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Effect of Novel High-fat Diet Feeding Methods on Food Wastage, Weight Gain, Hair Coat Grease Accumulation, and Scratching Behavior in C57BL/6NCrl Mice

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Soft-pelleted, high-fat diets (HFD) are greasy and crumble easily leading to food wastage and hair coat grease accumulation when mice are fed using commercially available feeders. The ideal HFD feeder design should reduce food wastage, facilitate mouse weight gain, and minimize variables such as hair coat grease accumulation that have the potential to alter scratching behaviors. Our study compared the feeding efficiency of 2 commercially available feeders (feeders A and E) to 4 novel feeder designs (feeders B, C, D, and F). Novel feeders had alterations in feeding aperture size, feeding surface area, feeder configuration, and level of food presentation. Male C57BL/6NCrl mice ($n = 120$; 4/cage) were randomly assigned to cages containing one of the 6 feeder types and were fed HFD for 12 wk. Feeders and cage bottoms were weighed before use and then weekly at the time of cage change. Mice were weighed before starting the HFD and then biweekly. Scratching behavior was video recorded at 0, 4, 8, and 12 wk. Hair coat grease accumulation was visually scored biweekly. Feeder A use was associated with the highest feed cost due to HFD wastage ($36.98 ± 1.54/cage/wk). Mice fed using Feeder A had the highest average weight gain (23.75 ± 0.8 g, $P < 0.005$). However, mice also had significantly higher hair coat grease accumulation scores ($P < 0.05$) and significantly increased scratching frequency at 4 wk ($P < 0.05$) when compared with mice fed using other feeder types. Novel feeder designs utilized 10 to 21 times less HFD dispensed when compared to feeder A. Mice fed using novel feeders also displayed improved welfare, as evidenced by low hair coat grease accumulation scores, and no significant differences in scratching frequency when compared with baseline behavior.

Abbreviations and Acronyms: DIO, diet-induced obesity; HFD, high-fat diet; UD, ulcerative dermatitis

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Introduction

Obesity has led to a major strain on healthcare systems worldwide and a need for research into prevention and treatment.[13,24] Obesity in modern society often results from decreased energy expenditure (lack of exercise) and increased energy intake (high-fat diets [HFDs]).[10,21] To better understand societal obesity, researchers have attempted to match Western eating habits by using wild-type rodents that become obese from experimental manipulation of their diet or what is commonly referred to as a diet-induced obesity (DIO) model.[9,14,20,22] DIO often utilizes a formulated soft-pelleted, rodent HFD to create standard, reproducible models of obesity.[14,23] A frequently employed model of DIO uses 4- to 6-week-old male C57BL/6 mice because of their high susceptibility to body fat accumulation, fast rate of weight gain, and reproducible disruptions in glucose metabolism.[5,9] While there is no universally recognized marker to identify obesity in DIO models, researchers often rely on parameters such as a 15% weight gain or a 20-g difference in body weight when compared with age-matched control animals.[5]

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At our institution, we have found that many of the commonly used soft-pelleted HFDs are greasy and crumble easily. Due to poor pellet consistency, HFD often falls through the wire bar lid feeders and mixes with the bedding substrate. As a result, wire bar lid feeders containing HFDs may need to be replenished 2 to 3 times more often than feeders containing standard, hard-pelleted rodent diets. Feeding the HFD (Modified Western Diet 100244 with 3 g/kg cholesterol; Dyets, Bethlehem, PA) used in this study cost $27.00/kg. In contrast, the cost of feeding a standard rodent diet (PicoLab Rodent Diet 5053; LabDiet, Richmond, IN) was approximately $3.00/kg. The frequency of HFD replenishment further exacerbated the cost of the experimental diet.

