

Effect of inulin addition in fermented cassava (growol) cookies on the glycemic control and lipid profile in STZ-NA-induced diabetic rats

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ABSTRACT:

Introduction: Diabetes mellitus (DM) is an emergency health problem that occurs globally. The condition of DM contributes to an increase in morbidity due to complications, mortality rates, and health costs. Improving diet is a solution for treating DM. The addition of inulin to growol cookie products is known to reduce the glycemic index of cookies to 41 (low glycemic index). The addition of inulin, a dietary fiber, is thought to be able to provide antihyperglycemic and antihyperlipidemic effects.

Objectives: This study aims to examine the effectiveness of inulin addition into growol cookies to improve glycemic control and lipid profiles.

Methods: Induction of DM was carried out in 20 male Sprague Dawley rats by administering a high-fat diet for three weeks followed by injection of 110 mg/kgBW of nicotinamide (NA) and 45 mg/kgBW of streptozotocin (STZ). Rats with DM were randomly divided into 4 groups, namely negative control (KN), positive control (glibenclamide 0.09 mg/200 grams of rat weight) (KP), giving control growol cookies as a snack (CGK), and giving inulin-modified growol cookies as a snack (CGI). Measurements of glucose, insulin, and lipid profiles were carried out before and after four weeks of intervention using GOD-PAP, ELISA, and CHOD-PAP techniques. Homa IR and Homa β measurements were carried out using a formula based on the results of glucose and insulin levels.

Results: There were significant differences in glycemic control and lipid profiles between groups ($p < 0.05$ and $p < 0.001$). The CGI group had glucose, insulin, and total cholesterol levels that were close to the healthy control group (KS) and better than the KP group. Homa IR values, triglyceride, HDL levels, and LDL levels in the CGI group were as good as those in the KP group. The best Homa β value was in the KS group, followed by the CGI group and the KP group.

Conclusions: The addition of inulin to growol cookies can improve glycemic control and lipid profiles in experimental animals.

1. Introduction

Diabetes mellitus (DM) is an emergency health problem that occurs globally (Al-Lawati, 2017; Antar et al., 2023; Bahri et al., 2023; Fortofoiu et al., 2022; Ong et al., 2023). More than half a billion people worldwide suffer from DM, and the International Diabetes Federation (IDF) estimates that there will be an increase in DM cases of 46% in 2021 (537 million), 2030 (643 million), and 2045 (783 million), with 90% the case is type 2 DM (Hossain et al., 2024; International Diabetes Federation, 2021b, 2021a). Indonesia, a developing country, ranks fifth in the world in the number of DM sufferers with a total of 19.5 million cases, and it is estimated that this number will continue to increase to 28.6 million. Meanwhile, in 2021 Indonesia be the third country in the world with 14.3 million undiagnosed DM sufferers (International Diabetes Federation, 2021b).

In 2019, DM contributed to 1.5 million deaths with almost 50% of deaths occurring in the population aged less than 70 years (Antar et al., 2023; Bahri et al., 2023). Possible complications include diabetic nephropathy, diabetic retinopathy, diabetic neuropathy, and cardiovascular disease (Ortiz-Martínez et al., 2022; Raghavan et al., 2019). Nearly 500 thousand cases of death are due to diabetes complications, especially diabetic nephropathy (Antar et al., 2023; Bahri et al., 2023). Furthermore, the incidence of DM can increase 2-4 times the death rate due to cardiovascular disease (Raghavan et al., 2019).

The 2021 IDF report states that the increasing prevalence of DM has an impact on increasing health spending (reaching almost \$1000 billion). The estimated surge in the number of DM cases in 2030 and 2045 will increase the health care costs by up to \$1028 billion and \$1054 billion (Hossain et al., 2024; International Diabetes Federation, 2021b).

DM emergencies do not only occur in adults, however, these emergencies have been seen since young people ≤ 18 years or early adulthood (19-39 years) or what is known as early-onset type 2 diabetes (Estina et al., 2020; Lascar et al., 2017; Misra et al., 2023). This urgency can also be seen in the form of the failure of glucose tolerance, namely 541 million cases (10.6%) in 2021 (International Diabetes Federation, 2021b). This condition will worsen diabetes complications and the possibility of premature death (Misra et al., 2023; Ong et al., 2023).

