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THE IMPROVEMENT OF INSULIN RESISTANCE AND THE ANTIOXIDANT CAPACITY IN TYPE 2 DIABETES MELLITUS RATS WITH WHITELEG SHRIMP SHELL POWDER (*LITOPENAEUS VANNAMEI*)

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ABSTRACT
As estimated having an increased incidence of about 50% until 2040, the diabetic condition could be augmented primarily from astaxanthin contained in carotenoids. This research examines and compares the influence of WSSP and AST complement on Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) level and Total Antioxidant Capacity (TAC) induced high-fat diet streptozotocin (HFD-STZ) in T2DM rats. WSSP 0.89gr/200gr/d (X1) and 1.77gr/200gr/d (X2) groups; and AST supplement 0.09mg/200gr/d (X3) groups were compared with both of positive (K1) and negative (K2) groups. The treatments were administered orally for 21 days to 25 Wistar rats which each group consisted of 5 rats. HOMA-IR and TAC levels were measured by ELISA and ABTs method respectively. TAC levels significantly increased in treatment groups than K1 group (p = 0.008). The increase in the TAC level of the X2 group was greater than the X1 group (p = 0.017). HOMA IR levels were significantly lower in treatment groups than K1 group (p = 0.009). X2 group had a greater decrease in HOMA IR levels compared to X1 (p = 0.016). In consequence, the research findings show a utilitarian effect of WSSP in increasing TAC and decreasing the HOMA-IR index.

Keywords: astaxanthin; HOMA-IR; antioxidant; T2DM; whiteleg shrimp shell powder

INTRODUCTION
Type 2 Diabetes Mellitus (T2DM) is a non-communicable disease that remains to be a global issue in which the incidence continues to increase every year. In 2015, the International Diabetic Federation (IDF) estimated that the number of people with diabetes will reach 415 million people with an estimation that will increase to 642 million people in 2040 **(IDF, 2015)**. The occurrence of diabetes in Indonesia reached 10 million in 2015, and it continuously increases each year for 4-6% of the world population until 2020, including Gestational Diabetes Mellitus (GDM) **(IDF, 2017; Primal, Putri and Meiriza, 2021)**. Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder characterized by hyperglycemia due to impaired metabolism of nutrients, as a result of decreased secretion and sensitivity toward insulin known as insulin resistance. Abnormality of metabolic nutrients induces increasing oxidative stress due to an imbalance between the levels of Reactive Oxygen Species (ROS) and Total Antioxidant Capacity (TAC) in the body **(Nolan, Damm and Prentki, 2011)**.

A decrease in TAC in the body results in damage of insulin receptor cell insulin resistance by activating several enzymes. The increase in circulating fatty acids in the liver and muscle results in increased activity of Protein Kinase C (PKC) followed by a disruption of phosphorylation in Insulin Receptor Substrate (IRS-1 and IRS-2) as an indication of liver and muscle insulin resistance. Circulating fatty acids can also activate the Hypoxia-Inducible Factor-1 (HIF-1) gene which will increase Jun N-terminal Kinase (JNK) and IkB Kinase (IKK) activity which will lead to insulin resistance to adipose tissue. The condition of chronic insulin resistance causes glucolipotoxicity to pancreatic β cells which results in a decrease in insulin secretion and an increase in blood glucose levels which is known as T2DM **(Sears and Perry, 2014; Samuel and Shulman, 2012; Kupsal et al, 2015)**.

The decrease of TAC in T2DM exacerbates insulin resistance which can be measured by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). This method illustrates the feedback response between insulin secretion by pancreatic β cells with glucose uptake from the circulation by insulin receptor cells into glucose target cells **(Antunes et al., 2016)**. Traditional medicine derived from nature is currently one of the most popular medical techniques. Whiteleg shrimp shells are part of unused shrimps in the shrimp processing industry, especially in Indonesia. The benefits of shrimp shells for health have not been widely known, especially for T2DM.