Mice that come in contact with soft-pelleted HFD can develop visible hair coat grease accumulation. Grease accumulation can significantly disrupt the ability of mice to perform normal grooming behaviors resulting in grossly visible hair matting. In addition, the use of HFD has been associated with an increased incidence of ulcerative dermatitis (UD) in C57BL/6 mice.[17] It is not clear whether the development of UD is a secondary side effect of HFD consumption or if UD development is associated with direct HFD contact. Hair coat grease accumulation may be irritating to the mice and has the potential to influence the frequency and intensity of scratching behaviors. An increase in scratching frequency has been linked to the progression of UD in C57BL/6 mice.[6,16]

In summary, the use of commercially available feeders for feeding HFD results in a 2-fold problem: diet wastage resulting in a significant, and potentially avoidable, cost and mouse hair coat grease accumulation with the potential to alter mouse grooming behavior. In an attempt to solve these problems at our institution, we collaborated with vendors (Process Control Solutions, St. Louis, MO and Tecniplast, West Chester, PA) to create novel feeder designs aimed at preventing food wastage and limiting direct contact with HFD. We hypothesized that if the feeder was modified in configuration and dimension to better contain soft-pelleted HFDs, then: 1) feeders would require less frequent refilling, 2) mice would have less direct contact with the HFD resulting in decreased hair coat grease accumulation, and 3) mice would exhibit less frequent scratching behaviors than mice fed HFD using a commercially available feeder. Our facilities predominantly house mice using Tecniplast GM500 and Lab Products Super Mouse 750™ shoebox cages. We therefore opted to use the commercially available feeders associated with these 2 cage types. Novel feeder designs were created to be compatible with the available caging.

Materials and Methods

Animals. All experimental procedures were approved by the New York University School of Medicine Institutional Animal Care and Use Committee. Animals were housed in AAALAC-accredited barrier facilities in accordance with the *Guide for the Care and Use of Laboratory Animals*.[8] Male C57BL/6NCrl mice ($n = 120$; age 6 wk; 19 to 24 g) were purchased from Charles River Laboratories (Wilmington, MA). Upon receipt, mice were randomly assigned to one of 2 commercially available ventilated caging systems located in 2 separate barrier housing facilities. Eighty mice were housed in Tecniplast GM500 ventilated cages (Tecniplast, West Chester, PA; 4 mice/cage). Forty mice were housed in Lab Products Super Mouse 750 ventilated cages (Lab Products, Seaford, DE; 4 mice/cage). All cages contained 300 g of corncob bedding (1/80-in. Bed-o'Cobs; Andersons Lab Bedding, Maumee, OH) and a nestlet (Ancare, Belmore, NY). Housing rooms were maintained at 72 ± 2°F (22 ± 1.5°C) with 30% to 70% humidity and a 12:12-h light:dark cycle (lights turn on at 0700 and off at 1900). Filtered and acidified (pH 2.5 to 2.9) water was provided ad libitum per facility standard operating procedures. Cages were changed weekly. During the 7-d acclimation period, mice were provided free access to PicoLab Rodent Diet 5053 (LabDiet, Richmond, IN) using the standard, commercially available feeders.

Based on vendor health reports and colony health surveillance, mice were free from mouse parvovirus, minute virus of mice, mouse hepatitis virus, mouse norovirus, Theiler murine encephalomyelitis virus, epizootic diarrhea of mice, Sendai virus, pneumonia virus of mice, reovirus, lymphocytic choriomeningitis virus, mouse adenovirus, ectromelia, K virus, polyomavirus, mouse cytomegalovirus, hantavirus, mouse thymic virus, lactate dehydrogenase elevating virus, *Encephalitozoon cuniculi*, *CAR Bacillus*, *Mycoplasma pulmonis*, *Clostridium piliforme*, pinworms, and fur mites.

Experimental groups. Following a 7-d acclimation period, established cages were randomly assigned to one of 6 experimental groups (5 cages/experimental group; 4 mice/cage). Each experimental group was fed HFD ad libitum (Modified Western Diet 100244 with 3 g/kg cholesterol; Dyets, Bethlehem, PA) for 12 wk using 6 different feeders. Feeders were distinguished by the size of feeding aperture and feeding surface area. The feeding aperture has been defined in our study as the size of the openings between the vertical and horizontal wire bars. The feeding surface area has been defined as the area of the feeder that contains feeding apertures. Mice acclimated using Tecniplast GM500 ventilated cages were randomly assigned one of 4 feeder designs: (A) a commercially available standard wire bar lid feeder with feeding apertures of 3.76 cm^2 and a feeding surface area of 261.8 cm^2 (Tecniplast, West Chester, PA); (B) a commercially available wire bar lid feeder (Tecniplast, West Chester, PA) with a novel wire bar insert with feeding apertures of 0.56 cm^2 and a feeding surface area of 261.8 cm^2 (Process Control Solutions, St. Louis, MO); (C) a modified wire bar lid feeder with feeding apertures of 0.8 cm^2 and a feeding surface area of 261.8 cm^2 (Tecniplast, West Chester, PA), or (D) a novel floor feeder with feeding apertures of 0.38 cm^2 and a feeding surface area of 68 cm^2 (Process Control Solutions, St. Louis, MO). Mice acclimated using Lab Products Super Mouse 750 ventilated cages were randomly assigned one of 2 designs: (E) a commercially-available standard modular feeder with feeding apertures of 1.4 and 2.1 cm^2 and a feeding surface area of 51.6 cm^2 (Lab Products, Seaford, DE); or (F) a commercially available standard modular feeder with novel insert and feeding apertures of 0.4 cm^2 and a feeding surface area of 51.6 cm^2 (Process Control Solutions, St. Louis, MO) (Figure 1).

