed AD II at 20 g/head/day; positive control (C2) and treatment groups (P1, P2, P3) administered with an high fat fructose diet (HFFD) for 21 days to condition metabolic syndrome rats. Rats were categorized as having metabolic syndrome of the Lee index >300, total cholesterol >129.52 mg/dL, low-density lipoprotein (LDL)>81.55 mg/dL, and fasting blood glucose >111.7 mg/dl [17]. The intervention was then administered by nasogastric tube for 28 days; P1 received striped catfish oil at a dose of 0.08 ml/200 gBW/day, P2 received turmeric extract at a dose of 5.04 mg/kg BW/day, and P3 received the combination of striped catfish oil at a dose of 0.08 ml/200 gBW/day and turmeric extract at a dose of 5.04 mg/kg BW/day. HFFD continued until the intervention's end in C2, P1, P2, P3. C1 group continued to receive a standard regular diet. The 2 ml of rat blood was collected from the retro-orbital vein after 6-10 h of fasting.

In a previous study, omega-3 reduced adipose tissue macrophages in human subjects with insulin resistance after fish oil supplementation of 4 g/day for 12 weeks. Therefore, after conversion to the rat dose, the striped catfish oil intervention was given as 0.08 ml/200 g BW/day [18]. Previous studies have shown that curcumin in turmeric extract significantly improved fasting plasma insulin (FPI) and HOMA index in overweight subjects at 200 mg/kg doses for eight weeks. Therefore, after conversion to the rat dose, the turmeric extract intervention was given as 5.04 mg/kg BW/day [19].

The technique for preparing HFFD is to mix 3 g lard (15%), 2 g duck egg yolk (10%), 15 g standard diet (75%), and add 2 ml fructose (1%). Striped catfish oil and turmeric extract were administered by mixing each ingredient according to dose with 0.5% carboxymethyl cellulose (CMC) solution up to 2 ml. The mixture was then stirred until homogeneous with a handheld high-shear homogenizer. The study was approved by The Ethical Committee of Medical Research of the Faculty of Medicine, Universitas Diponegoro, Indonesia (No.58/EC-H/KEPK/FK-UNDIP/VI/2023).

## 2.2. Preparation of striped catfish oil

Striped catfish oil was prepared from 7.5 kg samples of 5-6-month-old fresh striped catfish harvested in August 2023 from Kedung Ombo Purwodadi Reservoir, Grobongan Regency, Central Java, Indonesia. They were washed, filleted, and cut into small pieces, steamed at 80 °C for 30 min, and then pressed to separate the solid and liquid fractions. A liquid fraction (crude oil) was heated at 60 °C for 30 min, added bentonite 4% was heated and stirred at 80 °C for 30 min, and the last separate uses centrifuge at 3,000 rpm for 20 min to obtain 180 ml of pure fish oil [20], [21]. Fish oil is poured into a dark glass bottle, wrapped with aluminum foil, and stored at freezing. Analysis of omega-3 PUFA content used the gas chromatography (G.C.) method, while free fatty acids used the KOH titration method (AOCS 1998), and peroxide value used the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> titration method (AOAC 2005).

## 2.3. Preparation of turmeric extract

Extraction was carried out from 3 kg samples of 10-12-month-old fresh turmeric rhizomes harvested in May 2023 from Kranggan District, Temanggung Regency, Central Java, Indonesia. They were washed, thinly sliced, and dried using a vacuum oven at 500 C for two days. Dried turmeric rhizomes are blended into 350 g of simplicia powder. Simplicia powder was macerated in 95% ethanol (700 mL, three times) at room temperature for 24 hours. The 95% ethanol solutions were evaporated using a rotary evaporator at 45 °C speed 82-90 rpm for 3 hours 29 minutes to obtain 100.87 g of turmeric extract. The turmeric extract was put into a freeze dryer for two days to obtain 44.58 g of turmeric extract [22]. Turmeric extract was poured into a glass jar bottle, wrapped with aluminum foil, and stored at freezing. Determination of total flavonoid and tannin levels using the UV-Vis spectrophotometer (Shimadzu 1800, Japan) method, while determination of saponin levels using the gravimetric method.

