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Impact of dietary ingredients on the interpretation of various fecal parameters in rats fed inulin

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ABSTRACT

In the present study, the influences of diets (i.e. chow and AIN-93 diets) on the interpretation of various fecal parameters including viable microbiota, moisture, weight, and short-chain fatty acids in rats fed different amounts of inulin (0.5—2 g/kg). Eight groups of rats ($n = 8$/group) were fed, for 4 weeks, chow or AIN-93 diets with or without inulin supplementation. Fecal samples were analyzed for different fecal parameters. After a 2-week adaptation, apparent differences in some fecal parameters were observed between the chow and AIN-93 diet groups. Throughout the 4-week intervention period, significantly ($p < 0.05$) higher *Lactobacillus* spp. counts, fecal moisture (~2.7-fold), and fecal weight (~5.8-fold) were observed with chow diet over AIN-93 diet. More specifically, significant elevations in the levels of *Bifidobacterium* spp., *Lactobacillus* spp., fecal moisture, and fecal weight could be observed at low-dose (0.5 g/kg) of inulin in chow diet groups, while most of these changes could merely be seen at medium-dose (1 g/kg) in AIN-93 diet groups. These results demonstrated that the choice of experimental diets would affect the comparison of fecal parameters as well as the interpretation of effective dosage of prebiotic in intestinal health assessments.

1. Introduction

Rodent animal models are usually employed to assess the health of digestive tract [1]. Various physicochemical characteristics of feces including moisture, microbiota concentration, and short chain fatty acids (SCFAs) have been previously measured to determine the intestinal health [2].

Inulin is a popular prebiotic ideal for fermentation by a wide range of saccharolytic resident microbiota in the colon. The preferential fermentation of inulin by different beneficial gut flora, namely *bifidobacteria* and *lactobacilli*, has been extensively studied. A sufficient intake of inulin as prebiotic could help improve stool quality (e.g. microflora, pH, SCFAs, and frequency), lower the risk of intestinal infection, and

maintain intestinal health [3,4]. The beneficial physiological functions of this inulin-type prebiotic also included management of diabetes mellitus and obesity as well as improvement of serum lipids concentrations and mineral absorption [5].

Most efficacy evaluations on intestinal health have demonstrated that the usual effective concentration of inulin ranged from 5 to 10 g per 100 g of diet in rat assays [6], which were approximately equivalent to 10–20 g per day for 60 kg adults. Various criteria for judging the effective concentration of inulin have been involved in different studies. These criteria included various representable parameters such as gut microflora, fecal pH, and fecal SCFAs. Li et al. [7] have reported that dietary composition played a role in altering the equilibrium of gut *bifidobacteria*. There is still a gap about the influences of dietary composition on the interpretation of relationship between these parameters and inulin concentration.

Chow diet and the American Institute of Nutrition (AIN)-93 diet are common experimental diets in various animal studies. Chow is a fiber-rich diet containing complex carbohydrates and proteins with a variety of ingredients such as grain, alfalfa meal, beet pulp, soybean meal, and other crude materials. On the contrary, the ingredients of AIN-93 diet are relatively more refined and purified. Each ingredient (e.g. starch, vitamins, and minerals) in AIN-93 diet provides one main nutrient only [8]. The choice of animal diets and their corresponding ingredients might influence the fecal parameters and effective dose determination in intestinal health assessments.

The present study aimed to investigate the impact of dietary choices on the assessment of fecal parameters including viable fecal microbiota and various fecal characteristics in rats fed inulin. The changes in viable fecal microbiota including *Bifidobacterium* spp., *Lactobacillus* spp., *Escherichia coli*, and *Clostridium perfringens* were compared. Other fecal characteristics such as moisture, weight, pH, and SCFA levels were also determined. A comparison of different fecal parameters and effective dosage of inulin between chow and AIN-93 diet groups would be discussed.

2. Materials and methods

2.1. Sample

Inulin (cat. no. AF-01), a soluble dietary fiber (degree of polymerization > 23) from chicory root, was obtained from Exclusive Mark (M) Sdn. Bhd., Malaysia.

2.2. Experimental design and animal model

The study protocol was approved by the Animal Care and Use Committee of National Chung Hsing University and the laboratory animals were cared in accord with the institutional ethical guideline. Sixty four 7-week-old male Sprague Dawley (SD) rats, which were fed chow diet initially were purchased from BioLASCO Company, Taiwan. The animals were caged individually in a stainless steel cage and placed in an animal

room at a temperature of $22 \pm 1\,°C$, $60 \pm 5\%$ relative humidity, and 12-h light/dark cycle.