Of course, this problem must be resolved immediately. One way is by improving DM risk factors. One of the risk factors that cause DM is low activity, excessive food intake, and accumulation of fat cells (Hossain et al., 2024; Lascar et al., 2017; Misra et al., 2023; Ortiz-Martínez et al., 2022). Obesity is a real cause of DM, especially type 2 DM (Hossain et al., 2024). Intake of foods that are dense in calories and high in sugar contributes significantly to the incidence of obesity which will lead to DM (Lascar et al., 2017). In addition, foods with a high glycemic index (GI) followed by foods with low fiber, contribute to a twofold increase in the incidence of DM (Indrahadi et al., 2021; Kimura et al., 2021). However, a diet with low GI and high fiber helps control glycemic levels and reduce weight in individuals with prediabetes and diabetes (Vega-López et al., 2018; Zafar et al., 2019). Foods with low GI and high fiber are also able to reduce fasting blood glucose by 9.97-15.3 mg/dL and HbA1c by 0.26-0.55%. Dietary fiber can increase the potential of prebiotics which play a selective role in stimulating gastrointestinal microbes to produce short-chain fatty acids (SCFA), which are responsible for regulating energy metabolism, providing a feeling of fullness, increasing insulin sensitivity, and improving glycemic control and lipid profile (Everard & Cani, 2013; Vinelli et al., 2022).

Growol, a spontaneous-fermented product from cassava, is a food product with high fiber content (13.17 grams of dietary fiber in 100 grams of growol) (Puspaningtyas et al., 2019; Sari & Puspaningtyas, 2019). Growol has been developed being growol cookies, and it has been modified to use inulin to increase the effectiveness of the fiber in cookies. After the addition of inulin, the glycemic index of growol cookies reaches 41 (low GI), so growol cookies have the potential to be developed as a healthy food in the DM diet (Puspaningtyas et al., 2020, 2022).

The addition of inulin as a prebiotic to growol cookies has not been studied much before, especially in the context of DM management. Additionally, the use of STZ-NA-induced diabetic rats as an animal model provides a relevant approach for studying the effects of this functional food product on in vivo metabolic parameters. Further studies are needed to review the effectiveness of inulin-modified growol cookies as a snack for DM, especially in improving glycemic control and lipid profiles.

2. Objectives

This study aims to examine the effectiveness of adding inulin to growol cookies to improve the metabolic profile, especially in glycemic control and lipid profile. This study contributes as a basis for developing growol into a functional local food product that offers health benefits, especially for DM patients.

3. Methods

Research Preparation

Before the research was carried out, ethical clearance from the Health Research Ethics Committee of Universitas Respati Yogyakarta had been obtained. As for the ethical certificate number from this research is 092.3/FIKES/PL/VII/2024.

This research was in vivo study with pretest-posttest design and involved 25 rats Sprague Dawley 8 week-old males divided into 5 groups: 1) KS group (healthy control), 2) KN group (negative control), 3) KP group (positive control with the administration of glibenclamide dose of 0.09 mg/200 grams of rat weight), 4) CGK group (DM rats with the provision of snacks in the form of control growol cookies), 5) CGI group (DM rats given snacks in the form of inulin-modified growol cookies).

Before the intervention process was carried out, the experimental animals received adaptation first with the following conditions: 1) Individual cages (stainless steel) size 17.5 x 23.75 x 17.5 cm (7 x 9.5 x 7 inches), 2) Light-dark lighting cycle of 12 hours each, 3) Each cage has a drinking area and a feeding area, and standardized exhaust channels, 4) Room temperature 22–24°C with air humidity 50–70%, 5) The maintenance room is equipped with exhausted fan. The entire adaptation process of experimental animals meets the standards or principles of experimental animal welfare (Mutiarahmi et al., 2021; Wahyuwardani et al., 2020).

Animal Diabetes Induction Experiment

DM induction in rats was carried out by conditioning the rats to become obese by giving them a high-fat diet (*high-fat diet*) for three weeks. The protocol for administering a high-fat diet was in line with previous research procedures (Marques et al., 2016; Speakman, 2019). After three weeks of giving a high-fat diet, the experimental animals' body weight increased by 20–25% compared to their initial

body weight. Induction of DM in rats was continued by injection of NA at a dose of 110 mg/kgBW (dissolved in saline) followed by injection of STZ at a dose of 45 mg/kgBW (dissolved in citrate buffer) after 15 minutes. Proof of DM conditions in experimental animals was carried out three days after DM induction. Experimental animals are said to have DM when blood glucose levels are above 250 mg/dL (Furman, 2021; Ghasemi et al., 2014).

Animal Feed Preparation

The standard diet given during the intervention process was AIN-93M referring to previous studies (Co.KG, 2024; Reeves, 1997). The diet given is a standard diet (15 grams per day) and drinking *ad libitum*. **Table 1** presents a standard diet composition.

