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The Complementary Food for Intestine Microanatomy Amelioration of the Malnourished Rats (*Rattus norvegicus*)

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Abstract. Protein Energy Malnutrition (PEM) is insufficient animal or vegetable protein consumption in daily food. PEM can result in damage to intestinal cells, which causes villous atrophy, decreased crypt depth, and a decrease in the number of epithelial cells, which results in failure to absorb nutrients. CF can be used to stimulate growth, improve inflammation and improve intestinal microbiota. This study, crucial for understanding the impact of CF on PEM, aims to describe the microanatomy of small intestine (jejunum) mice (*Rattus norvegicus*) PEM after giving CF. The research method was experimental with a Randomized Block Design (RBD) using 24 mice. The experimental animals were divided into 6 groups: the control and treatment groups with complementary food with various carbohydrate sources. Treatment was carried out for 4 weeks. Data in microanatomy and small intestinal villi height resulted in the highest villous rate height in the K+P0 group and the lowest in the K- group. The results showed that administration of CF did not show a significant results ($P < 0.05$) on the height of the villi of the small intestine (jejunum).

1 Introduction

Protein-energy deficiency (PEM) is a condition of the body with low nutritional status, which is caused by a lack of consumption of animal or vegetable protein in daily intake [1,2]. The results of the Indonesian Nutrition Status Survey (SSGI) report that the stunting rate was 21.6% in 2022, and there was an increasing wasting rate to 7.7% in 2022, as well as the underweight rate increased in 2022 by 17.1% [3]. PEM causes delays in body growth and brain development, stunting, decreased body resistance to infectious diseases, and death in children under five [4].

One source of energy is obtained from protein in the form of essential amino acids, which are useful for growth and tissue formation, cell regeneration, maintaining the acid-base balance of fluids in the body, helping the body's metabolism, and maintaining cells and body tissues [5]. A body with a weak immune system can cause oxidative stress, which results in an unbalanced production of free radicals and antioxidants [6]. Oxidative stress occurs due to the increased production of free radicals in the form of Reactive Oxygen Species (ROS),

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which can cause cell damage [7]. The cell damage that occurs can affect various organ functions, including disruption of intestinal function.

The small intestine is the main organ for the absorption of nutrients in the body. The small intestine will process carbohydrates, fats and proteins into simpler substances with the help of enzymes from the pancreas [8]. The small intestine is approximately 5 m long and divided into 3 parts: the duodenum, jejunum and ileum [9]. The jejunum is an organ that makes up the small intestine with a complex role because most of the absorption processes, such as glucose, amino acids and fatty acids, occur in the jejunum [8,10]. One of the causes of absorption disorders in the intestine is cell damage, which is characterized by changes in the microanatomy of the small intestine itself. Changes in intestinal microanatomy result in villous atrophy, crypt hyperplasia, and a decrease in the number of epithelial cells due to decreased proliferation. These histological changes cause the failure of absorption and lead to diarrhea, dehydration, malabsorption, progressive malnutrition, and electrolyte disturbances [11].

CF can be used as a growth driver for PEM sufferers by improving plasma protein, bone growth, nerve development, inflammation, and intestinal microbiota. This innovative approach holds promise for significantly improving health outcomes in PEM cases.[13]. Previous research that has been carried out by Mostafa *et al.* (2022) states that the composition used is chickpea flour, peanut flour, soybean flour, green banana flour, sugar, soybean oil and a micronutrient mix. Indonesia is a tropical country with abundant natural resources that can be used as CF material. Using Indonesian biological food ingredients such as bananas, cassava, and corn could be a breakthrough in CF formulation because of their abundant availability and affordable prices. Banana fruit is a food ingredient that can be used as flour. Banana flour contains high levels of carbohydrates, protein, fat and starch, with an average of over 20% of its weight [15,16]. The use of cassava as a CF ingredient has a carbohydrate content of 34% with a starch content ranging from 15-25% with a water content of 18% [17]. Corn is a food source containing carbohydrates, fat and protein [18]. The main component contained in corn is 60% carbohydrates, followed by fat and protein [19].

Bananas, cassava, and corn are foods rich in resistant starch, which cannot be digested by digestive enzymes in the small intestine. Therefore, they are further processed in the large intestine to be fermented by probiotic bacteria. This fermentation produces Short-Chain Fatty Acid (SCFA), which is useful for helping lower intestinal pH, increasing calcium absorption, and reducing ammonia and amine absorption.[20]. Low intestinal pH causes the small intestine's villi to become taller and denser, increasing the absorption surface for nutrients [21]. Wangko (2020), explained that SCFA production by the intestinal microbiota has an impact on health status because it is an energy source for colonocytes and is estimated to provide 60-80% of energy for body cells and can fulfil 5-15% of the body's total calorie needs. The fulfilment of energy and calories has an impact on the function of SCFA, namely maintaining body homeostasis, regulating metabolism and the immune system, protecting pathogens in the body, and providing an anti-inflammatory impact on the digestive tract which can reduce the development of various inflammatory diseases, so it is hoped that it can be a solution for improving intestinal microanatomy. PEM sufferers. Based on this, this research was carried out to describe the microanatomy of the small intestine (jejunum) of PEM rats (*Rattus norvegicus*) after administration of CF.