In this experiment, the SD rats were first weighted and divided into eight weight classes of eight each prior to the adaptation period. The rats in each weight class were randomly assigned to the eight groups including two control groups and six sample groups. One control group was fed chow diet only while the other control group was just given AIN-93 diet. Three sample groups were fed chow diet (PMI Nutrition International, St. Louis, MO, USA) and given inulin (0.5, 1, and 2 g/kg, respectively) by oral gavage, while the other three sample groups were administrated AIN-93 rodent diet and given inulin (0.5, 1, and 2 g/kg, respectively) as well. Table 2S shows the composition of chow and AIN-93 diets. After an adaptation for two weeks without an intervention of inulin, the body weights of rats ranged from 217.1 to 332.9 g. There were no significant differences in body weights among the eight groups.

The animal dosage (mg/kg) was calculated from the multiplication of the human dosage (mg/kg for a normal 60 kg adult) by a factor of 6.2 (Food and Drug Administration, 2005). For examples, the daily consumption of inulin at low-, medium-, and high-dose in rats were approximately equivalent to 5, 10, and 20 g/kg, respectively, for a 60 kg adult. Throughout the 4-week experiment after adaptation, water and feed were provided *ad libitum*. Food intakes and body weights were recorded daily. Fecal samples were collected, weighed, and analyzed for routine measurements.

2.3. Determination of fecal pH and moisture

Fecal samples without urine and feed contamination were collected. Using the method as described by Chau et al. [9], fecal pH values were measured by homogenizing fecal samples with deionized water at a 1:4 (w/v) ratio, followed by a centrifugation at 1,006*g* for 10 min. As for the determination of fecal moisture content, the samples were dried in a 105 °C air-oven until reaching constant weight [10].

2.4. Determination of fecal SCFAs

According to the method described by Saw et al. [11] with slight modifications, fresh fecal samples were homogenized with cold saline (0.9% w/v) at a ratio of 1:10 (w/v), followed by a centrifugation at 1,006*g* for 10 min. Two milliliters of the supernatant was mixed with 10 μL of isocaporic acid (internal standard) and 20 μL of 50% (w/v) sulfuric acid. The SCFAs were then extracted using diethyl ether. Chromatographic analysis was performed using Agilent Technologies 7890A system equipped with flame ionization detector (FID). The ether layer (1 μL) was analyzed by a column (Agilent J and WHP-INNOWax GC Column, 30 m, 0.25 mm, 0.25 μm). Helium was supplied as the carrier gas at a flow rate of 7 mL/min. The conditions were as follows: the initial oven temperature at 80 °C was maintained for 1 min and raised to 140 °C at a rate of 20 °C/min, then held for another 1 min and raised again to 220 °C at a rate of 20 °C/min, and lastly held at 220 °C for 2 min; the temperatures of the

detector and the injector were 250 °C and 140 °C, respectively.

2.5. Determination of fecal bacterial counts

Fecal samples were collected immediately after defecation into an aseptic tube. Fresh fecal samples were analyzed by conventional microbiological methods within 20 min after collection. A series of ten-fold dilutions of the homogenized samples were made using sterile and anaerobic dilution solution. The enumeration of *Bifidobacterium* spp., *Lactobacillus* spp., *E. coli* and *C. perfringens* in the solutions were spread plated onto different selective and differential mediums, which were *Bifidobacteria* iodoacetate medium 25 (BIM-25), Rogosa agar, Levine eosin methylene blue (LEMB) agar, and tryptose-sulfite-cycloserine (TSC) agar medium (Merck KGaA, Darmstadt, Germany), respectively. *Lactobacillus* spp. and *E. coli* was aerobically cultivated at 37 °C for 72 h and 48 h, respectively, while *Bifidobacterium* spp. and *C. perfringens* were anaerobically cultivated at 37 °C for 48 h and 24 h, respectively [12–15].