Whiteleg shrimp shells contain several bioactive components such as AST of 101.7 ±11.2 µg / gr which is the main antioxidant in Whiteleg shrimp shells. The other bioactive components are chitosan, Mono Unsaturated Fatty Acid (MUFA), Poly Unsaturated Fatty Acid (PUFA), vitamin E, vitamin A, and calcium **(Hue et al, 2008; Nair et al, 2017; Cahú et al, 2012; Suptijah, Jacoeb and Deviyanti, 2012).**

Each bioactive component that is also found in WSSP content has been investigated for its protective role in T2DM. The protective mechanisms used are suppressing oxidative stress, suppressing the process of gluconeogenesis in the liver, increasing uptake of glucose into the tissue, and stimulating insulin secretion in pancreatic β cells **(Ambati et al, 2014; Liu, Chang and Chiang, 2010; Hodgkin, Hills and Squires, 2008).** Astaxanthin is an important component of WSSP and pure AST from many sources that have been studied for its protective role in T2DM and obese animal model **(Sila et al, 2015; Li et al, 2016; Chan, Pen and Yin, 2012)**. The treatment of shrimp extract oil is proven to be protective as indicated by improvement of HOMA-IR in HFD-STZ induced. Our study used WSSP to avoid decreasing levels of an active component during extraction. This study has never been done before; therefore, it is expected that this study can provide knowledge to the community that the whiteleg shrimp shell is the potential to improve T2DM conditions and it is expected to produce a product that can prevent T2DM using Whiteleg shrimp shell-based ingredients.

Scientific hypothesis

The research hypothesis hinged on the assumptions that the whiteleg shrimp shell powder (*Litopenaeus Vannamei*) could be positively improving the insulin resistance and antioxidant level of T2DM rats. As an object of the *in vivo* study in Wistar strain rats, WSSP in different doses and AST supplement has been approved as an improvement of total antioxidant capacity and insulin resistance.

MATERIAL AND METHODOLOGY

Samples

The drying process of shrimp shells using the freeze-drying method was aimed at maintaining the quality and quantity of bioactive substances found in shrimp shells **(Shofian et al., 2011**). The WSSP processing referred to the previous manufacturing technique which is suitable for maintaining the bioactive component in WSSP. Drying was carried out at -40°C for 3 – 4 days after separating each part of the shrimp shell (abdomen, head, and thorax). Dry shrimp shells were then mashed with a food processor for 2 – 3 minutes then sifted using a 60 mesh sieve. WSSP was stored in dark glass bottles coated with aluminum foil at 4°C. 19.20 The WSSP was added by suspending WSSP doses of 0.89gr/200gr/day and 1.77gr/200gr/day into Natrium Carboxymethylcellulose 0.5%. DPPH free radical scavenging and ABTs radical scavenging activity assays are used subjected to determine the antioxidant activity. WSSP and AST supplement in this study had IC50 mentioned in Table 1.

Chemicals

STZ (C8H15N3O7) and nicotinamide were obtained from Nacalai Tesque, Kyoto-Japan. Natrium Carboxymethyl Cellulose was obtained from Sigma-Aldrich, Japan. TAC examination used the ABTs method with TAC kit obtained from Randox Laboratory, United Kingdom. Insulin examination using ELISA reagents was obtained from Wuhan-China, Fine test. Blood glucose examination reagents were obtained from Diasys-German and measurement of insulin resistance used the HOMA-IR method.

Animals and Biological Material:

This study used 25 male white rats Wistar (Rattus novergicus) strain which was obtained from Central Food and Nutrition Laboratory, Gadjah Mada University-Yogyakarta, Indonesia with inclusion criteria of male, age of eight weeks, the weight of 150 – 200 grams, healthy and active.

Instruments

STZ-NA, ELISA test for fasting insulin measurements, ABTs test for TAC examination. A microtube and centrifuge for 4000 rpm spinning.

Laboratory Methods

The examination of the HOMA-IR and TAC levels was done twice which were three days after STZ-NA induction and at the end of the research. Before taking rats' blood, they have fasted for 8 – 10 hours. Two ml of blood from *plexus retroorbitalis* was taken and placed in a microtube then centrifuge and spin at 4000 rpm for 15 minutes. HOMA-IR index examination was obtained from fasting blood glucose levels and fasting blood insulin. Fasting glucose examination was performed by spectrophotometry and fasting insulin was measured by ELISA method in which TAC examination was carried out by ABTs method.