Table 1. Standard Diet Composition

No.	AIN-93M Standard Feed Composition	Weight (g/kg diet)
1.	Cornstarch	465
2.	Hydrolyzed corn starch (<i>dextrinized cornstarch</i>)	155
3.	Sucrose	100
4.	Casein	140
5.	Soybean oil	40
6.	Vitamin mix (AIN-93)	10
7.	Mineral mix (AIN-93)	35
8.	L-cystine	1,80
9.	Choline Bitartrate	2,50
10.	Alphacel	50
11.	TBHQ	0,008

Source: (Co.KG, 2024; Reeves, 1997)

Preparation for Experimental Animal Intervention

Products prepared in this study were the control growol cookies (CGK group) and inulin-modified growol cookies (CGI group). Growol cookies were given as a snack for experimental animals. Snack food was provided through a sonde system with an amount of 1.62 grams of cookies in 2 ml. The remaining feed was measured every day by weighing. Cookies formulation referring to previous studies (Puspaningtyas et al., 2023). The addition of inulin used was 10 grams in line with previous studies (Puspaningtyas et al., 2022).

Glycemic Control and Lipid Profiles Testing

Glycemic control testing consists of measuring glucose levels, insulin levels, Homa IR values, and Homa β values. Glucose levels and insulin levels were measured using the GOD-PAP enzymatic colorimetric technique (mg/dl) and the ELISA technique (pg/ml), respectively. Homa IR and Homa β measurements are carried out using a formula based on the results of glucose and insulin levels. Lipid profile testing consists of measuring total cholesterol, triglyceride, LDL, and HDL levels. Lipid profile levels were measured using the CHOD-PAP colorimetric enzymatic technique (mg/dl).

Statistical Analysis

The data normality test was carried out using the Shapiro-Wilk test, followed by a variance homogeneity test using the Levene test and the Brown-Forsythe test. The effectiveness of growol cookies with modification of inulin on the glycemic control and lipid profiles is proven by ANOVA analysis followed by Tukey analysis (if the data is normally distributed and has homogeneous variance). The Kruskal-Wallis test followed by the Mann-Whitney U Test is carried out when the ANOVA test requirements are not met. The level of significance used is 95%.

4. Results

The Shapiro-Wilk test showed that all metabolic profile data were normally distributed ($p > 0.05$). The homogeneity of variance test shows that the glucose and Homa β data do not have homogeneous variance. Therefore, the ANOVA test was used to determine the effectiveness of the intervention on insulin levels, Homa IR values, and lipid profiles and then tested with Tukey HSD to determine differences between groups. Meanwhile, the Kruskal-Wallis test followed by the Mann-Whitney U Test was used to determine the effectiveness of the intervention on glucose levels and Homa β .

Table 2. Effect of Giving Growol Cookies on Glycemic Control and Lipid Profile

Variable	KS	KN	KP	CGK	CGI	p
Glucose (mg/dl)						
Pretest	71.65±0.69 ^a	268.99±7.20 ^b	269.14±5.01 ^b	270.50±6.64 ^b	268.85±4.94 ^b	0.020 [#]
1 st Week	71.82±0.71 ^a	269.37±7.17 ^b	243.79±5.26 ^c	245.20±6.06 ^c	242.75±4.95 ^c	0.001 [#]
2 nd Week	72.04±0.73 ^a	269.51±7.18 ^b	151.97±1.56 ^c	222.68±1.79 ^d	141.06±1.70 ^e	<0.001 [#]
3 rd Week	72.50±0.80 ^a	270.21±6.52 ^b	128.50±1.56 ^c	200.28±1.95 ^d	117.28±1.93 ^e	<0.001 [#]
Posttest	73.15±0.93 ^a	270.45±6.34 ^b	99.72±1.28 ^c	168.51±1.88 ^d	88.24±2.09 ^e	<0.001 [#]
Insulin (pg/ml)						
Pretest	555.12±6.20 ^a	439.47±5.73 ^b	453.24±12.14 ^b	445.80±10.55 ^b	444.98±10.01 ^b	<0.001 [*]
Posttest	549.61±5.72 ^a	435.61±6.41 ^b	504.45±6.70 ^c	455.16±7.73 ^d	536.67±5.45 ^e	<0.001 [*]
Homa IR						
Pretest	2.95±0.04 ^a	8.76±0.28 ^b	9.03±0.16 ^b	8.93±0.23 ^b	8.86±0.15 ^b	<0.001 [*]
Posttest	2.98±0.05 ^a	8.73±0.26 ^b	3.72±0.06 ^c	5.68±0.14 ^d	3.51±0.17 ^c	<0.001 [*]
Homa β						
Pretest	696.23±55.77 ^a	23.06±0.80 ^b	23.77±1.13 ^b	23.23±1.11 ^b	23.36±1.00 ^b	0.012 [#]
Posttest	588.84±54.36 ^a	22.69±0.70 ^b	148.51±6.01 ^c	46.60±0.79 ^d	255.97±45.59 ^e	<0.001 [#]
Cholesterol (mg/dl)						
Pretest	88.94±3.27 ^a	193.90±3.19 ^b	193.19±2.99 ^b	190.50±5.71 ^b	189.22±4.58 ^b	<0.001 [*]
Posttest	90.92±3.36 ^a	195.13±2.98 ^b	107.60±2.97 ^c	147.31±1.78 ^d	98.01±1.42 ^e	<0.001 [*]
Triglyceride (mg/dl)						
Pretest	77.18±1.31 ^a	125.64±3.92 ^b	130.29±5.66 ^b	123.82±1.60 ^b	127.47±4.30 ^b	<0.001 [*]
Posttest	78.22±1.63 ^a	127.53±3.40 ^b	87.40±1.85 ^c	100.27±2.09 ^d	84.25±2.42 ^c	<0.001 [*]
HDL (mg/dl)						
Pretest	83.48±1.60 ^a	23.14±1.54 ^b	23.14±2.04 ^b	23.68±1.61 ^b	23.55±2.08 ^b	<0.001 [*]
Posttest	82.01±2.13 ^a	22.43±1.61 ^b	63.93±2.02 ^c	47.87±2.40 ^d	66.95±1.32 ^c	<0.001 [*]
LDL (mg/dl)						
Pretest	24.41±2.09 ^a	80.95±1.63 ^b	81.49±2.11 ^b	78.65±2.30 ^b	78.64±1.73 ^b	<0.001 [*]