2.6. Statistical analysis

All results expressed in mean ± standard deviation (SD) were analyzed by *t*-test or one-way ANOVA using the Statistical Analysis System (version 20.0; SPSS, Armonk, NY, USA). Values of $p < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Food, water, and nutrients intakes

All rats were healthy throughout the feeding experiment. The quantities of food, water, and nutrients intakes were measured for the comparison of dietary habits of experimental animals. Prior to the feeding period, there were no significant differences in average body weights (262.5–274.8 g) among the eight diet groups. At the end of the 4-week intervention of inulin, the final average body weights of the animals fed chow diet (419.3.1–431.3 g g) were comparable to those fed AIN-93 diet (432.0–439.2 g) (Table 3S). The weight gain between chow diet groups (5.6–6.0 g/day) and AIN-93 diet groups (5.8–6.2 g/day) showed no significant differences. Besides, inulin consumption at different doses made no apparent contribution to the body weight gain in animals with these two types of diets.

As shown in Table 1, the average food intakes of chow and AIN-93 diet groups were 24.9–25.6 and 25.8–26.6 g/day, respectively. As for water intakes, rats taking chow and AIN-93 diets were found to consume comparable amount of water (42.7–43.9 and 42.1–44.5 ml/day, respectively). There were no significant differences in food and water intakes among the eight diet groups after the experiment. Likewise, no significant differences in daily calorie and fat intakes among these diet groups were observed.

The average daily protein intakes among the chow diet groups (5.9–6.1 g) were 63% higher ($p < 0.05$) than those with the AIN-93 diets (3.6–3.7 g). On the contrary, the average daily carbohydrate intakes among the animals fed chow diets (14.7–15.1 g) were significantly ($p < 0.05$) lower (−27%) than those fed AIN-93 diets (18.6–19.1 g) (Table 1). These results were in an agreement with the other findings in which chow-fed animals also administrated higher quantity of protein and lesser carbohydrate as compared with AIN-93M diet [16]. It was probably attributed to the differences in the protein and carbohydrate composition between chow and AIN-93 diets (Table S2).

3.2. Viable fecal bacterial counts

Fecal *Bifidobacterium* spp., *Lactobacillus* spp., *E. coli* and *C. perfringens* are commonly used as biomarkers for intestinal evaluation. During the adaptation period of this experiment, these parameters have been first determined to make sure that the viable counts of these fecal bacteria attained a steady state while the rats were 8–9 weeks old, especially in *Lactobacillus* spp. (Table 1S). After two-week of adaptation, the feeding experiments were hence started until the rats reach nine weeks of age. At this point, the fecal *Lactobacillus* spp. (7.83–8.04 log CFU/g) in chow diet groups were initially higher ($p < 0.05$) than those in AIN-93 diet groups (5.35–5.45 log CFU/g). It was probably attributed to the different compositions between these two diets (Tables 2S and 4S).

Some previous studies have demonstrated that different dietary ingredients might affect the composition of gut microbiome in rats, thus altering the microflora in feces [17,18]. As shown in Table 2, after the feeding experiment, the fecal *Lactobacillus* spp. and *E. coli* counts (8.12–8.99 and 5.56–6.49 log CFU/g, respectively) in the four chow-fed groups were markedly ($p < 0.05$) higher than those fed AIN-93 diets (5.80–7.30 and 5.21–5.51 log CFU/g, respectively), while the counts of fecal *Bifidobacterium* spp. and *C. perfringens* showed no significant differences between these two diets.

As shown in Table 2, the oral administration of inulin at low-to high-dose resulted in an elevation in both fecal *Lactobacillus* spp. and *Bifidobacterium* spp. counts to different extents. As compared to the controls, significantly ($p < 0.05$) higher numbers of fecal *Lactobacillus* spp. in chow diet groups were observed with the administration of low-to high-dose of inulin (8.57–8.99 log CFU/g), whereas significant ($p < 0.05$) changes in rats fed AIN-93 diets were noted at medium-to high-dose of inulin (7.16–7.30 log CFU/g). A similar trend in fecal *Bifidobacterium* spp. was also observed in both the chow and AIN-93 diet groups as the doses of inulin increased. More specifically, statistically obvious changes at low-dose of inulin could be seen in chow diet groups rather than AIN-93 groups. Simply put, these results demonstrated that the feeding of chow diet was more effective in supporting the growth of these bacteria, especially *Lactobacillus* spp., with an intervention of inulin. Despite the daily carbohydrate consumptions with chow diets were lower ($p < 0.05$) than those with AIN-93 diets, the above phenomenon might be attributed to the higher fermentability of fibers available in chow diet than in AIN-93 diet [19].

