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Blood glucose homeostasis, birth weight, and pancreas of rat pups during pregnancy the mother is intervened with food substitution beef bone marrow

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ABSTRACT

Background: The growth of the intrauterine pancreas in the fetus is very dependent on the mother's nutritional intake during pregnancy. Previous research on animal models found that mother rats during pregnancy who were given low-protein feed gave birth to offspring with lower pancreatic weight compared to rat offspring given normal diet. This study aims to evaluate the potential of feed nutrients substituted for bovine bone marrow in increasing intrauterine pancreatic growth with biomarkers of glucose homeostasis, birth weight and pancreatic weight in rat offspring.

Methods: This study employs an experimental study with in vivo design. During pregnancy, the Sprague Dawley rat animal model used in this study was intervened with Low Protein Feed (LPF), Normal Feed (NF) and Bovine Bone Marrow Substitution Feed (BBMSF). All formulated feeds are made isocaloric.

Results: there was a significant difference ($\alpha < 0.05$) in the blood glucose of rat offspring from mothers who were intervened with LPF, NF and BBMSF at the age of 60 days. Rat pups whose mothers were intervened with LPF, NF, and BBMSF during pregnancy had significant differences in body weight ($\alpha < 0.05$). There was no significant difference ($\alpha > 0.05$) in the weight of the pancreas of rat offspring from mothers who were intervened with LPF, NF and BBMSF at the ages of 30 and 60 days.

Conclusion: Rat pups from mothers who were intervened during pregnancy with bovine bone marrow substitute feed had a better ability to maintain blood glucose homeostasis, had a higher average birth weight and pancreatic weight compared to rat pups from NF and LPF mothers.

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KEYWORDS

Dietary Supplementation, Fetal, Fetal Development.

INTRODUCTION

One of the organs most responsible for regulating blood sugar is the pancreas. The insulin hormone secreted by the organ's ß cells will be carried by the blood to the target cells where the insulin hormone then binds to the target cell receptor to open the glucose transporter (GLUT) gate so that glucose molecules can enter the cells[1]. Once in the cells, glucose will undergo further metabolism such as oxidation, glycolysis or glycogenesis[2]. An insufficient number of pancreatic beta cells will result in a decrease in the amount of insulin produced[3] causing disruption of blood glucose homeostasis resulting in hyperglycemia[4].

One of the factors that causes insulin production to be suboptimal is the insufficient number of pancreatic ß cells due to impaired cell proliferation that occurs during the intrauterine growth phase[3]. The factors causing disruption of cell proliferation are related to the mother's inadequate intake of nutrients needed to support this process (under nutrition) so that at birth the baby's ß-pancreatic cell number is also abnormal[5]. Change in people's dietary patterns will cause people with type 2 diabetes mellitus (T2DM) to continue to increase[6]. According to the International Diabetes Federation (IDF), the number of world people suffering from diabetes reached 537 million in

2021 and is expected to increase to 783 million in 2045[7]. The number of diabetes sufferers in Indonesia reached 10.7 million people in 2019 and of this number, 219,083 people were found in Central Sulawesi spread across 13 districts (Profile-Dinkes-Sulteng-TA.-2019).

Overweight and obesity are the main risk factors in T2DM cases, apart from several other factors which are also known to act as risk factors, namely lifestyle and genetic factors[8]. Epidemiological studies show that malnutrition in the fetus during pregnancy plays a role in the incidence of T2DM[9]. Low birth weight is considered a form of malnutrition that occurs during pregnancy[10]. A meta-analysis study states that every 1 kg increase in birth weight will reduce the risk of T2DM by 20% [11] 2023).

The thrifty phenotype hypothesis states that there is a relationship between poor fetal and infant growth and an increased risk of developing T2DM and metabolic syndrome due to poor nutritional intake early in life[12]. This condition will result in permanent changes in glucose-insulin metabolism[13]. These changes can take the form of reduced insulin secretory capacity and which, combined with the effects of obesity, aging and physical inactivity, are the factors that most determine the incidence of T2DM[8].