Posttest	25.87±2.55 ^a	82.22±2.14 ^b	33.33±1.25 ^c	53.17±2.69 ^d	31.75±2.17 ^c	<0.001*
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KS: healthy control, KN: negative control, KP: positive control, CGK: administration of control growol cookies, CGI: administration of inulin-modified growol cookies
 *Significant $p < 0.001$ in ANOVA test
 #Significant $p < 0.05$ and $p < 0.001$ in the Kruskal-Wallis test
 Source: Authors

5. Discussion

DM is a condition characterized by hyperglycemia which can be caused either by a lack of insulin production or failure of insulin function (Antar et al., 2023; Bahri et al., 2023; Galicia-garcia et al., 2020; Hossain et al., 2024; Ortiz-Martínez et al., 2022). Hyperglycemia is one of the acute complications that can occur in diabetes, and of course is one of the serious complications in DM (Bahri et al., 2023; Forțofoiu et al., 2022). Hyperglycemia conditions can cause complications in DM, including diabetic nephropathy, diabetic retinopathy, diabetic neuropathy, and cardiovascular disease (Ortiz-Martínez et al., 2022; Raghavan et al., 2019). Furthermore, hyperglycemia is a condition that further increases the risk of cancer in DM patients considering that hyperglycemia can cause DNA damage, increase the production of reactive oxygen species, and reduce the body's antioxidant potential, all of which are linked to the development of cancer (Tomic et al., 2022).

Handling DM emergencies by regulating food intake is the right step, especially in managing glycemic control and lipid profile. Consuming foods high in fiber can reduce glucose concentrations, especially postprandial glucose, and can increase insulin sensitivity (Pugh et al., 2023; Volpe, 2016). This can be seen from the research results which show that there are significant differences in levels of glucose, insulin, Homa IR, and Homa β between groups ($p < 0.05$ and $p < 0.001$). The CGI group had glucose levels and insulin levels that were close to the KS group and better than the KP group. In addition, the Homa IR value in the CGI group was as good as the KP group, and the best Homa β value was in the KS group, followed by the CGI group and the KP group.

Inulin-modified growol cookies are a cookie product with a low glycemic index (GI=41) with a high dietary fiber content, namely 9.69 grams in 100 grams of cookies (Puspaningtyas et al., 2022). Foods with a low glycemic index accompanied by high fiber levels play a role in controlling the level of carbohydrate absorption which will provide control over rising blood glucose levels (Previato, 2016). Inulin is a type of dietary fiber that acts as a prebiotic. Prebiotics play a role in maintaining digestive tract health (Abed et al., 2016; Wilson & Whelan, 2017). Studies conducted using growol show that the fiber content in growol can increase prebiotic potential in terms of increasing the number of good bacteria in the in vitro test (Sari & Puspaningtyas, 2019).

Inulin is known to be able to improve insulin sensitivity by increasing butyrate production from fiber fermentation in the colon, which has an impact on improving the insulin metabolic pathway which will have an impact on increasing HOMA- β control, which reflects improved pancreatic β -cell function. This is important considering that one of the main damages in type 2 DM is a dysfunction of pancreatic β cells which causes insulin secretion to be suboptimal (Guo et al., 2022; Mayorga-Ramos et al., 2022; Zou et al., 2021). This shows the importance of functional food innovation based on local food with the addition of bioactive components such as inulin for holistic DM management.