HFD dispensed. Feeders containing HFD were weighed before use (day 1) and before weekly cage change (day 7). Food levels were monitored daily. Supplementary HFD was added when feeders contained less than 20 food pellets. Supplementary food weight and frequency were recorded. The amount of HFD dispensed/week was calculated by adding the weight of HFD supplemented throughout the week to the baseline weight (total weight). The final weight of the feeder (day 7) was then subtracted from the total weight to determine the amount of HFD dispensed/week.

HFD wastage. Cage bottoms with bedding were weighed before mouse and feed occupancy (baseline weight) and at weekly cage change after removal of mice and feed (final weight). Cage feeders, cage tops, and water bottles were not included in the baseline or final weights. The amount of HFD wastage/week was calculated by subtracting the baseline weight from the final weight.

Mouse weight. Individual mice were weighed before starting the HFD (baseline weight). Mice were then weighed twice weekly between 1100 and 1500 for 12 wk. Total weight gain was calculated by subtracting baseline weight from the final weight taken at 12 wk. The average weekly rate of weight gain was calculated by subtracting the baseline weight from the final weight and dividing that value by 12. The percent difference in weight gain from week to week was calculated by subtracting the baseline weight from the weight of the subsequent week, dividing the result by the baseline weight, and then multiplying by 100. This process was repeated by calculating the difference between consecutive weeks over the 12-wk experiment. The weekly average percent change in weight gain was then reported as 4 quarterly 3-wk averages.

Hair coat grease scoring. Photographs of the dorsal and lateral view (including head, thorax, and abdomen) of each mouse were taken before starting HFD and then twice weekly. Photographs were grouped in pairs comprising one dorsal and one lateral photo of each individual mouse at each time point and were then randomized across all time points and individuals. Randomization of the dorsal and lateral photographs was performed by a designated member of the research team using simple randomization. Each photo was assigned a unique number and reordered according to a sequence generated by a random number generator. Hair coat grease scoring was