Beef bone marrow is a food ingredient with a fairly high nutrient density. The results of analysis of the marrow indicated that it contains several essential fatty acids, vitamins and minerals which are needed to support cell proliferation during intrauterine growth[14]. Previous research also succeeded in showing that cow bone marrow substitute feed can increase intrauterine kidney growth[15].

METHOD

This research is a laboratory experimental study using the Sprague Dawley rat animal model with a completely randomized design (CRD). The factors involved in this research are intrauterine growth feed which consists of: NF (normal feed), LPF (low protein feed), BBMSF (bovine bone marrow substitute feed) which is isocalorically formulated[15].

Stage I Formulation and Manufacturing of isocaloric feed

At this stage, calculations were performed to determine the quantities of ingredients required for the production of NF (normal feed), LPF (low protein feed), BBMSF (bovine bone marrow substitute feed), using the formulas presented in Table 1.

Table 1. Intrauterine Growth Intervention Feed Formulation

Composition	Feed composition during pregnancy gr/kg		
	NF (normal feed)	LPF (low protein feed)	BBMSF (bovine bone marrow substitute feed)
Casein	180	90	176.10
Marrow protein	0	0	3.90
Folic acid	1	1	1
Corn starch	425	482	421.46*
Sucrose	213	243	213
Choline	2	2	2
DL-Methionine	5	5	5
Vitamins1	5	5	5
Minerals2	20	20	20
Cellulose	50	50	50
Corn oil	100	100	23.50
Marrow fat	0	0	76.50

Stage II. Acclimatization, mating and intervention

Rats to be used as animal models in this study will be acclimatized for 2 weeks and treated with anthelmintics. Subsequently, once the female rats are in estrus, they will be mated by placing them in the same cage with male rats in a 2:1 ratio. During the mating process, vaginal checks will be conducted to confirm successful mating. If a vaginal plug is observed, the day of observation will be designated as day 0 of gestation. Pregnant dams will then be isolated in individual cages for intervention.

The gestation period of rats is approximately 21 days. After birth, the weight of each pup was recorded. Pups were weighed every 3 days to monitor their growth and development under different treatment conditions.

Phase III. Birth and rearing of stage IV rat pups, necropsy, pancreatic removal and blood sugar measurements.

Pups from each dam under the NF (normal feed), LPF (low protein feed), and BBMSF (bovine bone marrow substitute feed) treatments were monitored for growth and development by providing a standard commercial diet. At 1 and 2 months of age, blood samples were collected from the pups for glucose measurement using a glucometer. Prior to necropsy, the rats were anesthetized with ketamine-xylazine, and the pancreas was then excised and weighed.

Statistical analysis was performed using SPSS software, employing an Analysis of Variance (ANOVA) test. This research has received approval from the Ethics Commission No: 002503/KEPK POLTEKKES KEMENKES PALU/2024.

RESULTS

Blood glucose measurements of rat pups in this study were carried out after the rats were 60 days old. Blood glucose levels during 4-6 hours of fasting are one of the biomarkers to see the quality of the pancreas of rat offspring from mothers who were intervened during pregnancy with bovine bone marrow substitute feed. Data on the results of blood glucose measurements from each rat pup are presented in Table 2.

Table 2. Average blood glucose levels (mg/dL) of rat pups that were intervened with Normal Feed (NF) Low Protein Feed (LPF) Beef Bone Marrow Substitute Feed (BBMSF) at 60 days of age during pregnancy

Type of food	Blood glucose levels (mg/dL) (Mean ± SD)	p-value
Normal Feed (NF)	84.33 ± 16.64	0.027
Low Protein Feed (LPF)	120.90 ± 12.87	
Beef Bone Marrow Substitute Feed (BBMSF)	95.66 ± 17.60	

Table 2 presents the average blood glucose levels of 60-day-old rat pups whose mothers were fed different diets during pregnancy. The results indicate that there were significant differences in blood glucose levels among the three groups (p=0.027). Specifically, rat pups born to mothers fed a low-protein diet had significantly higher blood glucose levels compared to those born to mothers fed a normal diet. Interestingly, pups born to mothers fed a beef bone marrow substitute diet had intermediate blood glucose levels, suggesting a potential protective effect against hyperglycemia compared to the low-protein group.