Description of the Experiment

Sample preparation: Rats were acclimatized for seven days in individual stainless-steel cages at 21°C. It also had adequate air circulation and was exposed to light for 12 hours. Feed type of Comfeed II with the composition of 15% of crude protein, 3 – 7% of crude fat, 12% of moisture content, 6% of crude fiber, 7% of ash, 0.5% of phosphorus, and calcium 0.9 – 1.1% were given during the study. Rats in the K2 group received standard feed until the end of the study, while the remaining rats were induced to be T2DM by High-Fat Diet (HFD) followed by STZ-NA. HFD was given from the second week to the third week. The feed composition of HFD used was a mixture of 90% of Comfeed II, 10% of lard, and 1.25% of cholesterol of the total HFD feed weight. Comfeed II feed and HFD were given as much as 15 g/d. STZ-NA induction was carried out after rats received HFD for 14 days, STZ-NA dose was 45 mg/kg and 110 mg/kg (i.p). Rats induced by STZ-NA were declared to have T2DM if blood glucose levels were >250 mg/dl **(Ghasemi, Khalifi and Jedi, 2014)**.

Number of samples analyzed: 25 male white rats Wistar (Rattus novergicus) strain with the weight of 150-200 grams, healthy and active. This research is a true experiment containing a randomized pre-posttest with a

control group design. Rats were split up into five groups of which each group consisted of five. K1 group was a group of T2DM rats, while K2 group was a group of healthy rats which did not receive any treatment. X1 and X2 groups were groups of T2DM rats treated with WSSP in doses of 0.89 gr/200gr/d and 1.77gr/200gr/d respectively. Finally, X3 group was a group of T2DM rats treated with AST supplement in a dose of 0.09mg/200gr/d. WSSP and AST supplements were carried out for 21 days through the oral gavage.

Number of repeated analyses: 0

Number of experiment replication: 0

Statistical Analysis

Statistical analysis was used to see the effect of giving WSSP and AST supplements on HOMA-IR and TAC levels. Paired t-test analysis was utilized to see dissimilarity of the HOMA-IR and TAC level before and after WSSP and AST supplement treatment in the data with normal distribution, whereas the Wilcoxon test was used to see differences of the HOMA-IR and TAC level before and after WSSP and AST supplement treatment in the data with the abnormal distribution. Analysis of HOMA-IR and TAC level between groups used the ANOVA test followed by the Poshoc Bonferoni test for the normally distributed data, otherwise, the Kruskal Wallis test was followed by Mann-Whitney test for the abnormally distributed data. Statistical analysis was done by using SPSS 21 software, the difference was significant when the p-value is less than 0.05 and CI of 95%.

RESULTS AND DISCUSSION

The weighing of the rats was carried out every week upon which the average weight of the rat at the beginning of the study was 192.23 gr. The average weight and blood glucose of rats after the induction of HFD-STZ was 226.23 gram and 420.03 mg/dl; hence, this indicates that the induction of HFD-STZ can increase the blood glucose levels and weight of the rats.

The effect of WSSP and AST supplement treatments on the TAC of rats induced by HFD-STZ can be seen in Table 2. Both K2 and K1 groups had a reduced TAC level in the post-experiment and these differences were not significant ($p = 0.180$ and $p = 0.063$). All treatment groups (X1, X2, and X3) show increase TAC levels in the post-treatment, these were demonstrated by higher TAC levels in the post-treatment than those of pre-treatment, and these were significantly different ($p = 0.039$, $p = 0.001$, and $p = 0.041$). These findings indicated that WSSP in doses used in this study were affiliated with the rise of TAC levels in T2DM rats, this association was also observed in AST treatment.