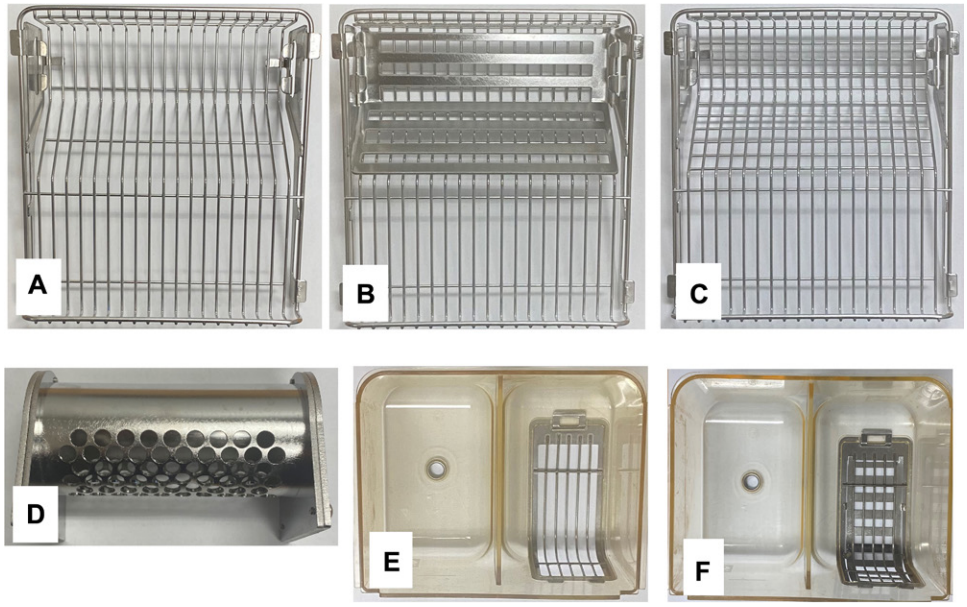

Figure 1. (A) Feeder A is a standard commercially available wire bar lid feeder design with a feeding aperture of 3.76 cm^2 and surface area of 261.8 cm^2. (B) Feeder B is a novel feeder design with a feeding aperture of 0.56 cm^2 and surface area of 261.8 cm^2. (C) Feeder C is a novel feeder design with a feeding aperture of 0.8 cm^2 and surface area of 261.8 cm^2. (D) Feeder D is a novel floor feeder design with a feeding aperture of 0.38 cm^2 and surface area of 68 cm^2. (E) Feeder E is a standard commercially available modular feeder design with feeding apertures of 1.4 and 2.1 cm^2 and surface area of 51.6 cm^2. (F) Feeder F is a novel feeder design with a feeding aperture of 0.42 cm^2 and surface area of 51.6 cm^2.

performed by a single blinded observer. A novel visual hair coat grease scoring system was used. For each set of photos (one dorsal and one lateral photo/mouse/time point), the observer assigned a single hair coat grease score ranging from 0 to 4; (0) smooth hair coat with no visible grease accumulation; (1) grease accumulation visible only on the neck, shoulders and/or face; (2) grease accumulation extending past the shoulders to the torso and abdomen; (3) grease accumulation visible diffusely throughout the hair coat resulting in matting; and (4) significant grease accumulation throughout the hair coat resulting in hair clumping and areas of visible skin (Figure 2).

Behavioral scratching assessment. Behavioral assessments were performed in a separate room located in the same facility where the mice were housed. Assessments occurred 72-h after cage change (0900 to 1200) at baseline (before starting HFD), 4, 8, and 12 wk. Mice were acclimated to the room for 15 min before the behavioral assessment. At the onset of the assessment, 4 mice, cohoused in the same cage, were individually transferred into 4 observation chambers. Observation chambers consisted of Tecniplast GM500 or Lab Products Super Mouse 750 clean cage bottoms consistent with the home cage housing. Each observation chamber contained 300 g of corncob bedding (1/8-in. Bed-o'Cobs; Andersons Lab Bedding, Maumee, OH) and a nestlet (Ancare, Belmore, NY). Behavior was video recorded (Canon, Tokyo, Japan) for 15 min starting 5 min after all 4 mice were placed in their individual observation chambers. Immediately following the assessment mice were promptly cohoused in their original home cage. Video recordings were randomized across all time points and individuals before being reviewed by a single blinded observer. Randomization of the video recordings was performed by a separate designated member of the research team using simple randomization methods. Each video was assigned a unique number and reordered according to a sequence generated by a random number generator. A single scratching bout was defined as use of a hindlimb to scratch any part of the body ending when the hindlimb paw is placed back on the floor.[16] The total number of scratching bouts/15 min were reported for each individual mouse.

Statistical analysis. Results are presented as mean ± SEM. Analysis was performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA). Before statistical analysis, data were assessed for normal distribution and equal variance (Shapiro-Wilks and Bartlett tests, respectively). Comparisons were performed with repeated measures ANOVA or one-way ANOVA followed by a Tukey test for multiple comparisons. Data that did not display normal distribution were analyzed using Kruskal-Wallis or Friedman test followed by a Dunn test for multiple comparisons. Statistical significance was set at a P value of less than 0.05.

Results

Animals. During the study, 7 mice were euthanized prematurely due to health concerns. Of these, two mice developed UD, and five mice presented with fight wounds. All 7 mice were excluded from statistical analysis for all variables. Upon completion of the study, the remaining mice were transferred to our division's training protocol.

HFD dispensed. The standard commercially available wire bar lid feeder (feeder A) resulted in a significantly higher average amount of HFD dispensed weekly (1,369.7 ± 57.1 g, $P < 0.0002$) when compared with all other feeder designs. Feeder A was the only feeder design that required HFD supplementation at a frequency of 2 to 3 times per week as a result of HFD wastage. HFD cost, when using feeder A, was 10 to 21 times higher than when mice were fed using one of the novel feeder designs. The standard commercially available modular feeder (feeder E) did not require HFD supplementation; however, this feeder design resulted in a significantly higher average amount of HFD dispensed weekly (195.1 ± 11.5 g, $P < 0.0001$) compared with feeder types B, D, and F. By contrast, feeder F did not require HFD supplementation resulting in significantly lower average weekly HFD dispensed when compared with all other feeder designs (62.6 ± 1.0 g, $P < 0.005$) (Table 1; Figure 3A).

