

The PHILIPPINE JOURNAL of

Veterinary Medicine

Volume 61

No. 2

July – December 2025

Published by the College of Veterinary Medicine
University of the Philippines Los Baños

The Philippine Journal of Veterinary Medicine

Volume 61

No. 2

July - December 2025

The Philippine Journal of Veterinary Medicine (ISSN 0031-7705 print; eISSN 2984-763X online) is a peer-reviewed international journal of basic, applied, and translational research in veterinary medicine and biomedical science. It is published semi-annually, for the periods January-June and July-December each year, by the College of Veterinary Medicine, University of the Philippines Los Baños. All articles are subjected to double-blind review. Authors of articles appearing in the journal are solely responsible for opinions expressed therein. All rights reserved. No article of the journal may be reproduced in any form and by any means without a written permission from the publisher or the Editor-in-Chief.

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Effects of Saba Banana [*Musa 'Saba'* (*Musa acuminata* x *Musa balbisiana*)] Peel Pectin Supplementation on Feeding, Fecal Weight and Adiposity Parameters of High-Fat Diet-Induced Obese Male ICR Mice

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Submitted: 06 Nov. 2024 Revised: 04 Mar. 2025 Accepted: 23 May 2025 Published: 19 Aug. 2025

Abstract

Background: Saba banana peels, often considered waste, are a rich source of pectin that can be used as a food ingredient and nutritional supplement. This study explored the potential of saba banana peel pectin in managing obesity, focusing on its impact on food intake and body fat.

Methods: *In vivo* experiments were conducted using high-fat diet (HFD)-induced obese male mice. Mice were divided into groups receiving either HFD supplemented with saba banana peel (SP) pectin or commercial citrus pectin (CP) for nine weeks. Control groups included HFD-fed mice and a normal diet (ND) group. **Results:** Results showed no significant difference in daily feed intake among HFD and pectin-supplemented groups, though water intake increased with pectin supplementation. Notably, the total adiposity index (TAI) in the HFD+SP group was significantly lower than the HFD group and comparable to the HFD+CP group. Fecal weight increased in pectin-supplemented groups, suggesting the binding of unabsorbed fats, which

likely contributed to weight reduction.

Conclusions: Supplementation with 10% saba banana peel pectin significantly improved obesity-related parameters in high-fat diet (HFD)-induced obese male ICR mice, including a 13.39% reduction in body weight, 3.66% decrease in abdominal circumference, and 76.72% lower total adiposity index, along with increased water and fecal output. With a human equivalent dose of 4.87g per day for a 60-kg adult, saba banana peel pectin shows strong potential as a natural anti-obesity agent, warranting further investigation through clinical studies.

Keywords

Adiposity, Body fats, Commercial citrus pectin, Pectin, Saba banana peels pectin

1. Introduction

The World Health Organization (WHO) declared in 2016 that over 650 million people are obese and more than 1.9 billion adults are

overweight. This phenomenon has reached epidemic proportions globally and its prevalence tripled between 1975 and 2016 [1]. Rapid growth in obesity was experienced in Asia and the Pacific, with magnitude varying among countries. The increasing prevalence worldwide has a huge societal effect on the economy like health care costs, national productivity such as the cost of days of work, higher employer insurance premiums and lower wages, physiological and psychological consequences including discrimination, depression, anxiety, and self-esteem, and lower quality of life [2,3,4]. Additionally, obesity is considered a major risk factor for non-communicable diseases (NCDs) which includes diabetes mellitus (DM), cardiovascular diseases (CVDs), musculoskeletal disorders, several cancers, and metabolic syndrome [1,5,6]. In 2013, however, the American Medical Association already classified obesity as a NCD [5]. Numerous factors contribute to the development of obesity. These are genetics, low physical activity and exercise, poor diet, and other unhealthy behaviors. Except for genetics, all are modifiable factors. Hence, obesity is a preventable and curable disease [1,8,9], and lifestyle modification and a balanced diet are recognized as recommended preventive strategies [10,11,12].

Among dietary constituents, dietary fiber has been the subject of increasing attention for its effects on multiple mechanisms such as regulating food intake and body weight, greater feelings of satiety, and changes in blood glucose and insulin [12]. Dietary fiber is classified either as soluble or insoluble, with pectin as one of the widely available sources of soluble fiber. Pectin is commonly found in fruits, vegetables, and seed extracts. Commercially, it is commonly extracted from citrus peels and apple pomace and developed into a variety of products, including food supplements, gelling and thickening agents, and as an all-around stabilizer used by the food industry [12,13].

Studies have shown that pectin, a soluble dietary fiber found in both natural and artificial sources, might help manage and prevent obesity by reducing food consumption and adiposity, which is one of the many ways that dietary fiber is known to affect obesity [12,14]. One of the recently identified novel sources of pectin is the saba banana peel [*Musa 'Saba'* (*Musa acuminata x Musa balbisiana*)]. Saba is abundantly grown in

the Philippines, but its peels currently have little to no use and, thus, are considered a waste product that can cause environmental problems. According to the study of Castillo-Israel *et. al.* [15], pectin from saba banana peels is comparable to commercial citrus pectin in terms of moisture content, gelling ability, and sensory qualities. Notably, it was found that ash content was much higher for the saba banana peels than that of commercial citrus pectin. These characteristics suggested the possible commercial use of extracted pectin from saba banana peels in food processing. However, studies are warranted to support its use as a nutraceutical product for obesity prevention and management.

Thus, the study investigated the anti-obesity potentials of pectin from saba banana peel via its effects on regulating food intake and adiposity *in vivo*. The results of this research hoped to provide bases to support initiatives promoting and utilizing saba banana peel pectin both as a functional food ingredient and an anti-obesity nutritional supplement. More importantly, this study is expected to contribute to the scant body of knowledge on the nutritional and health benefits of pectin from saba banana peels, specifically its role in obesity prevention and management.