The combination of using growol with inulin will further optimize the role of dietary fiber in glycemic control. This can be seen from the decrease in blood glucose levels in the group that received growol cookies (from 270.50 mg/dl to 168.51 mg/dl) and inulin-modified growol cookies (from 268.85 mg/dl to 88.24 mg/dl) from pretest conditions to posttest conditions. In addition, there was an increase in insulin levels (from 444.98 pg/ml to 536.67 pg/ml) and Homa β values (from 23.36 to 255.97) in the group that received the inulin-modified growol cookies. There was a decrease in the Homa IR value in the group that received the inulin-modified growol cookies, namely from 8.86 to 3.51.

Furthermore, dietary fiber plays a selective role in stimulating gastrointestinal microbes to produce short-chain fatty acids (SCFA), which are responsible for the regulation of energy metabolism, providing a feeling of satiety, increasing insulin sensitivity, and improving glycemic control and lipid profile (Everard & Cani, 2013; Vinelli et al., 2022). It can be seen that the group that received growol cookies experienced a decrease in total cholesterol levels (43.19 mg/dl), triglycerides levels (23.55 mg/dl), and LDL levels (25.48 mg/dl) and experienced an increase in HDL levels (24.16 mg/dl). A better effect was seen in the group that received dietary fiber sourced from inulin-modified growol cookies. There was a decrease in total cholesterol, triglyceride, and LDL levels by 91.21 mg/dl; 43.22 mg/dl; and 46.89 mg/dl. Meanwhile, there was an increase in HDL levels of 43.4 mg/dl.

The results of this study are in line with several previous studies. A study of 33 diabetic subjects showed that administration of inulin, especially inulin-type fructan (ITF) was able to reduce blood glucose levels by 0.42 mmol/L, total cholesterol by 0.46 mmol/L, and triglycerides by 0.21 mmol/L (Li et al., 2021). Another study conducted on experimental animals also tested the prebiotic potential of Pigeon Pea (*Cajanus cajan* L) on changes in several health indicators. Giving prebiotics for 18 weeks proved that giving prebiotics was able to reduce blood glucose levels and improve Homa IR values which provided an overview of the condition of insulin resistance in experimental animals (Shakappa et al., 2022).

A study conducted on 49 women with type 2 DM followed by overweight and obesity showed that giving 10 grams of inulin a day for two months was able to reduce fasting glucose levels and HbA1c. In addition, supplementation with 15 grams of inulin per day for six months can improve the condition of insulin resistance (Homa IR) in prediabetes patients (Iatcu et al., 2024).

Several preclinical studies conducted on male rats showed that giving inulin as much as 10% of the diet was able to reduce cholesterol and triglyceride levels. Meanwhile, studies on humans with hyperglycemia show that giving a dose of 20 grams of inulin per day for 3 weeks can reduce triglyceride levels (dos Reis et al., 2015).

6. Conclusions

The addition of inulin into growol cookies was able to improve glycemic control (especially in reducing fasting blood glucose levels and Homa IR as well as increasing insulin and Homa β levels) and lipid profile (especially in reducing total cholesterol, triglyceride, and LDL levels and increasing HDL levels) of experimental animals. These findings contribute to the development of local food-based dietary interventions for the prevention and management of metabolic complications in people with DM. The use of inulin-modified growol cookies can be an alternative DM management that is affordable, effective, and of course, based on local food ingredients.

This study only explored changes in the metabolic profile of experimental animals and did not explore changes in genetic expression in each group. Future studies can carry out more in-depth studies to see the complete picture of improving DM conditions, starting from the genomic, transcriptomic, proteomic, and metabolomic levels.

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Conflict of Interest

There is no conflict of interest in carrying out research and preparing this scientific manuscript. All authors have their contributions.