## 2.4. Metabolic syndrome indicators, TNF- $\alpha$ , and HOMA-IR measurements

The Lee index, fasting blood glucose, total cholesterol, and LDL measurements as metabolic syndrome criteria were performed after conditioning the rat with metabolic syndrome. The measurement of the Lee index used the formula:  $\{(\text{body weight (g)} / \text{naso-anal length (cm)}) \times 103\}$ . Fasting blood glucose and total cholesterol-LDL were analyzed using glucose oxidase-peroxidase aminoantipyrin (GOD-PAP) and cholesterol oxidase-peroxidase aminoantipyrin (CHOD-PAP) methods, respectively.

TNF- $\alpha$  levels and HOMA-IR were measured two times: after conditioning the rat with metabolic syndrome (pre-test) and after the rat was given intervention (post-test). According to the manufacturer's instructions, TNF- $\alpha$  levels were determined using ELISA-Kit (Fine Test, Wuhan Fine Biotech Co., Ltd., China). Blood glucose levels were determined by the glucose phenol 4-amino phenazone (GOD-PAP) (DiaSys Diagnostic Systems GmbH, Germany) method using a spectrophotometer. According to the manufacturer's instructions, insulin was determined using ELISA-Kit (Fine Test, Wuhan Fine Biotech Co., Ltd., China). Blood glucose and insulin levels were used to calculate insulin resistance using HOMA-IR. HOMA-IR was calculated using the formula:  $(\text{insulin (I.U./mL)} \times \text{glucose (mg/dL)}) / 405$ .

## 2.5. Data analysis

The data normality test used The Shapiro-Wilk Test. The Paired t-test was used to compare the body weight of the rats before and after the intervention. Differences in TNF- $\alpha$  levels before and after intervention were analyzed using the Kruskal-Wallis and Mann-Whitney tests to determine differences between groups. The difference in HOMA-IR before and after the intervention was analyzed using ANOVA followed by The Posthoc Tamhane Test to determine differences between groups.

# 3. RESULTS AND DISCUSSION

## 3.1. Characteristics of striped catfish oils and turmeric extract

Table 1 shows the characteristics of striped catfish oil. Our results show that striped catfish oil, extracted by the wet rendering method and purified with 4% bentonite, met the quality requirements for fish oil according to the International Fish Oil Standard (IFOS standard): free fatty acids <1.50% and peroxide value  $\leq 3.75$  mEq/kg. IFOS standards are the basis for the declaration that an oil is fit for consumption [23]. Free fatty acid content and peroxide value indicate deterioration of fish oil. The higher the free fatty acid and peroxide values, the lower the quality of the fish oil [24]. The striped catfish oil in our study contains EPA and DHA, which is more than the striped catfish oil from Bangladesh which contains EPA 2.72% and DHA 4.89% [25].

The characteristics of turmeric extract are shown in Table 2. Turmeric extract preserved by the freeze-drying method was analyzed quantitatively. The turmeric extract in our study contained flavonoids, tannins, and saponin. Flavonoids and tannins with higher levels compared to turmeric from Douala-Cameroon extracted by maceration method using methanol with flavonoid 48.50 mg QE/g and tannin 38.15 mg CE/g [26].