Table 2 shows that the administration of inulin did not affect the *E. coli* counts in fecal samples of rats fed AIN-93 diets. In contrast, an apparent ($p < 0.05$) reduction in fecal *E.*

Table 1 — Comparison of weight gain, food intake, calorie intake, and dietary intake of various nutrients in rats fed chow and AIN-93 diets after feeding different doses of inulin for 4 weeks.

Doses of inulin	Weight gain (g/day)[1,2]	Food intake (g/day)[1,2]	Calorie intake (kcal/day)[1,2]	Protein intake (g/day)[1]	Fat intake (g/day)[1,2]	Carbohydrate intake (g/day)[1]	Water intake (mL/day)[1,2]
Chow diet							
Control	5.7 ± 0.5	24.9 ± 2.0	94.6 ± 7.7	5.9 ± 0.5[a]	1.2 ± 0.1	14.7 ± 1.2[a]	43.3 ± 2.7
Low	5.6 ± 0.9	25.2 ± 2.0	95.8 ± 7.5	6.0 ± 0.5[a]	1.3 ± 0.1	14.9 ± 1.2[a]	43.9 ± 3.0
Medium	6.0 ± 0.8	24.9 ± 2.1	98.0 ± 10.4	5.9 ± 0.5[a]	1.2 ± 0.1	14.7 ± 1.2[a]	43.3 ± 2.8
High	5.6 ± 1.1	25.6 ± 2.4	97.2 ± 9.2	6.1 ± 0.6[a]	1.3 ± 0.1	15.1 ± 1.4[a]	42.7 ± 3.2
AIN-93 diet							
Control	5.9 ± 0.5	26.3 ± 0.8	99.9 ± 3.2	3.7 ± 0.1[b]	1.1 ± 0.0	19.0 ± 0.6[b]	43.5 ± 2.5
Low	5.8 ± 0.9	25.8 ± 1.0	98.1 ± 3.8	3.6 ± 0.1[b]	1.0 ± 0.0	18.6 ± 0.7[b]	42.6 ± 2.6
Medium	6.2 ± 0.8	26.1 ± 0.7	99.2 ± 2.7	3.7 ± 0.1[b]	1.0 ± 0.0	18.8 ± 0.5[b]	44.5 ± 2.4
High	5.8 ± 1.1	26.6 ± 0.6	100.9 ± 2.2	3.7 ± 0.1[b]	1.1 ± 0.0	19.1 ± 0.4[b]	42.1 ± 2.8

[1] Values (means ± SD, n = 8).
[2] No significant differences in weight gain, food intake, calorie intake, fat intake, and water intake between the chow and AIN-93 diet groups were observed.
[a–b] Protein and carbohydrate intakes in the same column with different superscripts are significantly different ($p < 0.05$).

coli counts from 6.49 log CFU/g down to 5.56 log CFU/g was noted at high-dose of inulin within the four chow diet groups. It was speculated that this significant reduction might be a

Table 2 — Comparison of fecal bacterial counts in rats fed chow and AIN-93 diets after feeding different doses of inulin for 4 weeks.

Doses of inulin	Viable counts (log CFU/g)[1]	
	Chow diet	AIN-93 diet
Lactobacillus spp.		
Control	8.12 ± 0.35[a,e]	5.80 ± 0.52[b,d]
Low	8.57 ± 0.34[a,d]	5.84 ± 0.25[b,d]
Medium	8.93 ± 0.21[a,c]	7.16 ± 0.23[b,c]
High	8.99 ± 0.27[a,c]	7.30 ± 0.29[b,c]
Bifidobacterium spp.[2]		
Control	6.35 ± 0.38[g]	6.36 ± 0.51[g]
Low	7.11 ± 0.71[f]	6.47 ± 0.64[g]
Medium	7.24 ± 0.46[f]	7.15 ± 0.29[f]
High	7.43 ± 0.48[f]	7.37 ± 0.26[f]
Escherichia coli		
Control	6.49 ± 0.39[a,h]	5.51 ± 0.37[b,h]
Low	6.26 ± 0.48[a,h]	5.50 ± 0.46[b,h]
Medium	5.95 ± 0.48[a, hi]	5.27 ± 0.41[b,h]
High	5.56 ± 0.43[a,i]	5.21 ± 0.25[b,h]
Clostridium perfringens[2, 3]		
Control	5.36 ± 0.44	5.66 ± 0.31
Low	5.28 ± 0.66	5.57 ± 0.37
Medium	5.15 ± 0.43	5.64 ± 0.29
High	5.22 ± 0.53	5.53 ± 0.36