References

- Abed, S. M., Ali, A. H., Noman, A., Niazi, S., Ammar, A.-F., & Bakry, A. M. (2016). Inulin as prebiotics and its applications in food industry and human health; a review. *International Journal of Agriculture Innovations and Research*, 5(1), 2319–1473.
- Al-Lawati, J. A. (2017). Diabetes mellitus: A local and global public health emergency! *Oman Medical Journal*, 32(3), 177–179. <https://doi.org/10.5001/omj.2017.34>
- Antar, S. A., Ashour, N. A., Sharaky, M., Khattab, M., Ashour, N. A., Zaid, R. T., Roh, E. J., Elkamhawy, A., & Al-Karmalawy, A. A. (2023). Diabetes mellitus: Classification, mediators, and complications; A gate to identify potential targets for the development of new effective treatments. *Biomedicine and Pharmacotherapy*, 168, 115734. <https://doi.org/10.1016/j.biopha.2023.115734>
- Bahri, S. L., Darmaningrat, C. I. A. A., Putra, I. W. M., Adzuba, K. K., Paramita, L. K. D. L., Alfarisi, M. D., & Irmayanto, T. (2023). Complications of diabetes mellitus: A review article. *Green Medical Journal*, 5(2), 128–136. <https://doi.org/https://doi.org/10.33096/gmj.v5i3.135>
- Co.KG, A. S. G. &. (2024). *AIN - 93 M - product data sheet AIN - 93 M purified diet , rats & mice*. info@altromin.de - www.altromin.com
- dos Reis, S. A., da Conceição, L. L., Rosa, D. D., Dias, M. M. dos S., & Peluzio, M. do C. G. (2015). Mechanisms used by inulin-type fructans to improve the lipid profile. *Nutricion Hospitalaria*, 31(2), 528–534. <https://doi.org/10.3305/nh.2015.31.2.7706>
- Estina, V. C., Suryawan, I. W. B., & Arimbawa, I. M. (2020). Two cases of type 2 diabetes mellitus (T2DM) in children. *Medicina*, 51(2), 142–149. <https://doi.org/10.15562/medicina.v51i2.695>
- Everard, A., & Cani, P. D. (2013). Diabetes, obesity and gut microbiota. *Best Practice and Research: Clinical Gastroenterology*, 27(1), 73–83. <https://doi.org/10.1016/j.bpg.2013.03.007>
- Forțofoiu, M., Vladu, I., Forțofoiu, M.-C., Pădureanu, R., Clenciu, D., Rădulescu, D., & Pădureanu, V. (2022). New strategies of diagnostic and therapeutic approach to emergencies in the evolution of patients with diabetes mellitus (Review). *Experimental and Therapeutic Medicine*, 23(2), 1–9. <https://doi.org/10.3892/etm.2021.11101>
- Furman, B. L. (2021). Current Protocols - 2021 - Furman - Streptozotocin-Induced Diabetic Models in Mice and Rats.pdf. *Current Protocols*, 1(doi: 10.1002/cpz1.78), 1–21.
- Galicia-garcia, U., Benito-vicente, A., Jebari, S., Larrea-sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martin, C. (2020). Pathophysiology of type 2 diabetes mellitus. *International Journal of*

- Molecular Sciences*, 21(6275), 1–34. <https://doi.org/10.3390/ijms21176275>
- Ghasemi, A., Khalifi, S., & Jedi, S. (2014). Streptozotocin-nicotinamide-induced rat model of type 2 diabetes (review). *Acta Physiologica Hungarica*, 101(4), 408–420. <https://doi.org/10.1556/APhysiol.101.2014.4.2>
- Guo, J., Zhang, M., Wang, H., Li, N., Lu, Z., Li, L., Hui, S., & Xu, H. (2022). Gut microbiota and short chain fatty acids partially mediate the beneficial effects of inulin on metabolic disorders in obese ob/ob mice. *Journal of Food Biochemistry*, 46(5), e14063. <https://doi.org/10.1111/JFBC.14063>
- Hossain, M. J., Al-Mamun, M., & Islam, M. R. (2024). Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. *Health Science Reports*, 7(3), 5–9. <https://doi.org/10.1002/hsr2.2004>
- Iatcu, O. C., Hamamah, S., & Covasa, M. (2024). Harnessing prebiotics to improve type 2 diabetes outcomes. *Nutrients*, 16(20), 1–31. <https://doi.org/10.3390/nu16203447>
- Indrahadi, D., Wardana, A., & Pierewan, A. C. (2021). The prevalence of diabetes mellitus and relationship with socioeconomic status in the Indonesian population. *Jurnal Gizi Klinik Indonesia*, 17(3), 103–112. <https://doi.org/10.22146/ijcn.55003>
- International Diabetes Federation. (2021a). IDF Diabetes Atlas 2021 _ IDF Diabetes Atlas. In *IDF official website* (pp. 1–4). <https://diabetesatlas.org/atlas/tenth-edition/%0Ahttps://diabetesatlas.org/data/en/world/>
- International Diabetes Federation. (2021b). *IDF Diabetes Atlas 10th edition 2021* (E. J. Boyko, D. J. Magliano, S. Karyanga, L. Piemonte, P. Riley, P. Saeedi, & H. Sun (eds.); Vol. 102, Issue 2). <https://doi.org/10.1016/j.diabres.2013.10.013>
- Kimura, Y., Yoshida, D., Hirakawa, Y., Hata, J., Honda, T., Shibata, M., Sakata, S., Uchida, K., Kitazono, T., & Ninomiya, T. (2021). Dietary fiber intake and risk of type 2 diabetes in a general Japanese population: The Hisayama Study. *Journal of Diabetes Investigation*, 12(4), 527–536. <https://doi.org/10.1111/jdi.13377>
- Lascar, N., Brown, J., Pattison, H., Barnett, A. H., Bailey, C. J., & Bellary, S. (2017). *Type 2 diabetes in adolescents and young adults*.
- Li, L., Li, P., & Xu, L. (2021). Assessing the effects of inulin-type fructan intake on body weight, blood glucose, and lipid profile: A systematic review and meta-analysis of randomized controlled trials. *Food Science and Nutrition*, 9(8), 4598–4616. <https://doi.org/10.1002/fsn3.2403>
- Marques, C., Meireles, M., Norberto, S., Leite, J., Freitas, J., Pestana, D., Faria, A., & Calhau, C. (2016). High-fat diet-induced obesity Rat model: a comparison between Wistar and Sprague-Dawley Rat. *Adipocyte*, 5(1), 11–21. <https://doi.org/10.1080/21623945.2015.1061723>
- Mayorga-Ramos, A., Barba-Ostria, C., Simancas-Racines, D., & Guamán, L. P. (2022). Protective role of butyrate in obesity and diabetes: New insights. *Frontiers in Nutrition*, 9(November), 1–9. <https://doi.org/10.3389/fnut.2022.1067647>
- Misra, S., Ke, C., Srinivasan, S., Goyal, A., Nyriyenda, M. J., Florez, J. C., Khunti, K., Magliano, D. J., & Luk, A. (2023). Current insights and emerging trends in early-onset type 2 diabetes. *The Lancet Diabetes and Endocrinology*, 11(10), 768–782. [https://doi.org/10.1016/S2213-8587\(23\)00225-5](https://doi.org/10.1016/S2213-8587(23)00225-5)
- Mutiarahmi, C. N., Hartady, T., & Lesmana, R. (2021). Kajian pustaka: Penggunaan mencit sebagai hewan coba di laboratorium yang mengacu pada prinsip kesejahteraan hewan. *Indonesia Medicus Veterinus*, 10(1), 134–145. <https://doi.org/10.19087/imv.2020.10.1.134>
- Ong, K. L., Stafford, L. K., McLaughlin, S. A., Boyko, E. J., Vollset, S. E., Smith, A. E., Dalton, B. E., Duprey, J., Cruz, J. A., Hagins, H., Lindstedt, P. A., Aali, A., Abate, Y. H., Abate, M. D., Abbasian, M., Abbasi-Kangevari, Z., Abbasi-Kangevari, M., ElHafeez, S. A., Abd-Rabu, R., ...