HFD wastage. When using feeder A, cages required more frequent changing (2 times per week) due to diet accumulation

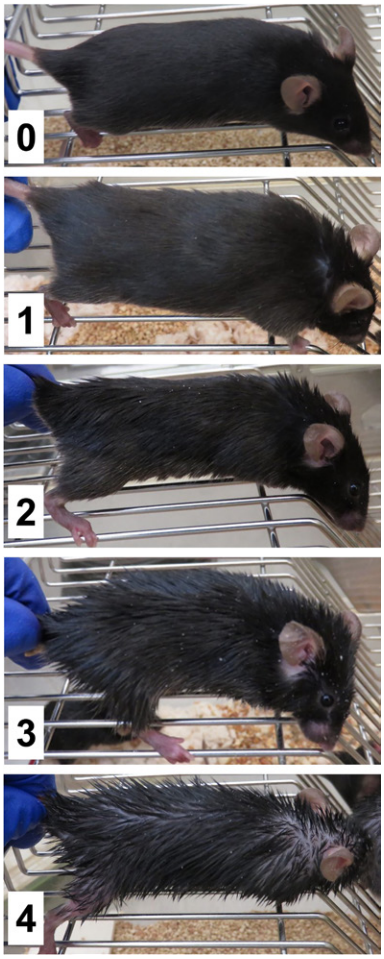

Figure 2. Hair coat grease scoring system: (0) smooth hair coat with no visible grease accumulation; (1) grease accumulation visible only on the neck, shoulders, and/or face; (2) grease accumulation extending past the shoulders to the torso and abdomen; (3) grease accumulation visible diffusely throughout the hair coat resulting in matting; and (4) significant grease accumulation throughout the hair coat resulting in hair clumping and areas of visible skin.

on the cage floor. Use of feeder A resulted in significantly higher average weekly HFD wastage (1,367.9 ± 61.6 g, $P < 0.001$) when compared with all other feeder types. Feeder E resulted in significantly higher average weekly HFD wastage (151.1 ± 11.8 g, $P < 0.0001$) when compared with feeders B, D, and F. In contrast, feeder F resulted in the lowest average weekly HFD wastage which was significantly lower compared with feeders A, B, C, and E (23.3 ± 0.9 g, $P < 0.01$) (Table 1; Figure 3B).

Mouse weight. The average baseline weight of mice across all experimental cohorts was 22.2 ± 0.1 g. At 12 wk, mice fed using

Table 1. Average HFD cost, amount dispensed, and diet wastage over 12 wk

Feeder	HFD cost cage/wk	HFD dispensed cage/wk (g)	HFD wastage cage/wk (g)
A	$36.98 ± 1.54	1,369.7 ± 57.1^{a}	1,367.9 ± 61.6^{a}
B	$2.41 ± 0.81	89.4 ± 3.0^{cd}	37.9 ± 2.1^{c}
C	$3.46 ± 1.97	128.0 ± 7.3^{bc}	77.9 ± 6.0^{b}
D	$2.14 ± 0.41	79.2 ± 1.5^{d}	34.8 ± 3.3^{cd}
E	$5.26 ± 0.31	195.1 ± 11.5^{b}	151.1 ± 11.8^{b}
F	$1.69 ± 0.03	62.6 ± 1.0^{e}	23.3 ± 0.9^{d}

$P < 0.005$ values with different superscript lowercase letters are significantly different.