[1] Values (means ± SD, n = 8).
[2] No significant differences in fecal *Bifidobacterium* spp. and *Clostridium perfringens* counts between the chow and AIN-93 diet groups were observed.
[3] Fecal *Clostridium perfringens* counts in the same column are not significantly different ($p < 0.05$).
[a–b] Fecal bacterial counts in the same row with different superscripts are significantly different ($p < 0.05$). [c–e] Fecal *Lactobacillus* spp. counts in the same column with different superscripts are significantly different ($p < 0.05$). [f–g] Fecal *Bifidobacterium* spp. counts in the same column with different superscripts are significantly different ($p < 0.05$). [h–i] Fecal *Escherichia coli* counts in the same column with different superscripts are significantly different ($p < 0.05$).

result of correspondingly higher fecal *Lactobacillus* spp. and *Bifidobacterium* spp. counts, which had an antagonistic effect on the growth of gastrointestinal pathogen like *E. coli* [20]. Throughout the experiment, no change was observed in the *C. perfringens* counts among all eight diet groups. Other authors have also reported that the *C. perfringens* counts in cecal contents remained unchanged after administrating xylooligosaccharides and fructooligosaccharides [21].

3.3. Assessments on fecal moisture, weight, pH, and SCFAs

At the beginning of feeding experiment, the fecal moisture contents in rats fed chow and AIN-93 diets were 60.4–62.4 and 22.7–23.4 g/100 g feces, respectively (Table 5S). Table 3 indicates that no further changes in fecal moisture in both control groups were observed after 4 weeks of feeding. A markedly ($p < 0.05$) higher fecal moisture content (approximately 2.7-fold) was achieved by feeding chow diet as compared with AIN-93 diet. The fecal moisture in rats fed AIN-93 diet was found to be about 17.8 ± 4.5 g/100 g in another study [22]. As shown in Table 3, the feeding of inulin at low-to-high-dose (0.5–2 g/kg) among the chow diet groups would lead to 5–9% increase ($p < 0.05$) in fecal moisture against their control. However, apparent ($p < 0.05$) increases in fecal moisture by 7–10% were merely seen in rats treated with medium-to high-dose of inulin among the AIN-93 diet groups.

It was speculated that the 2.7-fold higher moisture in feces with chow diet was partly attributed to the different fermentability of dietary fibers (e.g. cellulose, fermentable fiber) between chow and AIN-93 diets. As reported by Lu et al. [23], moisture content of feces differed significantly across various fiber-supplemented groups in the following descending order: arabinoxylan fiber > guar gum > wheat bran > cellulose. Chow diet contained a variety of dietary fiber sources (Table 2S), whereas AIN-93 diet was typically formulated to have only one type of fiber (i.e. highly refined cellulose, > 97% purity).

The fecal weights among the eight diet groups were presented in Table 3. After the 4-week feeding period, it was intriguing that a remarkably ($p < 0.05$) higher level of fecal weight (roughly 5.8-fold) was accomplished by feeding chow

Table 3 – Comparison of fecal moisture and fresh fecal weight in rats fed chow and AIN-93 diets after feeding different doses of inulin for 4 weeks.

Doses of inulin	Chow diet	AIN-93 diet
Fecal moisture (g/100 g feces)		
Control	$60.5 \pm 2.9^{a,d}$	$22.5 \pm 1.1^{b,d}$
Low	$63.4 \pm 3.6^{a,c}$	$22.6 \pm 1.2^{b,d}$
Medium	$63.8 \pm 3.8^{a,c}$	$24.0 \pm 1.4^{b,c}$
High	$65.7 \pm 3.9^{a,c}$	$24.7 \pm 1.5^{b,c}$
Fresh fecal weight (g/day)		
Control	$14.4 \pm 3.4^{a,f}$	$2.5 \pm 0.7^{b,f}$
Low	$16.9 \pm 4.2^{a,e}$	$2.9 \pm 0.4^{b,f}$
Medium	$17.1 \pm 4.5^{a,e}$	$3.1 \pm 0.6^{b,e}$
High	$18.8 \pm 4.3^{a,e}$	$3.5 \pm 0.6^{b,e}$

[a–b] Values (means $\pm$ SD, n = 8) in the same row with different superscripts are significantly different ($p < 0.05$).
[c–d] Fecal moisture contents in the same column with different superscripts are significantly different ($p < 0.05$).
[e–f] Fresh fecal weights in the same column with different superscripts are significantly different ($p < 0.05$).