- Vos, T. (2023). Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *The Lancet*, 402(10397), 203–234. [https://doi.org/10.1016/S0140-6736\(23\)01301-6](https://doi.org/10.1016/S0140-6736(23)01301-6)
- Ortiz-Martínez, M., González-González, M., Martagón, A. J., Hlavinka, V., Willson, R. C., & Rito-Palomares, M. (2022). Recent developments in biomarkers for diagnosis and screening of type 2 diabetes mellitus. *Current Diabetes Reports*, 22(3), 95–115. <https://doi.org/10.1007/s11892-022-01453-4>
- Previato, H. D. R. de A. (2016). Carbohydrate counting in diabetes. *Nutrition and Food Technology: Open Access*, 2(2), 1–4. <https://doi.org/10.4172/2155-9600.1000288>
- Pugh, J. E., Cai, M., Altieri, N., & Frost, G. (2023). A comparison of the effects of resistant starch types on glycemic response in individuals with type 2 diabetes or prediabetes: A systematic review and meta-analysis. *Frontiers in Nutrition*, 10(March), 1–11. <https://doi.org/10.3389/fnut.2023.1118229>
- Puspaningtyas, D. E., Nekada, C. D. Y., & Sari, P. M. (2022). Penambahan inulin terhadap indeks glikemik dan beban glikemik cookies growol: pengembangan makanan selingan diabetes. *AcTion: Aceh Nutrition Journal*, 7(2), 169. <https://doi.org/10.30867/action.v7i2.738>
- Puspaningtyas, D. E., Sari, P. M., Kusuma, N. H., & Helsius SB, D. (2019). Analisis potensi prebiotik growol: kajian berdasarkan perubahan karbohidrat pangan. *Gizi Indonesia*, 42(2), 83–90. <https://doi.org/10.36457/gizindo.v42i2.390>
- Puspaningtyas, D. E., Sari, P. M., Kusuma, N. H., & Helsius SB, D. (2020). Indeks glikemik cookies growol: studi pengembangan produk makanan selingan bagi penyandang diabetes mellitus. *Jurnal Gizi Klinik Indonesia*, 17(1), 34–42. <https://doi.org/10.22146/ijcn.54576>
- Puspaningtyas, D. E., Sari, P. M., Styaningrum, S. D., & Sucipto, A. (2023). Efektivitas cookies growol modifikasi inulin dan glukomanan untuk diet diabetes: Kajian pengembangan pangan lokal fungsional. In *Laporan Akhir Hibah Penelitian Fundamental*.
- Raghavan, S., Vassy, J. L., Ho, Y. L., Song, R. J., Gagnon, D. R., Cho, K., Wilson, P. W. F., & Phillips, L. S. (2019). Diabetes mellitus–related all-cause and cardiovascular mortality in a national cohort of adults. *Journal of the American Heart Association*, 8(4), 1–21. <https://doi.org/10.1161/JAHA.118.011295>
- Reeves, P. G. (1997). Components of the AIN-93 diets as improvements in the AIN-76A diet. *Journal of Nutrition*, 127(5 SUPPL.), 838–841. <https://doi.org/10.1093/jn/127.5.838s>
- Sari, P. M., & Puspaningtyas, D. E. (2019). Skor aktivitas prebiotik growol (makanan fermentasi tradisional dari singkong) terhadap *Lactobacillus* sp. dan *Escherichia coli*. *Ilmu Gizi Indonesia*, 02(02), 101–106. <https://doi.org/https://doi.org/10.35842/ilgi.v2i2.89>
- Shakappa, D., Talari, A., Naik, R., & Pradhan, S. (2022). Prebiotic potential and other health benefits of prebiotic mixture of Pigeon Pea (*Cajanus Cajan* L) in Wistar Nin Rats. *Journal of Veterinary Health Science*, 3(1), 88–96. <https://doi.org/10.33140/jvhs.03.01.06>
- Speakman, J. R. (2019). Use of high-fat diets to study rodent obesity as a model of human obesity. *International Journal of Obesity*, 43(8), 1491–1492. <https://doi.org/10.1038/s41366-019-0363-7>
- Tomic, D., Shaw, J. E., & Magliano, D. J. (2022). The burden and risks of emerging complications of diabetes mellitus. *Nature Reviews Endocrinology*, 18(9), 525–539. <https://doi.org/10.1038/s41574-022-00690-7>
- Vega-López, S., Venn, B. J., & Slavin, J. L. (2018). Relevance of the glycemic index and glycemic load for body weight, diabetes, and cardiovascular disease. *Nutrients*, 10(10), 1–27. <https://doi.org/10.3390/nu10101361>
- Vinelli, V., Biscotti, P., Martini, D., Del Bo', C., Marino, M., Meroño, T., Nikoloudaki, O., Calabrese, F. M., Turrioni, S., Taverniti, V., Caballero, A. U., Andrés-Lacueva, C., Porrini, M., Gobbetti, M.,