2. Materials and Methods

Extraction of Saba Banana Peel Pectin

The 'saba' banana peel samples were obtained from banana processing industries in Lipa City, Batangas. The extraction was done at the Institute of Food Science and Technology (IFST) of the College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB) following the established protocol of Castillo-Israel *et. al.* [15] with some modifications. Briefly, the saba banana peels were washed with running water to remove adhering dirt and were dried in an oven (Memmert, Germany) at 55°C for 24 h. The dried peels were then processed into flour using a grinding mill (Oster, USA) and stored in polyethylene bags. Extraction of pectin was then facilitated by adding 20 g of ground banana peel powder to 400 mL 0.45 N citric acid (Chemline Scientific, Philippines) solution at pH 1.5. The resulting mixture was then heated at 85°C for 5h with continuous agitation. After the extraction process, the mixture was cooled and filtered

through an ordinary wire screen with a 1-mm mesh size and a two-layer cheesecloth. The filtrate was collected and added with twice its volume of 95% ethanol (Checline Scientific, Philippines) to facilitate pectin precipitation. The precipitate was then recovered using a linen cloth lined on a Buchner funnel and was dried in an oven (Memmert, Germany) for 5h at 55°C. The dried pectin was then ground into powder and kept until used [16].

In Vivo Efficacy Test

All procedures performed in ICR mice for the efficacy study were approved by the UPLB Animal Care and Use Committee with approval no. CHE-2019-001.

Animals

A total of sixty (60) 6-week-old ICR male mice with initial weights between 22.0 ± 2 g obtained from the Research Institute for Tropical Medicine (RITM), Alabang, Muntinlupa City, were used in the study. Male ICR mice are an acceptable model for obesity and hyperglycemia studies [17], and being male prevents the interplay of sex with the variables of interest. Mice were housed individually in properly labeled polycarbonate cages with stainless steel tops (Techniplast, Italy) and maintained at 26°C, 50–60% humidity, and 12h: 12h light/dark cycle lights on at 7:00 AM and lights off at 7:00 PM at the laboratory animal experimental room, DBVS, CVM, UPLB. Commercial maintenance mice pellets (Altromin, Germany) and distilled drinking water were also provided *ad libitum* during the one-week acclimation period.

Components and Preparation of Diets

A regular diet (AIN-93G Purified Rodent Diet, Dyets, USA) and a custom high-fat (45% FDC) purified rodent diet were used for the normal and negative control groups (ND and HFD), respectively. The Dyets, Inc. (Pennsylvania, USA) feed formulation was used to develop the HFD, which contains 45 percent calories from fat. Pork lard used in the HFD was obtained by rendering fresh leaf fat surrounding the kidneys of swine—sourced from a certified local supplier—which was cleaned, chopped, and slowly heated using the dry heat method at 90–95 °C until golden cracklings formed, then filtered and stored at 4 °C for use in

diet formulation. The agar solution was microwaved for 3 min to prepare the HFD. Once it reached 40°C, pork lard was added and heated for another one min at the same temperature. The mixture was transferred to an electric mixer (KitchenAid Stand Mixer ,USA) and blended thoroughly. The agar-lard solution was added to the dry ingredients and mixed until fully combined. The agar-lard solution was then added to all the dry ingredients (i.e., Casein, L-Cystine, Sucrose, Cornstarch, Dytrose, t-Butyl hydroquinone, Cellulose, Mineral Mix #210025, Vitamin Mix #310025, Choline Bitartrate, Salt Mix #210088, Dicalcium Phosphate, Calcium Carbonate, Potassium Citrate H2O, Vitamin Mix #3000050), and everything was combined well. In the preparation of HFD pectin mixes, the same process was followed, except that the powdered pectin was added together with all the dry ingredients. The mice's food was then transferred into a clean container, covered, labeled, and kept in a freezer until it was needed. Table 1 shows the composition and caloric content of the formulated diets.

Commercial Citrus Pectin (CP) (L# 11612929, Alysons' Chemical Enterprises, Inc., Quezon City, Philippines) and Saba Banana Peels Pectin (SP) were added to the HFD at 10% w/w with a few modifications to achieve isonitrogenous and isocaloric diets with 4.60 kcal per gram and carbohydrate-protein-fat compositions of 36-18-46 percent, respectively. The 10% dosage was based on the *in vivo* studies of Adam *et. al.* [12,14] wherein supplementation of 10% pectin (w/w) was found to significantly reduce body weight and adiposity index when using the HFD control.

Induction Period

The obesity induction was done for three (3) week, wherein mice were fed with HFD to induce obesity. Mice with more than a 20% increase from their pre-induction weight were considered obese [18] and were included in the supplementation period.

Supplementation Period

After the induction phase, the mice were randomly allocated to four (4) groups (n=7 per group), namely: 1) the normal diet group (ND), given a normal diet and served as a normal control;

Table 1. Composition and caloric content of the different diets.

Ingredients	kcal per gram	ND (g)	HFD (g)	HFD+CP (g)	HFD+SP (g)
Casein	3.58	100.0	116.54	115.77	115.55
L-Cystine	4.00	1.50	1.75	1.75	1.75
Sucrose	4.00	50.00	100.69	100.69	100.69
Cornstarch	3.60	198.7	42.42	0.00	0.00
Dyetrose	3.80	66.00	58.27	57.00	57.50
Soybean Oil	9.00	35.00	0.00	0.00	0.00
t-Butyl hydroquinone	0.00	0.007	0.003	0.003	0.003
Cellulose	0.00	25.0	29.13	29.13	29.13
Mineral Mix #210025	0.88	17.5	0.00	0.00	0.00
Vitamin Mix #310025	3.87	5.00	0.00	0.00	0.00
Choline Bitartrate	0.00	1.25	1.17	1.17	1.17
Salt Mix #210088	1.60	0.00	5.83	5.83	5.83
Dicalcium Phosphate	0.00	0.00	7.57	7.57	7.57
Calcium Carbonate	0.00	0.00	3.20	3.20	3.20
Potassium Citrate H2O	0.00	0.00	9.61	9.61	9.61
Vitamin Mix #3000050	3.92	0.00	5.83	5.83	5.83
Pork Lard	9.00	0.00	118.00	117.72	117.35
Commercial citrus pectin	3.61	0.00	0.00	50.00	0.00
Saba banana peel pectin	3.59	0.00	0.00	0.00	50.00
Calorie		500.00	500.00	505.30	505.20
Total calories		1880.02	2295.23	2312.77	2309.54
Kcal per gram		3.76	4.59	4.58	4.57

ND – normal diet; HFD– high fat diet; HFD+CP– HFD w/10% commercial citrus pectin; HFD+SP– HFD w/10% Saba banana peels pectin

2) the HFD group, given with HFD, and served as a negative control; 3) the HFD + commercial citrus pectin group (HFD+CP), given with HFD + 10% (w/w) citrus pectin; and 4) the HFD + saba banana peel pectin group (HFD+SP), given with HFD + 10% (w/w) saba banana peel pectin. The ND group had mice with normal body weights, which served as a normal control, whereas the other three groups had obese mice. Animals received their respective diets *ad libitum* for nine (9) weeks of supplementation. Treatment-related effects were determined by measuring the feed and water intake, and adiposity parameters such as body weight, abdominal circumference, weight of body fats, total adiposity index (TAI), fecal weight, and adipocyte surface area.