Table 1. Characteristics of striped catfish oil

Parameter	Result
Free fatty acid (%)	0.46±0.000
Peroxide value (mEq O <sub>2</sub> /kg)	0.978±0.001
<i>Eicosapentaenoic acid</i> (EPA) (g/100 g)	4.771±3.25
<i>Docosahexaenoic acid</i> (DHA) (g/100 g)	6.093±5.50

Data are presented as the mean ± S.D. of duplicate measurements

Table 2. Characteristics of turmeric extract

Parameter	Result (g/100 g)
Total flavonoid	18.728±0.265
Tannin	1.752±0.015
Saponin	4.457±0.791

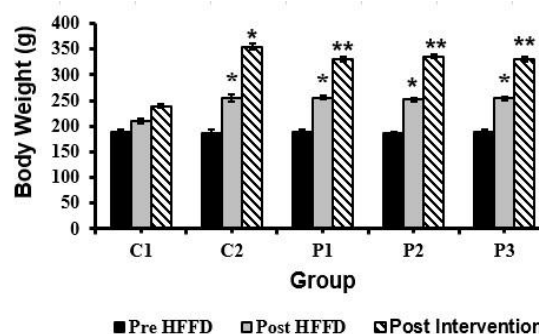
Data are presented as the mean ± S.D. of triplicate measurements

### 3.2. Body weight, TNF- $\alpha$ levels, and HOMA-IR in wistar rats with metabolic syndrome

Male Wistar rats were conditioned to develop metabolic syndrome for 21 days by administration of HFFD. The Lee index criteria for the obesity component, total cholesterol and LDL cholesterol for the dyslipidemia component, and fasting blood glucose for hyperglycemia were used in this study to determine metabolic syndrome criteria in rats. Table 3 shows that the metabolic syndrome can be induced in rats by administering HFFD for 21 days. Figure 1 also shows the body weight of rats in groups C2, P1, P2, and P3 could be significantly increased by administration of HFFD for 21 days. The increase in body weight in the C2, P1, P2, and P3 groups was on average 35.53% of the initial body weight. The standard diet group (C1) also showed an increase in body weight. However, the increase was only 11.50% of the initial body weight because the standard diet contained 15% crude protein, 5-7% fat, and some minerals for growth or survival.

Table 3. Metabolic syndrome criteria after 21 days of metabolic syndrome induction by feeding HFFD

Criteria for the metabolic syndrome	Normal value	Groups				
		C1	C2	P1	P2	P3
Lee index	>300	280.25	338.81	337.07	337.49	342.17
Total cholesterol (mg/dl)	>129.52	93.46	216.38	212.48	313.86	211.45
Cholesterol LDL (mg/dl)	81.55	25.00	81.57	79.57	81.22	78.16
Fasting blood glucose (mg/dl)	111.7	72.11	150.95	149.93	149.55	150.06



\*Significantly different with C1,  $p < 0.05$ ; \*\*significantly different with C1, C2,  $p < 0.05$

Figure 1. Comparison of body weight pre-and post-HFFD administration, and post-intervention

The HFFD diet is palatable for rats. This diet combination is used because it can describe the current high-calorie and high-fat diet that induces most metabolic syndrome metabolic disorders. A high-fat diet increases the formation of very low-density lipoprotein (VLDL). VLDL helps to distribute triglycerides. Fructose is a monosaccharide type, an intermediate glucose metabolism molecule. It is rapidly absorbed and quickly metabolized by the liver. When fructose enters the liver, it stimulates lipogenesis, accumulating triglycerides and cholesterol. It reduces insulin sensitivity, resulting in insulin resistance and glucose intolerance [27].

A recent study [28] has shown that 29 days of HFFD can induce metabolic syndrome markers, namely significant increases in obesity (the Lee index  $318.77 \pm 10.66$ ), glucose ( $147.35 \pm 34.07$  mg/dl), triglycerides ( $184.99 \pm 3.89$  mg/dl), total cholesterol ( $121.55 \pm 2.36$  mg/dl), LDL ( $69.78 \pm 1.97$  mg/dl) and decreased HDL-C ( $33.67 \pm 2.22$  mg/dl) in metabolic syndrome Wistar rats with an average daily calorie intake of  $61.03 \pm 6.45$  kcal/day, while the control group had  $33.83 \pm 6.37$  kcal/day. In our study, the administration of HFFD for 21 days resulted in an increase in body weight of 66.66 g (the Lee index  $337.07$ - $342.17$ ).