Because of Kruskal-Wallis test showed a significant difference of TAC level change ($p < 0.001$, Table 2), the Mann-Whitney test was then performed and can be seen in Table 3. TAC level change of K2 compared with K1 groups was not different ($p = 0.408$, Table 3). Both K2 and K1 groups were shown significant differences in TAC level change than each treatment group, i.e. X1, X2, and X3. The negative TAC level change in both control groups was significantly different than those of positive TAC level change in each treatment group (Table 2 and Table 3). The TAC level change of X2 group was higher than X1 group (ΔTACX2 = 0.86mmol/l $\pm$0.19, $p = 0.017$) and this was notably contrast ($p = 0.017$ Table 3). This indicated that the dose of 1.77gr/200gr/d WSSP showed a better effect on TAC level change than the dose of 0.89gr/200gr/d WSSP. The TAC level change of both X1 and X2 groups was lower than X3 groups (Table 2). This indicated that the effect of WSSP on TAC level change was lower than AST in T2DM rats. There is a slight decrease in TAC in the group of K2 (-0.15 mmol / l) which is followed by an increase in blood glucose levels ($p = 0.007$) and a decrease in insulin levels ($p = 0.012$) in Table 4; therefore, this indicated that there is an association between the decrease of TAC level with T2DM.

There is a declining number in blood glucose levels and an inclining in insulin levels after the WSSP and AST supplement treatment in T2DM rats ($p < 0.05$, Table 4). Both K2 and K1 groups had increase blood glucose levels in the post-experiment and this difference was significant ($p = 0.007$, $p = 0.013$, Table 4). K2 group has decreased blood insulin level in the post-experiment, and these differences were significant ($p = 0.012$, Table 4).

Because the Kruskall-Wallis test showed a significant difference in blood glucose and insulin change ($p = 0.000$, Table 4), the Mann-Whitney test was then performed (Table 5 and Table 6). Blood glucose level changes of K2 and K1 groups were different ($p = 0.009$, Table 5). Both K2 and K1 groups were shown significantly different blood glucose level changes than each treatment group i.e. X1, X2, and X3. The blood glucose level change of X2 group was higher than X1 group (Table 4), upon which this was significantly different ($p = 0.009$, Table 5). This indicates that the dose of 1.77gr/200gr/d WSSP showed a better effect on blood glucose level change than the dose of 0.89gr/200gr/d WSSP. The blood glucose level change of X2 group was higher than X3 group (Table 4), upon indicated that the effect of WSSP on blood glucose level change was better than AST supplement in T2DM rats.

The blood insulin level changes of K2 and K1 groups were different ($p = 0.008$, Table 6). Both K2 and K1 groups were shown significantly different blood insulin level changes than each treatment group. The blood insulin level change of X2 group was higher than X1 group (Table 4), this indicated that the dose of 1.77gr/200gr/d WSSP showed a better effect on blood insulin level change than the dose of 0.89gr/200gr/d WSSP. The blood insulin level change of X2 group was lower than X3 group (Table 4), this indicated that the effect of WSSP on blood insulin level change was lower than AST supplement.

The effect of WSSP and AST supplement treatment on the HOMA-IR of the T2DM rat is shown in Table 7. HOMA-IR index of K1 group was higher at the end of the experiment than those on the starting point and this was significantly different ($p = 0.001$, Table 7). Healthy rats in K-group showed no difference of HOMA-IR index in those to the final point ($p = 0.235$, Table 7). HOMA-IR index in each treatment group (X1, X2, and X3) was significantly lower in the post-treatment than in the pre-treatment.

Table 1. IC50 Value of WSSP and AST Supplement using DPPH and ABTs Radical Scavenging.

Method	Sample	IC50(ppm)
DPPH	WSSP	47,639.9
	AST Supplement	26,309.05
ABTs	WSSP	242,783
	AST Supplement	154,347.7

Table 2. Effect of Whiteleg Shrimp Shell Powder and Astaxanthin Supplement on TAC (mmol/l) Level.

Groups	Pre-Treatment Mean ± SD	Post-Treatment Mean ± SD	Difference (Δ) pre-post treatment	p^a	p^d
K2	2.06 (1.91 – 2.06)	1.91 ±0.18	-0.15 (0.15 – 0.15)	0.180[b]	0.000*
K1	0.32 ±0.12	0.15 (0.15 – 0.29)	-0.14 (0.01 – 0.15)	0.063[b]	
X1	0.29 ±0.15	0.74 (0.74-0.88)	0.59 (0.30 – 0.59)	0.039[b]*	
X2	0.44 (0.29 – 0.44)	1.24 ±0.17	0.86 ±0.19	0.001[c]*	
X3	0.26 ±0.12	1.91 (1.76 – 1.91)	1.61 (1.47 – 1.62)	0.041[b]*	