Feed Intake Measurement

Each mouse was given 7-8g of feed (pre-weighed feeds) per day starting at 7 a.m. After 24h, the leftover feeds were measured daily for nine (9) weeks using a digital top-loading balance

(Shimadzu, Japan). The leftover feeds were subtracted from the pre-weighed feeds (7-8g) to get the daily feed intake. Data were recorded to the nearest 0.01 gram. The caloric intake was computed by multiplying the daily feed intake by its equivalent calories per gram.

Water Intake Measurement

Each mouse was given 10 mL of distilled water daily, measured using a 60-mL syringe, and then placed in clean water bottles. The leftover water was measured using a 3 mL syringe cylinder starting at 7:00 a.m. daily for nine (9) weeks. The volume of leftover distilled water was deducted from the known volume of water given per mouse to get the daily water intake. Data was recorded to the nearest 1 mL.

Body Weight Measurement

Body weights of mice were measured weekly for nine (9) weeks using a digital top-loading

balance starting at 8:00 a.m. The measurement was done by placing the mouse in a container with a known weight and then placing the container with the mouse on the digital top-loading balance. The weight of the container was subtracted from the weight of the container + mouse to determine the weight of the mouse. The weight was recorded to the nearest 0.001 gram.

Abdominal Circumference Measurement

For abdominal circumference, mice were restrained by applying a scruff hold to the loose skin between ears using the thumb and forefinger with one hand while maintaining a grip on the tail with the other hand. The abdominal circumference was measured around the anterior abdomen of the mice [19], using a plastic non-flexible measuring tape with an accuracy of 0.1 cm.

Fecal Weight Measurement

Fecal samples were collected every week throughout the 9-week supplementation period. The collected feces were weighed using an analytical balance (Shimadzu, Japan) with the results recorded to the nearest 0.001g.

Euthanasia, Necropsy, and Tissue Collection, Processing, and Examination

Mice were euthanized via intraperitoneal injection with pentobarbital sodium using a 100 mg/kg dose. Necropsy was performed by licensed veterinarians, and any gross abnormalities in the pancreas and abdominal fat tissues were recorded. The weight of the pancreas and abdominal fats was also measured using an analytical balance. Pancreas and adipose tissue samples were obtained, fixed in 10% formalin for at least 72h, processed with routine paraffin technique, sectioned at 5 um thick, stained with H&E, and examined under the microscope. Histopathological changes in adipose tissues and the pancreas were assessed by a veterinary pathologist.

Total Adiposity Index (TAI)

The TAI, which accounts for the total fat tissues in the body of mice, served as a parameter of adiposity [20]. Different pad tissues were

dissected and weighed. Total body fat was calculated as the sum of the following individual fat pad weights, and the TAI was computed following the formula indicated below [21].

Total body fat: epididymal fat + retroperitoneal fat + visceral fat.

Adiposity Index = (total body fat / final body weight) × 100.

Mean Adipocyte Surface Area

The mean diameters of abdominal white adipocytes were calculated as an indicator of adipocyte hypertrophy in restricted view fields on a computer monitor using an automated image analysis system (Image J). The mean diameter was expressed in μm , and a minimum of three white adipocytes per fat pad was measured.

Animal Dose to Human Dose Conversion

The human equivalent dose (HED) was calculated using the km value. HED was calculated following the formula indicated in the study of Shin *et. al.* [22]:

HED (mg/kg) = animal dose (mg/kg) $\frac{\text{Animal Km}}{\text{Human Km}}$

Statistical Analysis

The data was analyzed using SPSS Statistics Version 26 [23]. All tests were evaluated with a significance level of 0.05. A one-way ANOVA followed by Tukey-Kramer HSD as a post-hoc test was used to determine whether there were any significant differences in the variables across the various groups. A paired t-test and a Wilcoxon signed rank test were employed to determine the significant difference between time points in each group, while the Pearson R correlation test was used to measure the strength and significance of the relationship between the two variables.

3. Results

Effect of SP on Feed Intake

Feed Intake

The mean daily feed intake of the mice groups from the start of the obesity induction period up to

the supplementation period shows that the ND mice group consistently consumed a significantly lesser amount of food than the HFD, HFD+CP, and HFD SP groups throughout the feeding duration (Fig. 1). The HFD groups showed a consistently similar daily feed intake from baseline to endline supplementation. The mean daily intake of HFD+CP was 5.22 ± 0.185 g and HFD+SP was 5.31 ± 0.119 g, which were lower compared to the HFD group but higher than the ND group. Notably, the HFD group had the highest mean daily food intake (5.38 ± 0.196 g), followed by HFD+SP and HFD+CP, but there was no significant difference between these groups.

Water Intake

Figure 3 shows the mean daily water intake of mice from the baseline of induction to the endline supplementation period. During the induction period, a significantly lower water intake was noted in the HFD group compared with the ND group. During the supplementation period, mice from the HFD group consistently consumed the least amount of water. The addition of pectin HFD, however, led to increased water intake, as evidenced by the significantly higher water intakes in the HFD+CP vs. HFD and HFD+SP vs. HFD groups ($p < 0.000$ and $p < 0.005$, respectively).