- De Angelis, M., Brigidi, P., Pinart, M., Nimptsch, K., Guglielmetti, S., & Riso, P. (2022). Effects of dietary fibers on short-chain fatty acids and gut microbiota composition in healthy adults: a systematic review. *Nutrients*, 14(13), 1–26. <https://doi.org/10.3390/nu14132559>
- Volpe, S. L. (2016). Resistant starch and type 2 diabetes mellitus: Clinical perspective. *ACSM's Health & Fitness Journal*, 20(5), 59–60. <https://doi.org/10.1111/jdi.14139>
- Wahyuwardani, S., Noor, S. M., & Bakrie, B. (2020). Etika kesejahteraan hewan dalam penelitian dan pengujian: Implementasi dan kendalanya. *WARTAZOA*, 30(4), 211–220. <https://doi.org/10.14334/wartazoa.v30i4.2529>
- Wilson, B., & Whelan, K. (2017). Prebiotic inulin-type fructans and galacto-oligosaccharides: definition, specificity, function, and application in gastrointestinal disorders. *Journal of Gastroenterology and Hepatology*, 32(Supp. 1), 64–68. <https://doi.org/https://doi.org/10.1111/jgh.13700>
- Zafar, M. I., Mills, K. E., Zheng, J., Regmi, A., Hu, S. Q., Gou, L., & Chen, L. L. (2019). Low-glycemic index diets as an intervention for diabetes: A systematic review and meta-analysis. *American Journal of Clinical Nutrition*, 110(4), 891–902. <https://doi.org/10.1093/ajcn/nqz149>
- Zou, J., Reddivari, L., Shi, Z., Li, S., Wang, Y., Bretin, A., Ngo, V. L., Flythe, M., Pellizzon, M., Chassaing, B., & Gewirtz, A. T. (2021). Inulin fermentable fiber ameliorates type I diabetes via IL22 and short-chain fatty acids in experimental models. *Cellular and Molecular Gastroenterology and Hepatology*, 12(3), 983–1000. <https://doi.org/10.1016/j.jcmgh.2021.04.014>