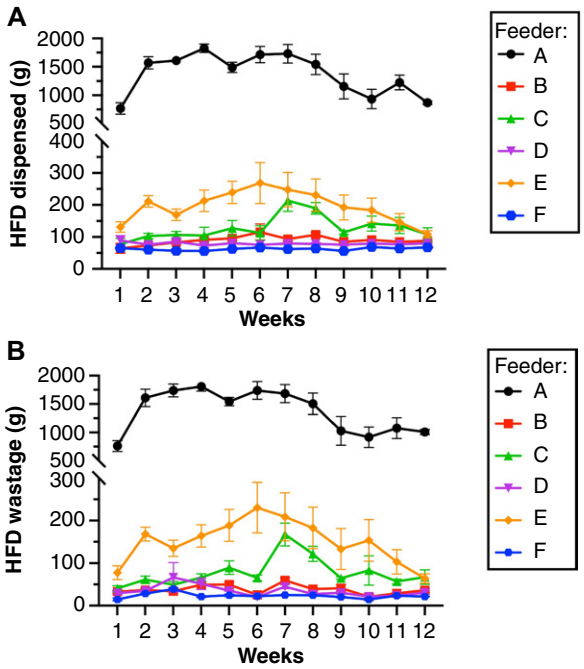

Figure 3. (A) Average HFD dispensed for different feeder designs. (B) Average estimate of HFD wastage for different feeder designs. Summary data represented as mean ± SEM.

feeder A gained significantly more weight (23.75 ± 0.8 g, $P < 0.005$) when compared with the weight gained by mice fed using other feeder types. Mice fed using feeder F (10.5 ± 0.8 g, $P < 0.001$) gained the least amount of weight when compared with feeders A, B, C, and E (Table 2; Figure 4A). Mice fed using feeders A, C, and E had a significantly higher average weekly percent increase in weight gain over the first 6 wk they were fed HFD when compared with the average weekly percent increase in weight gain the final 3 wk of the experiment ($P < 0.05$) (Figure 5). While the average weekly percent increase in weight gain for feeders B, D, and F trended downward after week 6, the difference was not statistically significant.

Hair coat grease scoring. Baseline hair coat grease scores were zero for all experimental groups. By week 1, all mice fed using feeder A displayed hair coat grease accumulation. By week 2, all mice in all experimental groups had visible hair coat grease accumulation. At 12 wk, mice fed using feeder A had significantly more hair coat grease accumulation (3.75 ± 0.1, $P < 0.05$) when compared to mice fed using all other feeder types. None of the mice in the other experimental groups averaged hair coat grease scores above 2.5 (Figure 4B).

Table 2. Average mouse rate of weight gain, total weight gain, and hair coat grease score at 12 wk

Feeder	Rate of weight gain/wk (g)	Total weight gain (g)	Hair coat grease score
A	2.0 ± 0.07	23.75 ± 0.8^{A}	3.75 ± 0.1^{a}
B	1.4 ± 0.1	16.3 ± 1.2^{BC}	1.6 ± 0.2^{bc}
C	1.6 ± 0.06	18.9 ± 0.8^{B}	2.4 ± 0.1^{b}
D	1.2 ± 0.08	14.0 ± 1.0^{CD}	1.0 ± 0.2^{c}
E	1.3 ± 0.08	15.8 ± 1.0^{BC}	2.1 ± 0.1^{b}
F	0.87 ± 0.07	10.5 ± 0.8^{D}	1.2 ± 0.1^{c}

$P < 0.05$ values with different superscript lowercase letters are significantly different.
$P < 0.005$ values with different superscript uppercase letters are significantly different.

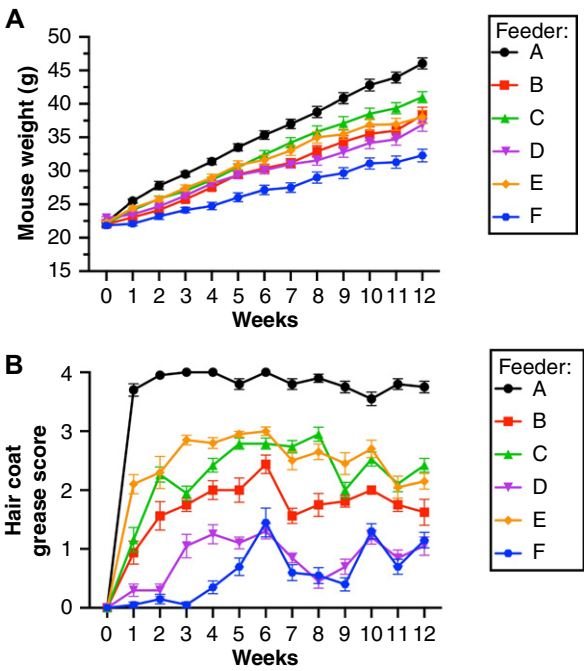

Figure 4. (A) Average mouse body weight for different feeder designs. (B) Average mouse hair coat grease scores for different feeder types. Summary data represented as mean ± SEM.