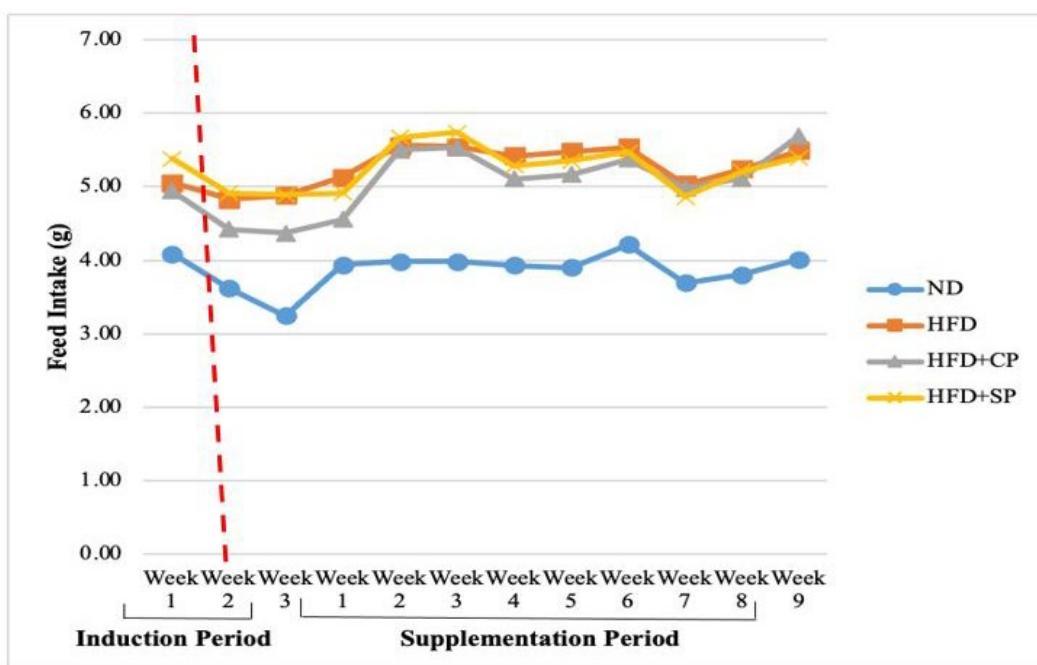


Figure 1. Mean daily feed intake of male ICR mice in the different treatment groups from induction to supplementation periods.

Caloric Intake

Figure 2 shows the mean daily caloric intake of all mice groups from the baseline of induction to the endline of the supplementation period. The same observation was made in the mean daily feed intake between groups in terms of caloric intake. Throughout the induction and supplementation periods, the ND group consumed significantly fewer calories than the HFD, HFD+CP, and HFD+SP groups ($p < 0.000$, $p < 0.000$, and $p < 0.000$, respectively). There was, however, no significant difference in the caloric intake among mice in the HFD, HFD+CP, and HFD+SP groups.

Effect of Pectin from Saba Banana Peels on Adiposity Parameters

This section shows the mean body weights, abdominal circumference, body fats, TAI, and adipocyte area of ICR mice during the supplementation period. Data have revealed that the body weights of HFD+CP and HFD+SP groups were comparable and significantly different to HFD groups at endline supplementation ($p < 0.003$, and $p < 0.023$, respectively). Notably, the HFD+CP and HFD+SP groups' abdominal circumference was lower than the HFD group, although no significant difference was noted. The % TAI of the

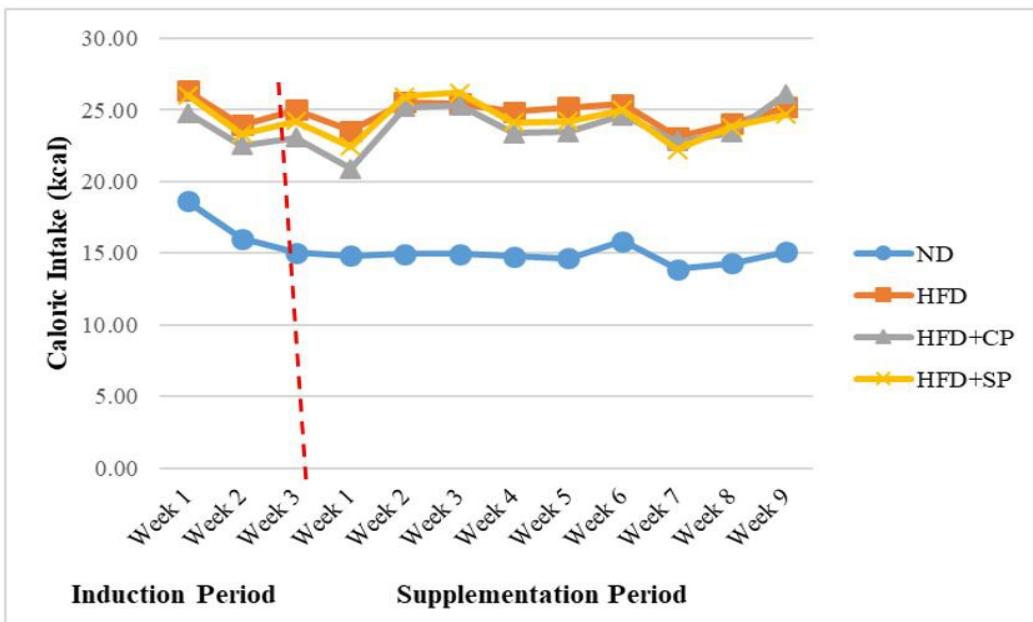


Figure 2. Mean daily caloric intake of male ICR mice in the different treatment groups from induction to supplementation periods.