Figure 1 also shows that after 28 days of intervention, the body weight of the metabolic syndrome rats in groups C2, P1, P2, and P3 continued to increase due to the HFFD, which was continued until the end of the intervention. However, the metabolic syndrome rats that received the intervention (P1, P2, and P3) experienced significantly less weight gain ( $329.50$ - $335.67$  g) than group C2 ( $354.67$  g). Groups P1 and P3 showed the greatest weight loss ( $329.50$ - $330.17$  g). A recent study [29] suggests that obese Wistar rats fed fish oil for 8 weeks had lower weight gain (gain 5.5 g) and adipocyte volume compared to the obese Wistar rats without fish oil (gain 6.8 g). The mechanisms by which omega-3 PUFAs (EPA and DHA) promote weight loss through stimulation of  $\beta$ -oxidation and inhibition of fatty acid synthesis and VLDL secretion also regulate gene expression. The other studies [30] mentioned that the administration of curcumin 50 mg/kg/day for two weeks in rats induced by a high-fat diet (16 weeks) can reduce the body weight of rats (15 g) in comparison to the group of rats that did not receive the intervention. Flavonoids in turmeric extract prevent adipogenesis by suppressing angiogenesis in adipose tissue, reducing preadipocyte differentiation, and reducing lipid accumulation in adipocytes and the liver. These contribute to reduced adipose tissue growth, weight loss, and obesity.

Table 4 shows the changes in TNF- $\alpha$  and HOMA-IR after 28 days of intervention. At the end of the intervention, the C2 group that did not have the intervention had a significant increase in TNF- $\alpha$  levels and an increase in HOMA-IR, although it was not significant. Meanwhile, TNF- $\alpha$  and HOMA-IR levels decreased significantly in the P1, P2, and P3 groups. From these results, it can be concluded that in our study, striped catfish oil, turmeric extract, and a combination of striped catfish oil and turmeric extract can significantly reduce the TNF- $\alpha$  levels and HOMA-IR in Wistar rats with metabolic syndrome. The P3 group (a combination of striped fish oil and turmeric extract) showed the greatest reduction in TNF- $\alpha$  levels. For the reduction in HOMA-IR, there was no significant difference between the intervention groups (P1, P2, and P3) but the P3 group lowered HOMA-IR more than the single administration.

Table 4. Serum TNF- $\alpha$ , fasting blood glucose, insulin levels, and HOMA-IR pre- and post-intervention