Mice fed using feeder D (1 ± 0.2) and feeder F (1.2 ± 0.1) had significantly lower hair coat grease scores when compared with feeder A (3.75 ± 0.1, $P < 0.0001$), C (2.4 ± 0.1, $P < 0.001$), and E (2.1 ± 0.1, $P < 0.05$) (Table 2; Figure 4B).

Behavioral scratching assessment. The baseline scratching frequency was 0.16 ± 0.04 for all mice. Mice fed using feeder A had a significantly higher scratching frequency (1.7 ± 0.3, $P < 0.05$) when compared with mice fed using all other feeder designs at the 4-wk time point (feeder B: 0.3 ± 0.2; feeder C: 0.1 ± 0.1; feeder D: 0.2 ± 0.1; feeder E: 0.5 ± 0.2; and feeder: F 0.1 ± 0.1). No significant differences in scratching frequency were observed between experimental groups at the 8- and 12-wk time points (Figure 6). Mice fed using feeder A had an increased scratching frequency at the 4, 8, and 12-wk time points when compared with baseline scratching frequency (1.7 ± 0.3, $P < 0.0005$; 1.7 ± 0.4, $P < 0.01$; and 1.7 ± 0.5, $P < 0.02$, respectively). No significant differences were observed between baseline and subsequent time points when mice were fed using any of the other feeder types.

Discussion

Formulated HFDs are greasy and crumble easily leading to wasted diet, increased cost, and mouse hair coat grease accumulation. Furthermore, hair coat grease accumulation may have the potential to alter normal mouse behaviors. We compared 3 novel wire bar lid feeders and one novel floor feeder to 2 standard commercially available wire bar lid feeder designs. Our study's focus was on how well each feeder prevented HFD wastage and whether the feeder design succeeded at limiting direct contact with HFD. The data presented here support our hypothesis that feeders can be modified in configuration and dimension to better contain a soft-pelleted HFD resulting in a decrease in HFD wastage, a decrease in mouse hair coat grease accumulation, and a reduction in scratching behavior when compared with a standard commercially available wire bar lid feeder (feeder A). However, an unexpected result was that the mice fed using feeder A gained significantly more weight when compared to mice fed using all other feeder designs.

The *Guide for the Care and Use of Laboratory Animals* (8th ed.) states that "Feeders should be designed and placed to allow easy access to food and to minimize contamination with urine and feces."[8] All novel feeders described in this study generated less food wastage on the cage floor and, therefore, had less fecal and urine contamination when compared with the commercially available wire bar lid feeders. The ability of the novel feeders to better contain the soft-pelleted HFD led to a subsequent decrease in the amount of HFD dispensed thereby significantly reducing HFD cost. Throughout the study, HFD cost associated with novel feeder use averaged $1.70 to $3.50/cage/wk. HFD cost associated with the commercially available feeder A averaged $36.98/cage/wk. This difference highlights the potential cost savings when working with expensive specialized diets such as the soft pelleted HFD. As a result of increased food wastage, all cages containing feeder A created additional work for animal husbandry staff because feeders required supplementation 2 to 3 times weekly, and supplemented cages had to be changed a minimum of twice weekly. Additional staff time was needed because cages that needed feed supplementation twice weekly needed an additional cage change due to the volume of diet wastage. The combination of bedding substrate and diet waste build-up resulted in a substantial increase in bedding volume. These frequent cage level disruptions, introduced by the addition of feed and increased cage changes, represent a recognized stressor.[1,15]

Due to the absence of a specific marker and a scientific consensus on what defines the presence of obesity in mice, our study defined obesity as 15% weight gain when compared with age-matched control animals. Based on data provided from a published growth curve, male C57BL/6NCrl mice fed a standard diet (Purina diet 5008; PMI, Richmond, IN), averaged 30 g by 19 wk.[2] For mice in our study to achieve the 15% weight gain target, they would have needed to weigh a minimum of 34.5 g at 19 wk. Mice fed using feeders A, B, C, D, and E averaged weight gain greater than 34.5 g and, therefore, exceeded our weight gain target. Feeder F was the only feeder design that resulted in an average mouse weight gain that did not meet our 15% weight gain target when compared with age-matched controls. The significant difference in total weight gain when mice were fed using feeder A, compared to the total weight gain of mice that were fed using all other experimental and commercially available feeder designs, could affect study variables associated with the development of rodent obesity models and obesity-related metabolic disease outcomes. Our study underscores the importance of evaluating the effect of feeder design before feeding soft-pelleted HFDs.