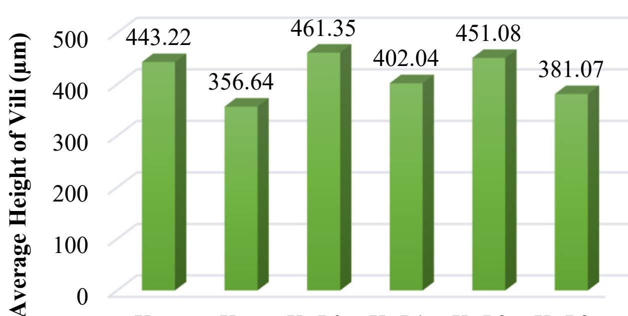
2 Method

This research is an experimental study with a Randomized Group Design. This research was carried out at the Animal and Human Physiology Laboratory and the Green House, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang as a place for raising and treating experimental animals from May to August 2023. Intestinal preparations were carried out at the Animal Anatomy Laboratory, Faculty of Veterinary Medicine, Brawijaya University. The experimental animals used were male Wistar strain rats with an average weight of 95 g and an age of 3-4 weeks. 24 mice were divided into 6 treatment groups and 4 replications, with group division as follows. The mice were given a low protein diet containing 2% of protein for 3 weeks. Afterwards, they were treated with complementary diets with various carbohydrate sources. Normal mice were fed with standard food as a negative control (K+), and LPD mice were fed as a positive control (K-). The other groups were malnourished mice, which were given standard food (K+P0), the mice were fed CF with a variation of banana (K+P1), the mice were fed CF with a variation of cassava (K+P2), and the mice fed CF with a variety of corn (K+P3). This research has passed the ethical approval test with Reg. No. code: 802/KEPK-POLKESMA/2023.

Making CF feed with the main composition of 3.75 g tempeh flour, 3.75 g chickpea flour, 3.75 g peanut flour, 4 ml corn oil, 5.625 g sucrose, and 0.015 g micronutrient mix. All ingredients were mixed and various additives were added as much as 9.375 g and the main composition and each additional variation were homogenized evenly then formed into a ball and weighed 30 g.

3 Results and Discussion

Microscopic measurements were taken of small intestine (jejunum) preparations with the average height of the villi presented in Figure 1.



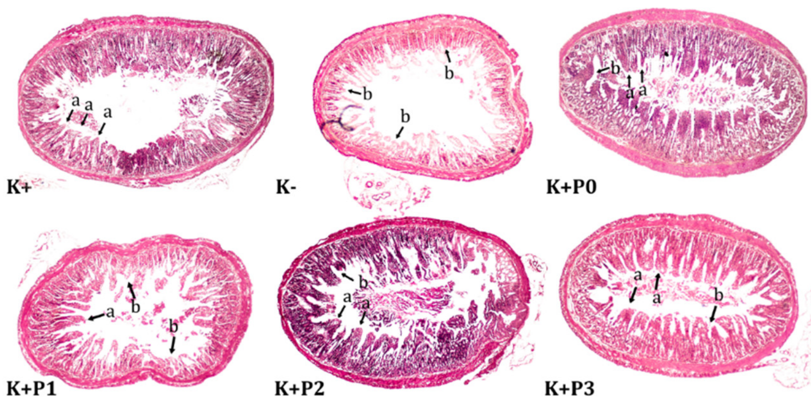
Description: K+: Positive Control; K-: Negative control; K+P0: Standard Feed; K+P1: CF Banana; K+P2: CF Cassava; K+P3: CF Corn.

Fig. 1. Average Height of Small Intestinal Villi (Jejunum) After Treatment

Figure 1 shows the average height of the small intestine villi (jejunum) in the K+, K-, K+P0, K+P1, K+P2, and K+P3 groups, respectively, was 443.22 μm, 356.64 μm, 461.35 μm , 402.04 μm, 451.08 μm, and 381.07 μm. The lowest mean height of the villi of the small intestine (jejunum) was in the K- K-treatment group, and the highest was K+P0. The short height of the villi is an indication of low health status in mice, this was proven in the K-treatment group, the PEM model treatment group [23]

Villi are part of the structure found in the intestinal mucosa layer, and their role is to expand and increase the efficiency of absorption of nutrients [24]. The increase in villi height is influenced by the increase in SCFA resulting from probiotic fermentation which functions for the proliferation of intestinal epithelial cells [25,26]. SCFA comes from persistent liver or digestive waste products that are resistant to the hydrolysis of amylolytic enzymes in the small intestine, which will then be fermented in the large intestine. Resistant starch fermentation can help improve colon health by acting as a nutrient to help the growth of

beneficial microbiota, reduce symptoms of diarrhea, stimulate the immune system, and increase the absorption of Fe, Ca and Mg ions.[27]. The longer the villi that the intestine has a wider absorption area and vice versa. The increase in villi height is directly proportional to the absorption of nutrients, improved health status, the effectiveness of enzyme work, and smooth transportation of nutrients in the digestive tract.[28].
Microscopic observation of the small intestine (jejunum) of mice with 4x magnification is presented in Figure 2.