Note: K2 = healthy rat + standard feed; K1 = T2DM + standard feed; X1 = T2DM + WSSP 0.89 gr/200 gr/d; X2 = T2DM + WSSP 1.77 gr/200 gr/d; X3 = T2DM + AST supplement 0.09 mg/200 gr/d; data with normal distribution represented as mean ± SD, otherwise it was written as median (min-max); a =*p* value of the differences between pre- and post-treatment; b = Wilcoxon; c = paired t-test; d = *p* value for the differences between pre- and post-treatment among five groups (Kruskal Wallis); * = *p* value significant.

Table 3. Mann Whitney Test of TAC Level Change (mmol/L) Pre-Post Treatment.

Groups	*p* value				
	K2	**K1**	**X1**	**X2**	**X3**
K2	-	0.408	0.006*	0.007*	0.007*
K1		-	0.008*	0.008*	0.008*
X1			-	0.017*	0.008*
X2				-	0.008*
X3					-

*p<0.05 = significant level

Table 4. Effect of Whiteleg Shrimp Shell Powder and AST Supplement on Blood Glucose (mg/dl) and Insulin (µIU/ml) Level.

Groups		Pre Mean ± SD	Post Mean ± SD	Difference (Δ) pre-post treatment	*p* value	p^c
K2	Blood Glucose (mg/dl)	76.41 ±6.01	77.49 ±5.79	1.08 ±0.48	0.007[a]*	0.000
	Blood Insulin (µIU/ml)	17.32 ±0.20	16.49 ±0.30	-0.38 ±0.20	0.012[a]*	0.000
K1	Blood Glucose (mg/dl)	433.62 ±22.37	440.17	6.55 ±3.41	0013[a]*	
	Blood Insulin (µIU/ml)	12.21 ±0.13	±22.42	0.35 (0.09-0.35)	0.042[b]*	
			12.49 ±0.20			
X1	Blood Glucose (mg/dl)	391.74 (383.76-423.93)	138.08 ±3.39	-258.11 ±15.16	0.000[a]*	
	Blood Insulin (µIU/ml)	12.46 ±0.24	13.88 ±0.32	1.41 ±0.14	0.000*	
X2	Blood Glucose (mg/dl)	477.78 (393.45-484.90)	107.70 ±1.91	-370.25 (-287.51 – -376.95)	0.043[b]*	
	Blood Insulin (µIU/ml)	12.28 ±0.15	14.68 ±0.20	2.40 ±0.32	0.000[a]*	
X3	Blood Glucose (mg/dl)	405.87 ±27.78	97.24 ±1.24	-308.63 ±28.09	0.000[a]*	
	Blood Insulin (µIU/ml)	12.18 ±0.12	15.49 ±0.18	3.31 ±0.07	0.000[a]*	

Note: K2 = healthy rat + standard feed; K1 = T2DM + standard feed; X1 = T2DM + WSSP 0.89 gr/200 gr/d; X2 = T2DM + WSSP 1.77 gr/200gr/d; X3 = T2DM + AST supplement 0.09 mg/200 gr/d; data with normal distribution were represented as mean ± SD, otherwise it was written as median (min-max); a = paired t-test; b = Wilcoxon; c = p value for the difference between pre-post treatment among five groups (Kruskal Wallis); * = *p* value significant.

Table 5. Mann Whitney Test of Blood Glucose Change Pre-Post Treatment.

Groups	*p* value				
	K2	K1	X1	X2	X3
K2	-	0.009*	0.009*	0.009*	0.009*
K1		-	0.009*	0.009*	0.009*
X1			-	0.009*	0.028*
X2				-	0.175
X3					-

Note: *p<0.05 = significant level.

Table 6. Mann Whitney Test of Blood Insulin Change Pre-Post Treatment.

Groups	*p* value				
	K2	K1	X1	X2	X3
K2	-	0.008*	0.009*	0.009*	0.009*
K1		-	0.008*	0.008*	0.008*
X1			-	0.009*	0.009*
X2				-	0.009*
X3					-

Note: *p<0.05 = significant level.