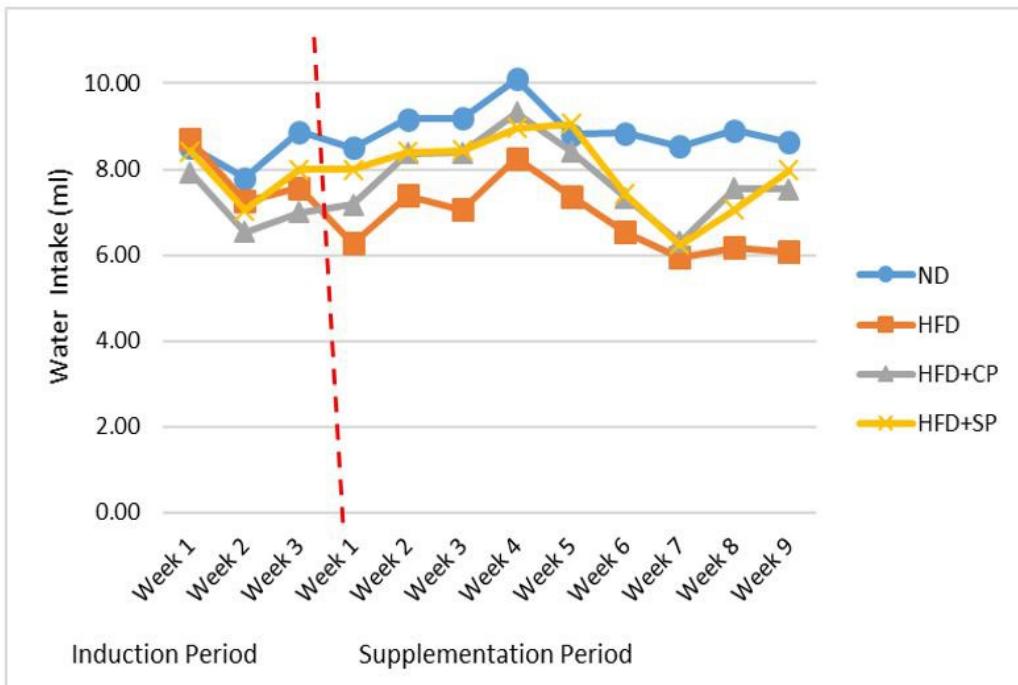


Figure 3. Mean daily water intake of male ICR mice in the different treatment groups from induction to supplementation periods.

HFD+CP and HFP+SP groups were comparable and significantly different from the HFD group ($p<0.001$ and $p<0.010$, respectively). The abdominal fat (A. fat), mesenteric fat (M. fat), and heart fat (H. fat) were lower in the HFD+SP group compared to the HFD+CP group and HFD group. In comparison, subcutaneous (S. fat) and

epididymal fat (E. fat) in HFD+CP were significantly lower than in HFD+SP and HFD groups ($p<0.004$ and $p<0.006$, respectively). Lastly, the mean adipocyte surface area of HFD was higher compared to the HFD+SP and HFD+CP groups.

Bodyweight

The body weight of all mice groups increased throughout the supplementation period, as shown in Table 2. This indicates the normal growth of

weight gains of both pectin-supplemented groups showed no significant difference with the ND group while statistically lower than the HFD group ($p<0.005$). These findings may suggest the anti-obesity potential of both saba pectin and commercially available citrus pectin.

Table 2. Mean body weight of male ICR mice in the different treatment groups during the supplementation period.

Animal Groups	Baseline Supplementation (g)	Endline Supplementation (g)
ND	29.42 ± 0.580 a,*	32.31 ± 1.115 a,†
HFD	31.40 ± 0.566 b,*	39.19 ± 0.977 b,†
HFD+CP	31.40 ± 0.566 b,*	33.19 ± 0.889 a,†
HFD+SP	31.40 ± 0.566 b,*	34.56 ± 1.202 a,†

ND – Normal Diet; HFD – HFD; HFD+CP – HFD w/ commercial citrus pectin; HFD+SP – HFD w/ Saba Banana peels pectin

Values represent the mean \pm S.E. (ND n = 7; HFD n= 7; HFD+CP n=7; HFD+SP n=7)

Means in the same column followed by a different letter are significantly different at $p<0.05$.

Means in the same row followed by a different symbol are significantly different at $p<0.05$.

mice as they age. Also, the HFD+CP and HFD+SP groups had a steady weight gain, demonstrating no obvious treatment-related harm from pectin supplementation. As observed in the HFD+SP and HFD+CP groups, supplementation with pectin had almost the same effects on body weight except for the remaining four (4) weeks of supplementation. During this period, there was a modest increase in body weight among mice in the HFD+SP group. It was also noted that the HFD+SP and HFD+CP groups showed a clear steady body weight gain from week 1 to week 5, where mice were 10-14 weeks old. This was despite continued access to a high-fat diet as compared with the HFD group, which had a sharp increase in body weight.

At the start of the supplementation period, the HFD group and pectin-supplemented (HFD+CP and HFD+SP) groups had comparable mean body weights and were significantly higher than the ND group ($p<0.005$). But at the end of the supplementation, the body weights of the HFD+CP and HFD+SP groups were significantly lower than the HFD group ($p<0.003$, and $p<0.023$, respectively) and statistically comparable with the ND group despite continued access to unlimited high-fat food. In terms of weight gain, the HFD+CP group had the lowest body weight gain and percent weight change (1.79 g and 5.69%, respectively), which were even lower than the ND group (2.89 g and 9.84%, respectively). The HFD+SP group's mean body weight gain and percent weight change (3.16 g and 10.05%, respectively) were a little higher than both the ND group and the HFD+CP group. Nonetheless, the

Abdominal Circumference

At baseline, the mean abdominal circumference of all mice groups was statistically comparable (Table 3). At the endline, the HFD group had the highest abdominal circumference and ND had the lowest value, but no significant difference was found between the control (ND and HFD groups) and pectin-supplemented (HFD+CP and HFD+SP groups). Interestingly, HFD+SP had a significantly greater reduction in mean difference and percent change in abdominal circumference, followed by the ND group and the HFD+CP group. Given that continued and prolonged access to a high-fat diet significantly increased the abdominal circumference in the HFD group ($p<0.000$), pectin supplementation therefore has positive effects on abdominal fats, thereby reducing the risk of abdominal obesity related to high-fat diet intake. These are interesting findings that can be explored further given the lack of published studies yet on the effects of pectin supplementation on abdominal obesity *in vivo*.

Body fats

Table 3 shows that the ND had the lowest A. Fat, M. Fat, and H. Fat, whereas the HFD+CP had the lowest S. Fat and E. Fat. Among HFD groups, HFD+SP had the lowest A. Fat, M. Fat, and H. Fat. Meanwhile, the HFD group consistently had the highest levels of fat in all body parts analyzed. The HFD group had the highest mean TAI, followed by

Table 3. Mean abdominal circumference during the supplementation period, mean body fats, and TAI% of male ICR mice in the different treatment groups at endline supplementation.