Description: K+: Positive Control; K-: Negative control; K+P0: Pellet Feed; K+P1: CF Banana; K+P2: CF Cassava; K+P3: CF Corn.

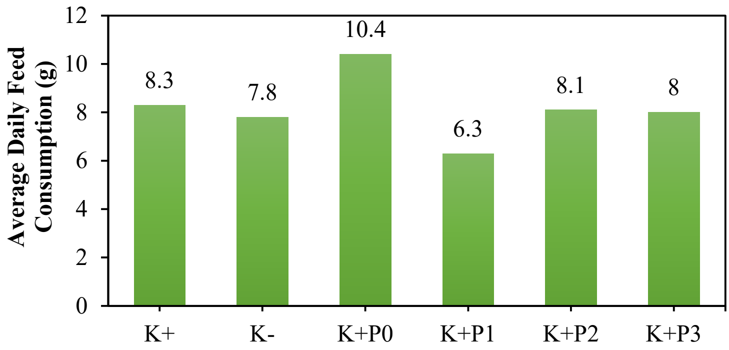
Description: Normal Villi (a), Villous Atrophy (b).

Fig. 2. Microanatomy of the Small Intestine (Jejunum) with 4x

Figure 2 is data from microanatomical observations of the small intestine of mice (jejunum) in the control group. The K+ villi looked dense and tall, indicating that the mice's small intestine (jejunum) was healthy. In the K+P0, K+P1, K+P2, and K+P3 groups, it was seen that some of the villi had atrophied, but several others had experienced improvement, as indicated by the villi looking tall and dense. Group K- is a group of PEM mice that experience atrophy of the small intestine's villi (jejunum). In the condition of PEM, the intestine will experience villous atrophy, crypt hyperplasia, and a decrease in the number of epithelial cells characterized by cell degeneration [11], [29]. Villous atrophy is a condition where the villi become blunted, which results in digestive enzyme deficiencies and damage to intestinal epithelial cells, causing malabsorption and maldigestion of nutrients [23]. Villous atrophy can occur due to a reduction or absence of nutrients, which causes decreased cell proliferation and increased cell apoptosis to reduce energy use [11].

Administration of CF showed improvements in the villi of mice's small intestine (jejunum), which were microscopically characterized by a denser and taller arrangement of villi than in the K- group. There was an improvement in villi in the CF treatment group because the CF composition was rich in nutrients derived from tempeh flour, chickpea flour, peanut flour, corn oil, sugar, micronutrient mix and added variations of banana flour, cassava flour and corn flour. Tempeh flour is a source of vegetable protein containing 19.5% protein [30]. Chickpea flour contains 60.2% carbohydrates, 23.8% protein, and 17.4% fiber [31]. Peanut flour contains 25-30% protein, 40-50% fat, 12% carbohydrates and vitamin B1 [32]. Corn oil is high in vitamin E which acts as an antioxidant and can prevent the formation of free radicals in the body[33]. Sugar contains 400 calories in every 100 grams [34]. Bananas contain 23% carbohydrates, 1% protein, 0.5% fat and 2.6% fiber [35]. Cassava contains 34% carbohydrates, 15-25% starch, and 18% water content [17]. Corn contains protein of 10.57%, fiber 2.41%, and fat 4.60% [36].

The results of the average height of the villi and microanatomy of the small intestine are supported by the influence of daily feed intake.The feed intake that was consumed during the treatment was weighed every day for 4 weeks and the average daily consumption was obtained which is presented in Figure 3.



Description: K+: Positive Control; K-: Negative control; K+P0: Pellet Feed; K+P1: CF Banana; K+P2: CF Cassava; K+P3: CF Corn.

Fig. 3. Average Daily Feed Consumption During Treatment

Figure 3 shows the average feed consumption per day. During treatment. The average consumption of the K+ group was 8.3 g, K- was 7.8 g, K+P0 was 10.4 g, K+P1 was 6.3 g, K+P2 was 8.1 g, and K+P3 was 8. Based on the calculation of average feed consumption, the results show that it is directly proportional to the height of the small intestine's villi (jejunum). Treatment with much feed consumption showed good morphology and average villi height, and vice versa.

However, based on the results of ANOVA calculations ($P < 0.05$), the villi's height did not significantly affect the CF treatment. It is suspected that several other factors are causing this, such as duration of treatment, composition of diet feed, and composition of treatment feed, so further research is needed regarding the effect of giving CF to rats (*Rattus norvegicus*) with PEM on repair of the small intestine (jejunum).

4 Conclusion

Providing normal feed and CF improves the microanatomy of the small intestine (jejunum). However, CF feeding had insignificant results ($P < 0.05$) on the height of the villi of the small intestine (jejunum).

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