Variable		C1 (n-6)	C2 (n-6)	P1 (n-6)	P2 (n-6)	P3 (n-6)	p-value
TNF- $\alpha$ (pg/ml)	Pre	$7.20 \pm 0.23b$	$18.27 \pm 0.35a$	$18.44 \pm 0.44a$	$18.23 \pm 0.38a$	$18.06 \pm 0.30a$	0.000*
	Post	$7.54 \pm 0.28d$	$19.48 \pm 0.24a$	$11.10 \pm 0.32b$	$10.71 \pm 0.18b$	$9.37 \pm 0.25c$	0.000*
	$\Delta$	$0.34 \pm 0.10b$	$1.20 \pm 0.20a$	$-7.33 \pm 0.26c$	$-7.51 \pm 0.52c$	$-8.65d$ $(-8.90) - (-8.61)$	0.000*
	p-value	0.000*	0.000*	0.000*	0.000*	0.000*	
Fasting blood glucose (mg/dl)	Pre	$72.11 \pm 2.46^b$	$150.95 \pm 2.86^a$	$149.93 \pm 2.60^a$	$149.53 \pm 3.35^a$	$150.06 \pm 2.78^a$	0.000*
	Post	$73.19 \pm 2.32^d$	$159.26 \pm 3.12^a$	$122.96 \pm 1.96^b$	$118.82 \pm 2.31^b$	$114.16 \pm 3.54^c$	0.000*
	$\Delta$	$1.08 \pm 0.38^b$	$8.31 \pm 0.60^a$	$-26.97 \pm 1.00^c$	$-30.73 \pm 2.14^c$	$-35.90 \pm 1.38^d$	0.000*
	p-value	0.001*	0.000*	0.000*	0.000*	0.000*	
Insulin levels (IU/ml)	Pre	$16.80 \pm 0.17^a$	$13.71 \pm 0.29^b$	$13.56 \pm 0.22^b$	$13.60 \pm 0.32^b$	$13.77 \pm 0.16^b$	0.000*
	Post	$16.97 \pm 0.18^a$	$13.23 \pm 0.19^d$	$14.61 \pm 0.23^b$	$14.48 \pm 0.21^c$	$14.83 \pm 0.08^b$	0.000*
	$\Delta$	$0.16 \pm 0.12^b$	$-0.47 \pm 0.46^c$	$1.05 \pm 0.15^a$	$0.88 \pm 0.36^a$	$1.05 \pm 0.18^a$	0.000*
	p-value	0.022*	0.003*	0.000*	0.002*	0.000*	
HOMA-IR	Pre	$2.99 \pm 0.11b$	$5.10 \pm 0.13a$	$5.02 \pm 0.6a$	$5.02 \pm 4.99a$	$5.10 \pm 0.12a$	0.000*
	Post	$3.06 \pm 0.11d$	$5.20 \pm 0.09a$	$4.43 \pm 0.05b$	$4.31c$ $4.08 \pm 4.36$	$4.18 \pm 0.13c$	0.000*
	$\Delta$	$0.75 \pm 0.02a$	$0.09 \pm 0.08b$	$-0.58 \pm 0.06c$	$-0.77 \pm 0.09c$	$-0.92 \pm 0.07c$	0.000*
	p-value	0.027*	0.058	0.027*	0.027*	0.028*	

Values are mean  $\pm$  standard deviation. p=One-way ANOVA if data were normally distributed and Kruskal-Wallis test if not normally distributed. P=Paired t-test if data were normally distributed and Wilcoxon if not normally distributed. \*=significant at  $p < 0.05$  or  $P < 0.05$ . Pre = before the intervention of the striped catfish oil (P1), turmeric extract (P2), and the combination of both (P3). Post = after the intervention of the striped catfish oil, turmeric extract, and the combination of both for 28 days.

Omega-3 PUFAs (EPA and DHA) have an anti-inflammatory effect. This anti-inflammatory effect is mediated through the activation of GPR120. GPR120 is expressed in adipocytes and macrophages. The G protein-coupled receptor 120 (GPR120) has a receptor/activator function for omega-3 PUFAs. Omega-3 PUFAs (EPA and DHA) activate GPR-120, which will inhibit the regulation of the nuclear factor kappa B (NF- $\kappa$ B) pathway and thus reduce the production of inflammatory mediators such as TNF- $\alpha$ . This in turn inhibits the development of insulin resistance [31]. This is in line with a recent study [32] mentioned above in

which fish oil supplementation for 12 weeks in schizophrenia patients with metabolic syndrome was able to reduce TNF- $\alpha$  levels by 4.47 pg/ml compared with placebo ( $72.47 \pm 14.81$  pg/ml) and reduce glucose levels ( $6.10 \pm 1.47$  mmol/L) compared with placebo ( $5.94 \pm 1.24$  mmol/L). The study reported a positive correlation between the reduction in TNF- $\alpha$  levels and a 0.62 mmol/L reduction in triglyceride levels. By reducing peripheral TNF- $\alpha$  levels, omega-3 PUFAs improve triglyceride concentrations.