Animal Groups	Abdominal Circumference (cm)		Body Fats (g)				TAI (%)	
	Baseline Supplementation	Endline Supplementation	A. Fat	S. Fat	E. Fat	M. Fat		
ND	7.31± 0.25 a,*	7.26± 0.15 a,*	0.29± 0.09 a	0.90± 0.19 a	0.93± 0.11 a	0.40± 0.06 a	0.15± 0.01 a	2.65± 0.30 b
HFD	7.11± 0.10 a,*	7.64± 0.11 a,†	1.00± 0.80 a	1.87± 0.38 b	1.99± 0.23 b	0.87± 0.12 a	0.19± 0.06 a	5.92± 0.50 a
HFD+CP	7.27± 0.11 a,*	7.37± 0.18 a,*	0.56± 0.24 a	0.59± 0.17 a	0.98± 0.23 a	0.63± 0.16 a	0.13± 0.01 a	2.84± 0.57 b
HFD+SP	7.61± 0.22 a,*	7.37± 0.13 a,*	0.49± 0.18 a	0.87± 0.17 ab	1.26± 0.18 ab	0.62± 0.18 a	0.11± 0.03 a	3.35± 0.58 b

ND – Normal Diet; HFD – High Fat Diet; HFD+CP – HFD w/ commercial citrus pectin; HFD+SP – HFD w/ Saba Banana peel pectin

A.Fat. –Abdominal Fat; S.Fat. - Subcutaneous Fat; M.Fat.-Mesenteric Fat; H.Fat.-Heart Fat; TAI- Total Adiposity Index

Values represent the mean ±S.E. (ND n = 7; HFD n= 7; HFD+CP n=7; HFD+SP n=7)

Means in the same column followed by a different letter(s) are significantly different at p<0.05.

HFD+SP, HFD+CP, and the lowest in ND group. There were no significant differences in adiposity indexes between the ND group and the HFD+CP and HFD+SP groups. However, the TAI in HFD+CP was relatively lower than in HFD+SP. There were no significant differences in adiposity indexes between the ND group and the HFD+CP and HFD+SP groups. However, the TAI in HFD+CP was relatively lower than in HFD+SP.

Fecal Weight

Table 4 shows that at baseline, fecal weight was significantly higher in the HFD group and pectin-

supplemented groups than in the ND group (p<0.002). At the end of supplementation, HFD+CP had the highest fecal weight, followed by HFD+SP, HFD, and the lowest in the ND group. The fecal weight of the HFD+CP group was significantly higher than that of ND, HFD, and HFP+SP. Among the HFD groups, HFD+CP had the greatest increase in fecal weight from baseline to endline (75.47%; 2.43g), followed by HFD+SP (26.65%; 0.89g), with the lowest increase observed in the HFD group (10.34%; 0.36g). A significant difference was observed between HFD and HFD+CP, as well as between HFD+CP and HFD+SP.

Table 4. Mean fecal weight of male ICR mice in the different treatment groups during the supplementation period.

Animal Groups	Mean Fecal Weight (g)	
	Baseline Supplementation (Week 0)	Endline Supplementation (Week 9)
ND	2.56 ± 0.16 a,*	3.05 ± 0.20 a,†
HFD	3.48 ± 0.19 b,*	3.84 ± 0.13 a,*
HFD+CP	3.22 ± 0.14 b,*	5.65 ± 0.53 b,†
HFD+SP	3.34 ± 0.12 b,*	4.23 ± 0.21 a,†

ND – Normal Diet; HFD – High Fat Diet; HFD+CP – HFD w/ commercial citrus pectin; HFD+SP – HFD w/ Saba Banana peel pectin

Values represent the mean ±S.E. (ND n = 7; HFD n= 7; HFD+CP n=7; HFD+SP n=7)

Means in the same column followed by a different letter(s) are significantly different at p<0.05.

Means in the same row followed by a different symbol are significantly different at p<0.05.

Histopathology of Pancreas and Fats

Pancreas

Figure 4 shows the results of histological analysis of the pancreas from the ND, HFD, HFD+CP, and

in the diameter of the adipocytes ($31.78\mu\text{m}$). Similar to HFD, HFD+SP also showed an increase in the diameter of adipocytes ($29.24\mu\text{m}$) but to a lesser extent. Meanwhile, the HFD+CP ($21.75\mu\text{m}$) and the ND group ($17.05\mu\text{m}$) showed normal

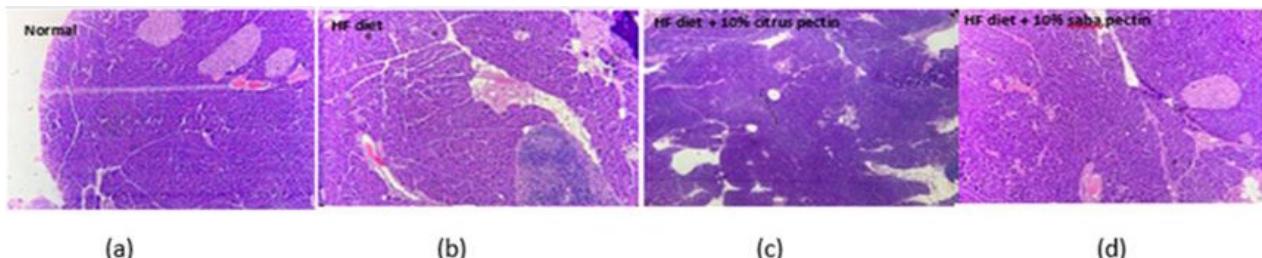


Figure 4. H&E image of a pancreas tissue: (a) normal diet (ND) group; (b) high-fat diet (HFD) group; (c) high-fat diet plus commercial pectin (HFD+CP) group and (d) high-fat diet plus saba banana peel pectin (HFD+SP) group.

HFD+SP groups. Histological analysis from all the groups showed a normal architecture of the pancreas characterized by oval or elongated pancreatic islets surrounded by a thin connective sheath. No inflammatory or fibrotic changes were noted in any of the islets examined. The ND group did not show any adipocyte deposits and appeared normal. Several sections from the HFD group (Figure 4b) showed mild deposition of adipocytes in the parenchyma, while HFD+CP (Figure 4c) and HFP+SP (Figure 4d) showed occasional fat deposition.

Abdominal Fat

In Table 5, representative histological sections of the abdominal fat from HFD showed an increase

architecture of the adipocytes (Fig. 5) and were significantly lower in adipocyte diameter. Essentially, the histology of adipose tissue revealed that commercial pectin in HFD+CP was able to effectively ameliorate adipose tissue hypertrophy in obese diet-induced mice compared to HFD+SP.

