2ND ICBC
INTERNATIONAL CONGRESS ON BIOACTIVE COMPOUNDS
3rd International Workshop on Bioactive Compounds:
Personal Nutrition, Ageing and Food Science
November 9-10th, 2022 at Unicamp

GERMINATED MILLET (*PENNISETUM GLAUCUM* (L.) R. BR.) FLOUR IMPROVED THE GUT FUNCTION AND ITS MICROBIOTA COMPOSITION IN RATS FED WITH HIGH-FAT HIGH-FRUCTOSE DIET

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Keywords: Intestinal health; Prebiotic; Whole grain

Summary: Diets rich in sugar and fat contribute to the progression of metabolic changes such as intestinal dysbiosis. On the other hand, diets rich in dietary fiber and protein are associated with increased intestinal cell proliferation, which benefits intestinal health. The germinated millet (*Pennisetum glaucum* (L.) R. Br.) is a whole grain abundant in proteins, dietary fiber, minerals, and bioactive compounds that has potential prebiotic action. Therefore, the aim of the present study was to evaluate the effect of germinated pearl millet flour on gut function and its microbiota composition in rats fed with a high-fat high-fructose (HFHF) diet. In the first phase, lasting eight weeks, the experiment consisted of two groups: AIN-93M (n = 10) group and HFHF group (diet

rich in saturated fat: 31% and fructose: 20%, n = 20). In the second phase, which lasted ten weeks, the AIN-93M group (n = 10) were kept, and the HFHF group was divided into HFHF group (high-fat high-fructose diet, n = 10) and the HFHF + millet group (high-fat high-fructose diet added with 286.3g of germinated millet flour. After the 18th week, the animals were euthanized and were collected proximal colon for histological analysis by hematoxylin and eosin technique and the images were processed using the ImagePro-Plus® software version 4.5. The cecum content was used for analysis of fecal pH, concentration of SCFA (short chain fatty acid) by HPLC, and extraction and sequencing of genomic DNA by Illumina MiSeq platform. The Mothur v.1.44.3 software was used for data processing and analysis. In addition, an analysis of intestinal permeability by lactulose and mannitol excretion was also performed by HPCL. Germinated millet flour increased ($p<0.05$) beta diversity, cecum weight, cecum/body weight ratio and improved gut histological parameters by increasing ($p<0.05$) the depth and thickness of the crypt and the number of goblet cells ($p<0.05$). Furthermore, germinated millet reduced ($p<0.05$) the fecal pH and mannitol urinary excretion and increased ($p<0.05$) the propionate short-chain fatty acid concentration. The lower pH in group fed with germinated millet flour may have improved the microbial diversity and provided substrate for the growth of SCFA-producing bacteria**.** The dietary fiber and undigested proteins present in germinated millet flour were probably fermented in the intestine and produced SCFAs, such as propionate**.** The largest production of propionate may have stimulated the growth of beneficial bacteria, such as Actinobacteria, which increases mucus secretion and the ability to promote hypertrophy of intestinal mucosa cells, consequently increasing the area of the surface of the intestine. These results can be

related to the reduction of intestinal permeability by less urinary mannitol excretion in the group treated with millet, which improved the dysfunction caused by the HFHF diet. These results demonstrate that the germinated millet flour improves the morphology, function, and gut microbiota composition in rats fed with high-fat high-fructose diet. Therefore, the present study highlights the potential of this flour to improve intestinal health in vivo.

Enter the project / scholarship number: CAPES and CNPq [Grant number: 001], CAPES-PrInt [Grant number: 88887.511858/2020-00], Embrapa Food Technology [Grant number: 13.16.05.043. 00.00] and FAPERJ [Grant number: E-26 / 202.848 /2017].