Table 3 presents the average birth weight of rat offspring born to mothers fed different diets during pregnancy. The results indicate that there were significant differences in birth weight among the three groups (p=0.03). Specifically, rat pups born to mothers fed a normal diet had a significantly higher birth weight compared to those born to mothers fed a low-protein diet. Interestingly, pups born to mothers fed a beef bone marrow substitute diet also had a significantly higher birth weight compared to those fed a low-protein diet, although the difference was not as pronounced as compared to the normal diet group. The pancreas is a gland that is capable of secreting various types of hormones. One hormone that plays a very important role in regulating blood glucose is insulin which is secreted by the ß cell group of the gland. The ability of these glands to secrete the hormone in question decreases with increasing age[5]. In connection with the above, in this study evaluation of intrauterine pancreatic growth was carried out when rat pups were 30 and 60 days old.

Table 3. Average birth weight of rat offspring from mothers who were intervened with Normal Feed (NF) Low Protein Feed (LPF) Beef Bone Marrow Substitute Feed (BBMSF) during pregnancy

Type of food	Birth weight (gram) (Mean ± SD)	p-value
Normal Feed (NF)	6.46 ± 0.51	0.03
Low Protein Feed (LPF)	5.98 ± 0.45	
Beef Bone Marrow Substitute Feed (BBMSF)	6.82 ± 0.49	

After the mice were 30 days old, a necropsy was performed, the pancreas of each mouse was taken and weighed. The average weight of the pancreas of rat offspring from each mother who was intervened with NF, LPF and BBMSF during pregnancy is presented in Table 4.

Table 4. Pancreas weight of rat offspring from mothers who were intervened with NF, LPF and BBMSF feeds during pregnancy after 30 days of age

Type of food	Pancreas weight (gram), (Mean ± SD)	p-value
Normal Feed (NF)	0.16 ± 0.03	0.323
Low Protein Feed (LPF)	0.14 ± 0.05	
Beef Bone Marrow Substitute Feed (BBMSF)	0.17 ± 0.02	

Table 4 presents the average pancreas weight of rat offspring at 30 days of age whose mothers were fed different diets during pregnancy. The results indicate that there were no significant differences in pancreas weight among the three groups (p=0.323), suggesting that the type of maternal diet during pregnancy did not have a significant impact on the development of the offspring's pancreas.

After the other rat pups were 60 days old, stage 2 necropsy was carried out and data on the weight of the pancreatic of rat pups from each mother who was intervened with NF, LPF and BBMSF feeds is presented in Table 5.

Table 5. Average pancreatic weight of 60-day old rat pups from mothers who were intervened with NF, LPF and BBMSF during pregnancy

Type of food	Pancreatic weight (gram), (Mean ± SD)	p-value
Normal Feed (NF)	0.51 ± 0.06	0.341
Low Protein Feed (LPF)	0.44 ± 0.16	
Beef Bone Marrow Substitute Feed (BBMSF)	0.55 ± 0.13	

Table 5 presents the average pancreatic weight of 60-day-old rat pups whose mothers were fed different diets during pregnancy. The results indicate that there were no significant differences in pancreatic weight among the three groups (p=0.341), suggesting that the type of maternal diet during pregnancy did not have a significant impact on the development of the offspring's pancreas at 60 days of age.

DISCUSSION

The highest average blood glucose levels were found in rat offspring from mothers who were intervened with LPF during pregnancy, followed by rat offspring from mothers who were intervened with BBMSF, and NF (Table 1). Referring to Jensen T, et al (2013)[16] mice that are healthy and not diabetic will have fasting blood glucose (4-6 hours fasting) between 80-100 mg/dL, this means that rat offspring from mothers who were intervened with BBMSF have high levels of blood glucose of 95.66 mg/dL is still within the normal range or there is no hyperglycemia/diabetes (>135mg/dL). Even though the blood glucose levels of rat pups from mothers who were intervened with LPF have not yet entered the hyperglycemia range, they can be used as an indication of a disturbance in the regulation of blood sugar by insulin. According to Chen M, et al (1985)[17] as age increases, the ability of the pancreas will also experience a functional decline in producing insulin.