Animal Dose to Human Dose Equivalent

The study demonstrated that pectin supplementation at 10% (w/w) of the total diet is beneficial in the prevention of weight gain and reduction of body fats in HFD-induced obese mice. When compared to the HFD group, SP supplementation at 10% (w/w) of the diet resulted

Table 5. Mean adipocyte surface area of different treatment mice after the supplementation period.

Animal Groups	After Supplementation Period (μm)
ND	$17.05 \pm 3.07^{\text{b}}$
HFD	$31.78 \pm 13.04^{\text{a}}$
HFD + CP	$21.75 \pm 7.37^{\text{b}}$
HFD + SP	$29.24 \pm 11.20^{\text{a}}$

ND – Normal Diet; HFD – High Fat Diet; HFD+CP – HFD w/ commercial citrus pectin; HFD+SP – HFD w/ Saba Banana peel pectin

Values represent the mean (ND n = 7; HFD n= 7; HFD+CP n=7; HFD+SP n=7)

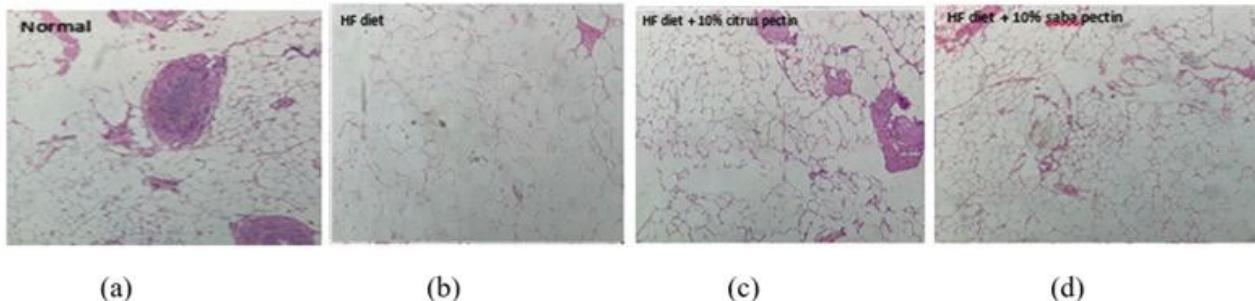


Figure 5. H&E image of Abdominal fat tissue: (a) Normal diet (ND) group (b) High-fat diet (HFD) group (c) High-fat diet plus commercial pectin (HFD+CP) group and (d) High fat diet plus saba banana peel pectin (HFD+SP) group.

in 14.61% higher water intake, 13.39% lower body weight, 3.66% lower abdominal circumference, and 76.72% lower TAI. The effective dose of HFD+SP at 10% (w/w) when converted to a human dose using the Reagan-Shaw formula [24] is equivalent to 4.87g per day for a 60-kg typical adult.

4. Discussion

The results of this study confirmed the ability of SP to reduce food intake and adiposity parameters, which play an important role in the prevention of fat accumulation and obesity. Specifically, this study demonstrated that SP added to a high-fat diet did not decrease the food but resulted in increased water intake and fecal weight, reduced body weight, and body fats in nine (9) weeks of supplementation. These findings are similar to the reports of several rat trials indicating that a high-fat diet supplemented with pectin from various sources has shown a lower body weight [14,33], and suppressed the development of adipose tissue [33]. The pectin added to the diet had resulted in increased water-holding capacity and therefore increased the bulk of the diet. Thus, it can be considered that weight and adiposity regulation in SP, like in CP can possibly be due to the increased water-holding capacity of pectin brought about by its gelling ability or increased viscosity, which both intakes and reduces the absorption of nutrients, including fats, thus resulting in lower body weight gain.

With body weight being an index of adiposity, the significant reductions observed in body weight of SP-supplemented groups may indicate the reduction of body fats in obese mice despite continued access to high-fat food. Similar to the findings of Adam *et. Al.* [14] dietary pectin

supplementation in rats induced 23% body fat loss, leading to 12% lower final body weight and 44% lower total body fat mass than controls. Also, their study confirmed that pectin-supplemented rats showed that the decrease in body weight was associated with significant body fat loss. The highest reduction observed in HFD+SP in terms of abdominal circumference and lower weights of abdominal fats than HFD and HFD+CP suggest that SP may have positive effects on reducing the risk of abdominal obesity related to high-fat diet intake.

Furthermore, it was found in this study that SP, similar to CP, was also effective in increasing the fecal weight of mice. The significant increase in fecal weight seen in both pectin-supplemented groups may reflect the presence of pectin in the diet and may indirectly and partially indicate the amount of fat removed from the body, which may contribute to their anti-obesity effects. As previously noted in the study of Slavin [28], pectin binds to cholesterol and bile acids in the gut and promotes their excretion, as well as inhibiting fat deposition by removing them through bile production and stool [29]. The bacterial mass formed from fermentable substances such as pectin and the retained water is responsible for the increase in fecal bulk [30]. These effects are assumed to decrease fat accumulation, increase fecal weight, decrease body weight, increase water intake, and lower TAI, indicating that it may indirectly and partially increase the amount of fat removed from the body, which may contribute to the anti-obesity effects of pectin.

In terms of the association between feed intake and body weight, a positive correlation was noted in pectin-supplemented groups. However,

the effect was more pronounced in commercially available citrus pectin, where a significantly strong positive correlation was noted. Likewise, in the study of Adam *et. al.* [14], it was also determined that there is a strong positive correlation between food intake and weight gain in rats in a four-week pectin-based dietary intervention. It was also found that the body weight was lower in rats fed with pectin-containing diets compared with rats on high-fat diets. Based on these findings, it can be said that the pectin from saba peels when supplemented with high-fat diets, is effective in lowering body weight and adiposity similar to its commercially available counterpart, citrus pectin.