Table 7. Effect of Whiteleg Shrimp Shell Powder and AST Supplement on HOMA-IR Index.

Groups	Pre-Treatment Mean ± SD	Post-Treatment Mean ± SD	Difference (Δ) pre-post treatment	p^a	p^d
K2	2.94 ±0.26	2.92 ±0.26	-0.03 ±0.04	0.235[c]	0.000*
K1	11.77 ±0.66	12.21 ±0.63	0.45 ±0.12	0.001[c]*	
X1	10.97 ±0.36	4.26 ±0.08	-6.71 ±0.42	0.000[c]*	
X2	12.20 ±1.26	3.51 ±0.06	-9.41 (7.22,9.63)	0.043[b]*	
X3	10.98 ±0.73	3.35 ±0.08	-7.64 ±0.74	0.000[c]*	

Note: K2 = healthy rat + standard feed; K1 = T2DM + standard feed; X1 = T2DM + WSSP 0.89 gr/200 gr/d; X2 = T2DM + WSSP 1.77 gr/200 gr/d; X3 = T2DM + AST supplement 0.09 mg/200 gr/d; data with normal distribution were represented as mean ± SD, otherwise it was written as median (min-max); a = p value of the difference between pre and post treatment; b = Wilcoxon; c = paired t-test; d = p value for the difference between pre and post treatment among five groups (Kruskal Wallis); * = p value significant.

Table 8. Mann Whitney Test of HOMA-IR Index Change Pre-Post Treatment .

Groups	*p* value				
	K2	K1	X1	X2	X3
K2	-	0.009*	0.009*	0.009*	0.009*
K1		-	0.009*	0.009*	0.009*
X1			-	0.016*	0.028*
X2				-	0.175
X3					-

Note: *p <0.05 = significant level.

This indicated that WSSP and AST supplement treatments were reduced insulin resistance in T2DM rats. Additionally, T2DM rats without treatment showed an increase in insulin resistance at the end of treatment. There are differences in HOMA-IR among the groups after WSSP and AST supplements treatment (p = 0.000, Table 7). The statistical analysis of the HOMA-IR index change was shown in Table 7.

The increase in HOMA-IR index change found in K1 group was significantly different than those of a decrease in the HOMA-IR index change in the treatment group (X1, X2, and X3) (Table 7 and Table 8). This indicated that WSSP and AST supplement treatment was associated with reduced insulin resistance in T2DM rats. Decreasing the index in the HOMA-IR change of the X2 group was more obvious than those in X1 group, and this difference was significant (p = 0.016, Table 8). This suggested that WSSP

in a dose of 1.77gr/200gr/d has a better effect on insulin resistance than the dose of 0.89gr/200gr/d on T2DM rats. Interestingly, decreasing HOMA-IR index change of X2 group was not different than those of X3 group ($p = 0.175$, Table 8). This suggested that the dose of 1.77gr/200gr/d WSSP has no different than AST supplements in their ability to reduce insulin resistance.

The most interesting finding in this study was WSSP and AST had no different abilities in reducing insulin resistance in T2DM rats. This was shown by HOMA-IR index change analysis (Table 7 and Table 8). It was previously hypothesized that WSSP treatment has a higher ability than AST supplement in reducing insulin resistance. To our knowledge, this study was the first that shows this interesting finding. Previous studies open the possibility of many mechanisms involved in reducing insulin resistance.