Table 4 – Comparison of fecal pH[1,2] and fecal short chain fatty acids[1,2] in rats fed chow and AIN-93 diets after feeding different doses of inulin for 4 weeks.

Doses of inulin	Chow diet	AIN-93 diet
Fecal pH value		
Control	6.28 ± 0.12^{a}	6.26 ± 0.10^{a}
Low	6.25 ± 0.08^{a}	6.26 ± 0.07^{a}
Medium	6.11 ± 0.14^{b}	6.12 ± 0.12^{b}
High	5.96 ± 0.11^{c}	5.89 ± 0.12^{c}
Fecal total SCFAs[3] (μmol/g)		
Control	162.3 ± 14.3^{e}	171.7 ± 24.2^{e}
Low	172.9 ± 22.2^{e}	162.7 ± 20.4^{e}
Medium	197.5 ± 22.5^{de}	182.6 ± 23.6^{de}
High	211.0 ± 22.3^{d}	202.1 ± 30.5^{d}
Fecal acetic acid (μmol/g)		
Control	95.0 ± 12.8^{h}	98.8 ± 17.1^{h}
Low	100.2 ± 16.9^{gh}	98.4 ± 15.6^{gh}
Medium	112.9 ± 17.6^{fg}	109.6 ± 10.7^{fg}
High	117.5 ± 15.9^{f}	122.3 ± 15.7^{f}
Fecal propionic acid (μmol/g)		
Control	38.6 ± 5.0^{j}	38.0 ± 14.1^{j}
Low	42.2 ± 5.9^{j}	34.3 ± 8.6^{j}
Medium	49.3 ± 9.4^{i}	37.7 ± 5.7^{i}
High	51.2 ± 7.2^{i}	48.8 ± 8.7^{i}
Fecal butyric acid (μmol/g)		
Control	28.6 ± 3.6^{m}	26.0 ± 7.1^{m}
Low	30.5 ± 5.0^{m}	27.9 ± 6.8^{m}
Medium	35.3 ± 4.5^{l}	35.4 ± 6.4^{l}
High	42.3 ± 6.5^{k}	35.6 ± 8.4^{k}

[1] Values (means $\pm$ SD, n = 8).
[2] No significant differences in fecal pH and short chain fatty acids between the chow and AIN-93 diet groups were observed.
[3] Total SCFAs = acetic acid + propionic acid + butyric acid.
[a–c] Fecal pH values in the same column with different superscripts are significantly different ($p < 0.05$). [d–e] Fecal total SCFAs in the same column with different superscripts are significantly different ($p < 0.05$). [f–h] Fecal acetic acids in the same column with different superscripts are significantly different ($p < 0.05$). [i–j] Fecal propionic acids in the same column with different superscripts are significantly different ($p < 0.05$). [k–m] Fecal butyric acids in the same column with different superscripts are significantly different ($p < 0.05$).

diet (14.4 g/day) between the control groups. Kao and associates likewise have shown that the administration of chow diet would result in fecal weight approximately 13.7 g/day in rodents [24]. The results obtained in AIN-93 control group (2.5 g/day) were comparable with the values (1.29–3.19 g/day) as reported in other studies [22,25]. A higher fecal output with chow diet was probably associated with the sources of nutrients in chow diet which supported a moderate microbial growth in gut [26].

As shown in Table 3, significant increases in fecal weight were observed at all three doses (low to high) in chow diet groups (by 17–31%), but only medium-to high-dose groups with AIN-93 diet could lead to significant increases (by 24–40%). It was inferred that the fermentable ingredients in chow diet seemed more efficient to work with inulin in increasing the fecal weight.

In Table 4, the fecal pH values of the control groups fed chow and AIN-93 diets (6.28 and 6.26, respectively) were comparable to each other after the feeding period. It was noted that the consumption of inulin at medium- and high-dose would lead to significant ($p < 0.05$) decrease in the fecal pH down to a range between 5.89 and 6.12 upon consumption of both the chow and AIN-93 diets.