Comparing the two (2) pectin sources, the current study revealed no significant difference in the effect of SP and CP as pectin sources in the variables that were investigated, except in terms of subcutaneous fat, epididymal fat, and fecal weight. Both CP and SP supplementation resulted in higher water intake and a lower mean abdominal circumference compared to the HFD diet after the supplementation period. Also, the mean adipocyte surface area was lower in HFD+CP and HFD+SP compared to HFD at endline supplementation. However, the effect of CP supplementation tended to reduce daily feed and calorie intakes; lower body weight; lesser body weight gain; higher fecal weight; lesser TAI; subcutaneous fat and epididymal fat; and adipose tissues. On the other hand, SP supplementation resulted in higher water intake and lower fat accumulation in the pancreas, abdominal fat, mesenteric fat, and heart fat. These may suggest that saba peel pectin in HFD+SP could have a more pronounced effect on reducing the degree of body fat in some body parts, such as A. Fat, M. Fat, and H. Fat, whereas commercial pectin in HFD+CP has a more pronounced effect on S. Fat and E. Fat. Differences in their effects can be partly attributed to differences in the source of pectin, as backed up by several studies [31,32,33,34]. In certain mice studies, data have revealed that pectin lyase-modified red ginseng extract supplementation reduced the size of adipocytes from epididymal adipose tissue in a dose-dependent manner when compared to HFD-obese mice [34]. In arabinoxylan supplementation, the arabinoxylan treatment normalized the subcutaneous adipose tissues as compared to HFD, which increases the amount of subcutaneous adipose tissues [6]. In inulin intake, the HFD-

induced increase in subcutaneous and epididymal white adipose tissue [25] was observed; in an arabinoxylan-supplemented diet, a lower fat mass development was observed through the weight of epididymal, subcutaneous, and visceral adipose tissues [32]. Among other sources of DF, (1-4) Beta-D-glucan from oats was shown to have a lower adiposity index (visceral fat/final body weight) in high-glucan-fed mice than the low glucan-fed mice [30]. In the study of Schroeder *et. al.* [26], it was mentioned that the lowest epididymal fat pad weight was observed in high-viscosity fiber, whereas the cellulose group had the heaviest epididymal weight. In a gum arabic supplementation study, the adipose tissues of the supplemented group, such as mesenteric fat, perinephric fat, and periovarian fat, relative to body weight and mean adipocyte area, tended to be smaller compared to a normal group [32].

The chemical properties and purity of pectin from CP and SP may partially explain their closely similar effects on food intake and adiposity parameters. According to Castillo-Israel *et. al.* [15], pectin from banana peels is comparable to commercial citrus pectin in terms of gelling ability, sensory qualities, moisture content, and degree of esterification. However, the more pronounced effect of CP in ameliorating some of the adiposity parameters could be attributed to the significantly higher methoxyl content (9.09%) as compared to SP (5.25%) [15]. The higher methoxyl content indicates greater solubility in water [35], which may increase the gelation capacity of the pectin, thus promoting gastric distention once ingested in the body. The relatively low purity of pectin extracted from Saba peels, as indicated by its lower % Anhydrogalacturonic Acid (AUA) (39.68% vs. 74.26% in commercial pectin) and higher ash content exceeding the 10% maximum limit for good gel formation [37], may have diminished its effectiveness in regulating feed intake, body weight, and adiposity biomarkers [15]. These impurities suggest that residual substances in SP may interfere with efficient gelation, thereby potentially diminishing its effectiveness in regulating feed intake, body weight, and adiposity biomarkers [15], which are key mechanisms in anti-obesity activity attributed to pectin's gelling properties.

Taken collectively, it can be said that the pectin from saba peels, when supplemented in high-fat diets, is effective in lowering body weight

and adiposity to a level closely similar to its commercially available counterpart, citrus pectin. Pectin is considered viscous and capable of holding water and forming gels, resulting in higher viscosity, gastric distention, and inhibition of gastric emptying, thereby altering food digestion and fat absorption [29,38]. All these mechanisms could be responsible for the observed anti-obesity effects of SP supplementation.

5. Conclusion

In general, when compared to the HFD group, SP supplementation at 10% (w/w) of diet resulted in 14.61% higher water intake, 13.39% lower body weight, 3.66% lower abdominal circumference, 76.72% lower TAI and 9.22% higher fecal weight. The study demonstrated that pectin supplementation at 10% SP fraction of the total diet is beneficial in the prevention of weight gain and reduction of body fats in HFD-induced obese male ICR mice. The human dose equivalent of 10% (w/w) saba banana peel pectin is 4.87g per day for a typical adult weighing 60 kg.. Given these findings, saba banana peels, a considered waste material, are a cheap source of pectin that has the potential to regulate feed intake and adiposity parameters. These interesting findings merit further investigations through clinical studies to fully establish the anti-obesity potential of saba banana peel pectin supplementation.

Availability of Data and Materials

All data are available in this study.

Author Contributions

Conceptualization - L.M.A., M.A.C.E., and K.A.C.I.; Methodology - L.M.A., E.M.F.O., P.A.A.B., C.J.M., M.A.C.E., K.A.C.I., R.P.G., J.R.C., and P.J.V.G.; Validation - L.M.A., M.A.C.E., and K.A.C.I.; Formal analysis - L.M.A., M.A.C.E., and K.A.C.I.; Investigation - E.M.F.O., P.A.A.B., C.J.M., M.A.C.E., K.A.C.I., L.M.A., R.B.N., R.P.G., J.R.C., and P.J.V.G.; Resources - E.M.F.O., P.A.A.B., C.J.M., M.A.C.E., and K.A.C.I.; Data curation - L.M.A., M.A.C.E., and K.A.C.I.; Writing - Original Draft - L.M.A., E.M.F.O., and A.D.F.; Writing - Review and Editing - L.M.A., E.M.F.O., and P.A.A.B.; Visualization-L.M.A., M.A.C.E., and K.A.C.I.; Supervision - L.M.A., M.A.C.E., and K.A.C.I.;

Project administration - L.M.A., M.A.C.E., and K.A.C.I.

Ethics Approval and Consent to Participate

All animal experiments conducted were authorized by the University of the Philippines Los Baños Animal Care and Use Committee (UPLB IACUC) under approval number CHE-2019-001.

Acknowledgment

The authors would like to extend their heartfelt thanks to the National Research Center of the Philippines of the Department of Science and Technology and the Commission on Higher Education for funding this research.

Funding

This research was funded by the National Research Council of the Philippines (NRCP) under the Department of Science and Technology (DOST). Other resources were supported by the dissertation grant from CHED K to 12 Scholarship.

Conflict of Interest

The authors declare no conflict of interest.

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