Although the results of the activity test of antioxidant WSSP was a low category, the bioactive component in WSSP will be digested by digestive enzymes including the pepsin and lipase enzyme in the body. This digestive process causes WSSP to be more representative when digested in the body. Pepsin is a hydrolase enzyme that catalyzes the hydrolysis reaction of a substrate using water molecules as a mediator. Pepsin can break down protein molecules to be smaller; therefore, the AST can separate itself from its bonds, in this case, are amino acids. In addition to the enzyme pepsin, the lipase enzyme, which is one of the enzymes that play a role in breaking down fat molecules into fatty acids, also plays a pivotal role in the release of AST from fatty acid bonds. The digestive process of AST follows the process of fat metabolism in the body. The absorption of AST greatly depends on the steps of food digestion to ease the absorption of AST into the intestinal lumen starting from the mechanical and chemical digestion of the digestive tract, emulsification assisted by bile salts, absorption by microvilli, and binding of AST with some lipoproteins. The emulsion of the fat types can increase the bioavailability of AST by expanding the surface for the release of AST. This emulsion's works resemble the mechanism of release of bile salts during lipolysis and help the absorption of lipophilic compounds that will increase the solubility of AST. By formulating AST in the form of fat-based emulsions, AST absorption can get into the lymphatic circulation without having to follow fat metabolism from the beginning. Polar carotenoids such as zeaxanthin, lutein, AST are transported by High-Density Lipoprotein (HDL). The fat composition in WSSP and digestive processes in animal bodies can predictably increase AST bioavailability in WSSP, hence it can improve TAC and insulin resistance **(Ayudiarti, 2011; Affandi, Julianto and Majeed, 2012; Ambati et al., 2014; Benarroch et al., 2016)**.

An increase in the production of ROS was accompanied by a decrease in TAC levels results in the release of fatty acids into the blood circulation. This mechanism impacts the activation of HIF-1 gene. The gene will have an impact on an increase in the activity of several enzymes such as JNK and IKK which are known to cause damage to insulin receptor cells in adipose tissue **(Ye, 2013)**. Oxidative stress also results in insulin resistance in the liver and muscles by increasing Diasyl Glycerol (DAG) which increases Protein Kinase C (PKC), this is followed by a disruption of phosphorylation in Insulin Receptor Substrate (IRS-1 and IRS-2) as an indication of liver and muscle insulin resistance. An increase in fatty acids in the circulation due to oxidative stress also causes an increase in the formation of ceramide which will produce large amounts of Nitric Oxide (NO). This substance is toxic to pancreatic β cells and results in the death of pancreatic β cells which is known as T2DM **(Al-Goblan, Al-Alfi and Khan, 2014; Sears and Perry, 2015)**.

Whiteleg Shrimp Shell Powder contains several bioactive components that can improve T2DM. One of the main antioxidants in the carotenoid category found in WSSP is AST, AST is known as a powerful antioxidant that works from inside and outside the cell **(Ambati et al., 2014)**. Astaxanthin acts as a powerful antioxidant by the mechanism of supplying electrons and reacting with free radicals. One of which is ROS which will form more stable free radicals and stop the systemic reaction of free radicals. Astaxanthin can inhibit the production of ROS that occurs in mitochondria in antioxidant activity from AST. Therefore, it will increase antioxidant status in the bodies of rats induced by T2DM **(Yang et al., 2015)**. The previous study shows that giving AST extract from shrimp waste increases the levels of Catalase (CAT), Superoxide Dismutase (SOD), and Glutathione Peroxide (GPx), particularly GPx in diabetic induced rats (**Sila et al., 2015)**. Astaxanthin supplements resulted in an increase in TAC in overweight and obese humans **(Choi et al., 2011)**. Giving HFD-STZ aims to form oxidative stress in the body especially pancreatic β cells which are known as one of the cells of the body that contains a little antioxidant **(Savu et al., 2012)**. Giving HFD-STZ results in a decrease in TAC levels which is caused by several things, namely an increase in the production of ROS and inadequate endogenous antioxidants due to competition between endogenous antioxidants and pro-oxidants in the body **(Asmat, Abad and Ismail, 2016)**. There was a significant decrease in the levels GPx, Glutathione Redoxin (GR), and SOD when they were compared to the non DMT2 group **(Aouacheri et al., 2015)**. A decrease in oxidative stress caused by the activity of AST antioxidant results in the improvement to insulin receptors which will lead to the improvement of the T2DM condition. There is a slight decrease in TAC in the group of K2 (-0.15 mmol / l) which is followed by an increase in blood glucose levels ($p = 0.007$) and a decrease in insulin levels ($p = 0.012$). This condition was predictably caused by psychological stress experienced in K2 group. The lack of antioxidant composition in the standard feed also predictably leads to a decrease in TAC in the group of K2. It illustrates the importance of exogenous antioxidant intake to maintain body health **(Shiloah et al., 2003)**.