Tannins and saponins also reduce intestinal glucose absorption by inhibiting  $\alpha$ -amylase and  $\alpha$ -glucosidase activity. They induce  $\beta$ -cell regeneration, thereby increasing insulin activity [33]. This is in line with a recent study [13], which found that the administration of 900 mg turmeric extract tablets for 12 weeks in subjects with overweight or prehypertension/mild hypertension can reduce the levels of TNF- $\alpha$  ( $-0.153 \pm 1.718$  pg/mL) versus placebo ( $0.215 \pm 0.342$  pg/mL), blood glucose ( $-0.1 \pm 6.6$  mg/dl) versus placebo ( $2 \pm 5$  mg/dl), and HbA1c ( $-0.033 \pm 0.125\%$ ) versus placebo ( $0.016 \pm 0.103\%$ ). Flavonoids work by suppressing the NF- $\kappa$ B pathway in turmeric extract. The development of insulin resistance is inhibited by inhibiting NF- $\kappa$ B activation. People with chronic low-grade inflammation, turmeric extract has the potential to improve systemic glucose metabolism.

The combination of striped catfish oil and turmeric extract significantly affects TNF- $\alpha$  levels and HOMA-IR. However, no complementary effects were observed in this study. Kuszewski *et al.* [34] has argued the need to identify the optimal dose when combining these two bioactive, while Thota *et al.* [35] has argued that the interaction between the two bioactive must still be studied in long-term trials.

Figure 2 shows there is a significant correlation between TNF- $\alpha$  and HOMA-IR ( $p=0.000$ ). Spearman's  $r=0.908$  indicates a very strong positive correlation. The F-Simultaneous test in this study showed  $p=0.000$  and  $F=86.621$ . The adjusted R-squared value is 74.7%. This means that TNF- $\alpha$  levels explain 74.7% of the variability of HOMA-IR. Based on simple linear regression analysis, TNF- $\alpha$  levels independently affected HOMA-IR values and obtained the equation  $\text{HOMA-IR} = 0.146 \times \text{TNF-}\alpha \text{ levels} + 2.526$ . The results of this study indicated that TNF- $\alpha$  levels had a positive effect on HOMA-IR ( $R^2=75.6\%$ ). An increase in TNF- $\alpha$  levels leads to an increase in HOMA-IR. The results of this study are in line [36] that TNF- $\alpha$  is positively correlated with HbA1c and HOMA-IR in patients with type 2 diabetes mellitus. TNF- $\alpha$  reduces the expression of GLUT-4. GLUT-4 is mainly found in adipocytes, skeletal muscle, and heart. TNF- $\alpha$  can induce the expression of the substrate IRS-1. This inhibits the action of insulin in the periphery, leading to insulin resistance.

This study has weaknesses and limitations. These may cause the study's results to be inconsistent with previous studies, namely the less than optimal dosage in combining the two ingredients to determine the complementary effect. This study was carried out on animals in a laboratory setting. Further research is needed to see the same effect in humans.

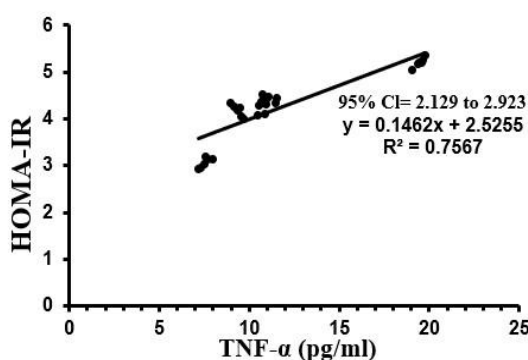


Figure 2. Simple linear regression analysis of HOMA-IR and TNF- $\alpha$

#### 4. CONCLUSION

Our study found that striped catfish oil, turmeric extract, and their combination were able to significantly reduce TNF- $\alpha$  levels and HOMA-IR in Wistar rats with the metabolic syndrome. No complementary effects were observed for striped catfish oil and turmeric extract. However, the combination of striped catfish oil and turmeric extract can reduce TNF- $\alpha$  levels and HOMA-IR to a greater extent than the single dose. This study hopes that freshwater striped catfish oil and turmeric extract can be used as a nutraceutical to improve metabolic syndrome by reducing inflammatory responses and insulin resistance.

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