The results of the ANOVA analysis showed that the blood glucose levels were significantly different (α<0.05), this is an indication that the cow bone marrow substitute feed intervention given to the mother during pregnancy had a significant effect on the ability of the offspring born to maintain blood glucose homeostasis. The ability to maintain glucose homeostasis is an illustration of the quality of the pancreas which can function optimally in secreting the hormone insulin. The increase in the quality of the pancreas is related to the effectiveness of cell proliferation that occurs during pregnancy due to the support of macro and micro nutrients contained in the intervention feed[13]. Macro and micro nutrients in beef bone marrow such as amino acids, fatty acids, fat soluble vitamins and macro and micro mineral content have an important role during the process of pancreatic organogenesis.

Beef bone marrow contains 16 types of amino acids, and 7 of them are essential amino acids (histidine, isoleucine, leucine, lysine, phenylalanine, threonine, phenylalanine) and 9 non-essential amino acids (arginine, tyrosine, serine, valine, aspartic acid, arginine, glycine and glutamic acid)[15]. The macronutrients integrated in beef bone marrow come from the essential fatty acid group such as α-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid[15]. Previous research stated that α-linoleic acid has the ability to maintain and support cell proliferation and differentiation processes[18]. α-linoleic acid is an important precursor in the formation of DHA and EPA fatty acids in the body. According to[19] fertile women have the ability to convert α-linoleic acid into DHA and EPA which are needed to support intrauterine growth. Apart from DHA and EPA which are the result of the lipogenesis process during intrauterine growth in mothers, DHA and EPA contained in cow bone marrow also contribute to increasing the growth of the pancreas in rat offspring (Tangkas et al., 2018).

The contribution of bovine bone marrow nutrients to intrauterine growth is also seen in the birth weight of rat offspring. The average birth weight of rat pups that came from mothers who were intervened with BBMSF food during pregnancy was significantly heavier than rat pups that came from mothers who were intervened with LPF feed.

The research results obtained in this study are in accordance with the results of research conducted by[20], which states that pregnant women in China have a higher risk of giving birth to babies with low birth weight compared to women who receive protein intake. the high one. In experiments using animal models, it was also found that rat mothers during pregnancy were intervened with low-protein feed which had an impact on the low birth weight of their offspring[21].

Even though it was not proven to be statistically significant, the average weight of the pancreas showed a different trend, in this case the rat offspring from mothers who were intervened with BBMSF had the heaviest pancreas compared to the offspring of LPF and NF rats. The unit used in measuring weight is grams, so if you compare and trace the number of cells, the difference will be very large.

The average weight of one mammalian cell is between 3 and 4 ng, therefore the difference between the weight of the pancreas of NF, LPF and BBMSF rat offspring can be calculated by the difference in the number of beta cells. The difference between the weight of the NF pancreas and the LPF when the rats were 60 days old was 0.0683 grams. After converting to nanograms, the difference became 68,300,000ng. If the weight of each cell is 3 ng, then NF rat pups have an excess number of pancreatic cells of 22,766,666 cells compared to the number of pancreatic cells in LPF rat pups. Furthermore, the difference in pancreatic weight between BBMSF and LPF rat pups is 0.1133 grams. If converted into nanograms, the difference in pancreatic weight is 113,300,000 ng. If calculated, BBMSF rat pups have an excess number of pancreatic cells of 37,766,666 cells. As many as 75% of the excess number of cells are pancreatic beta cells which function to synthesize and secrete insulin to regulate blood glucose. Differences in the number of cells between the pancreas of rat offspring from BBSMF, NF and LPF parents have an impact on the ability of these glands to secrete pancreas and regulate blood glucose.

CONCLUSION AND RECOMMENDATIONS

Rat pups from mothers who were intervened during pregnancy with bovine bone marrow substitute feed had a better ability to maintain blood glucose homeostasis, had a higher average birth weight and pancreatic weight compared to rat pups from NF and LPF mothers.

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