The other study shows that there was a decrease in blood glucose levels in diabetic rats after the intake of AST with a dose of 50 mg/kg Body Weight and 100 mg/kg Body Weight for 14 days **(Li et al., 2016)**. Shrimp waste oil can lower blood glucose levels in rats that were given HFD after glucose tolerance testing **(Nair et al, 2017)**. The previous study shows that AST increases the production and secretion of insulin in T2DM rats. Regeneration of pancreatic β cells is caused by suppression of oxidative stress biomarkers such as H_2O_2 and MDA as well as an increase in total antioxidant capacity which results in a decrease in blood glucose levels in the circulation.

Therefore, it gives them time for β cells to repair to increase insulin secretion **(Al-Bulish et al., 2017)**.

An increase in TAC resulted in an improvement in the HOMA-IR index. This research finds that there was an increase in TAC and a decrease in the HOMA-IR index after the treatment of WSSP and AST supplements. Astaxanthin is not the only bioactive substance contained in WSSP, chitosan is a chitin deacetylation product which is a polymer of glucosamine long-chain (β-1, 4-2 amino-2-deoxy-D-glucose) which has an impact on the HOMA-IR index. Chitin and chitosan are known to have several activities such as antioxidants and antidiabetic. The mechanism of the improvement in T2DM done by chitosan through suppressing the process of gluconeogenesis in the liver is by suppressing the expression of Phosphoenolpiruvate Carboxykinase (PEPCK), p38 phosphorylation, and an increase in the activities of AMP-kinase (AMPK) in the liver so that the store of glucose in the liver can be maintained. Chitosan also increases kinase Protein Akt / PKB which is the main mediator in the translocation of GLUT4. This can increase the uptake of glucose by expediting the translocation of GLUT4 from the circulation into glucose target tissues such as the liver and muscles **(Liu, Chang and Chiang, 2010)**. The mechanism of T2DM repair done by chitosan provides an opportunity for Pancreatic β cells and insulin receptors to carry out cell regeneration due to a decrease in glucolipotoxicity and low calories from the standard feed. An increase in insulin secretion results in a decrease in blood glucose levels which leads to an improvement in the HOMA-IR index **(Sathananthan et al., 2015)**.

Another component that contains WSSP is calcium which has a role in insulin secretion by pancreatic β cells. The condition of T2DM leads to a decrease in calcium levels both in extracellular and intracellular. Hypocalcemia was caused by the accumulation of Advanced Glycation End Products (AGEs) in bone collagen fibers. Accumulation of AGEs increases the process of calcium resorption that results in Osteoporosis under the condition of T2DM. As many as 85.49% of the calcium content in shrimp shells is known to be able to meet calcium needs to stimulate insulin secretion by pancreatic β cells when the condition of pancreatic β cells has improved and insulin secretion by β cells is still normal **(Miyata et al., 1997)**. There was an increase in insulin secretion by pancreatic β after calcium supplementation for diabetic patients and those at risk of diabetes **(Mitri et al., 2011)**. The other study shows that there was a decrease in the HOMA-IR index in diabetic patients that were given fortified yogurt of vitamin D and Calcium **(Nikooyeh et al., 2011)**.

The results of this study showed that a higher increase of TAC was found in the treatment group of AST supplement, otherwise, the HOMA-IR index change showed that X2 group had better improvement compared to X1 and X3 treatment group. It indicated that WSSP has an advantage in improving the condition of T2DM. Bioactive substances contained in WSSP, such as AST, chitosan, and calcium, as well as various other substances, that get together with different mechanisms can reduce the HOMA-IR index as a form of improvement in T2DM conditions and insulin resistance in peripheral tissues. The results of this study provide a new insight into which WSSP can increase TAC and decrease the HOMA-IR index in T2DM conditions. Further researches need to be carried out to determine the in-depth mechanism for T2DM improvement by WSSP so it can make WSSP one of the products for prevention of T2DM disease and progressive prevention of T2DM itself.

CONCLUSION

In a conclusion, the results of this study show a beneficial effect of WSSP in increasing TAC and decreasing the HOMA-IR index in Wistar rats induced by HFD-STZ. For further investigation of the proper use of pathohistological preview of the pancreatic tissue amelioration, it would be advisable to investigate the tissue remodeling and hematological profile in different stages of diabetic tissue damage.

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