Table 4 reveals the fecal SCFA profiles among the eight diet groups. Total SCFAs were a sum of various SCFA moieties (i.e. acetate, propionate, and butyrate) produced in the colon. Regarding the control groups, there were no apparent differences in the total SCFA concentrations (162.3–171.7 μmol/g) between the chow and AIN-93 diets. A similar pattern in the average concentrations of acetate, propionate, and butyrate (95.0–98.8, 38.0–38.6, and 26.9–28.6 μmol/g, respectively) was observed between the above two control groups. These SCFAs could play an essential role in maintaining intestinal lining integrity and suppressing the growth of undesirable bacteria [27,28].

SCFA analysis indicated that the feeding of inulin at medium- and high-dose in both chow and AIN-93 diet groups would result in significant ($p < 0.05$) increases in all three SCFA moieties including acetate, propionate, and butyrate

(Table 4). These results demonstrated that the statistically effective doses of inulin among different fecal parameters (Tables 2–4) were varied. The quantity of SCFAs produced was associated with the consumption levels of fermentable carbohydrate (i.e. inulin) as well as a diversity of key SCFA-producing bacterial species in addition to *Lactobacillus* spp. and *Bifidobacterium* spp. [29,30]. Furthermore, it was inferred that the decrease in fecal pH after taking medium-to high-dose of inulin was related to the levels of SCFAs produced by symbiotic intestinal bacteria [31].

In terms of the SCFA profile (Table 4), the levels of acetic acid: propionic acid: butyric acid in fecal samples collected from rats fed the chow and AIN-93 diets were found to have a similar ratio of 10:4:3. This ratio was basically in agreement with previous studies in which the ratio of these three fecal SCFAs in rats fed chow was 60:20:20 [32], while that with AIN-93 diet was 68:22:10 [33]. Our results demonstrated that the ratio of SCFAs would basically remain unaltered even after feeding inulin at different dosages.

Based on the above findings in this study, the types of experimental diets (i.e. chow and AIN-93 diets) might affect the judgement of the effectiveness of inulin in altering different fecal parameters (including viable bacterial counts, moisture, and weight). It was interesting to note that more significant changes in these fecal parameters could be observed at low-dose of inulin in rats fed chow diet, while most of significant changes could merely be seen at medium-dose of inulin in respect of AIN-93 diet. This implied that different conclusion would be drawn solely from the results of low-dose of inulin between these two diets.

It was certainly difficult to reach a harmonized consensus on the effective inulin dose from a variety of significant results in different parameter assays or even different studies. It also posed a question of which parameters should be considered or otherwise which parameter should be rated higher for the same effective dose of inulin. In the present rat study, low-dose of inulin (0.5 g/kg) in chow diet model or the other way medium-dose of inulin (1 g/kg) in AIN-93 diet model would be suggested. To extrapolate these doses to human equivalent doses, it meant that a different amount of inulin (5 or 10 g, respectively) would be suggested to achieve comparable efficacy. Diet choices and their corresponding ingredients might possibly influence the comparison of different fecal parameters as well as the interpretation of effective dosage of a particular prebiotic in intestinal health assessments. It was further postulated that the composition of foods that people consumed might in some way affect the effective dosage of prebiotic (e.g. inulin).

4.   Conclusion

The present study revealed that the uses of chow and AIN-93 diets would result in remarkably ($p < 0.05$) differences in various fecal parameters, specifically a higher *Lactobacillus* spp. counts, fecal moisture, and fecal weights in rats after the 2-week adaptation. A significantly ($p < 0.05$) higher levels of fecal moisture (~2.7-fold) and fecal weight (~5.8-fold) could be obtained with chow diet over AIN-93 diet at the beginning and end of the feeding experiment. After a 4-week intervention, relatively more significant changes in these fecal parameters (e.g. *Bifidobacterium* spp., *Lactobacillus* spp., fecal moisture, and fecal weights) could be observed at low-dose (0.5 g/kg) of inulin in rats fed chow diet, while most of significant changes could merely be seen at medium-dose (1 g/kg) of inulin in respect of AIN-93 diet. The choices of experimental diets might influence the interpretation of effective dosage of inulin. There is still much comprehension gaps to be filled in understanding the potential impact of dietary composition on the efficacy assessment of prebiotic ingredients.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfda.2019.06.005.

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