Our study demonstrated that the size of the feeding apertures affects the rate of weight gain thereby resulting in differences in total weight gained. The feeding apertures must be long enough, vertically or horizontally, to accommodate the incisors. Smaller feeding apertures may have increased the difficulty in obtaining food. Mice fed using feeder A, which had the largest feeding apertures (3.76 cm^2), gained the most weight when compared with those fed by all other feeder designs. Mice fed using novel feeders B and C, with added horizontal bars resulting in smaller feeding apertures (0.56 and 0.8 cm^2, respectively), had slower rates of weight gain when compared to mice fed using a commercially available wire bar lid feeder with larger feeding apertures (feeder A). Mice fed by a commercially available modular feeder (feeder E) with feeding apertures of 1.4 and 2.1 cm^2 gained significantly more weight than mice fed by

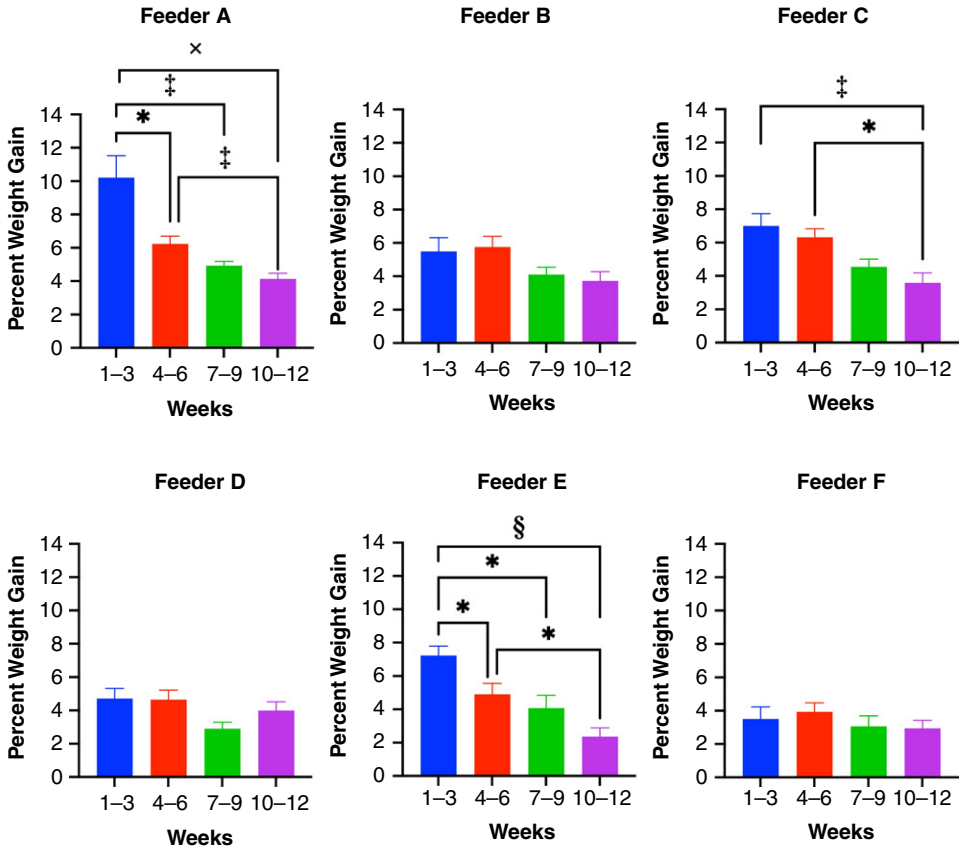

Figure 5. Percent weekly increase in weight gain represented as four 3-wk averages for each feeder design. Summary data represented as mean ± SEM. *, $P < 0.05$; ‡, $P < 0.001$; §, $P < 0.0001$; ×, $P < 0.0005$.

novel feeder F, which had smaller feeding apertures (0.42 cm^2) due to the addition of a feeder insert. Feeders D and F had the smallest feeding apertures (0.38 and 0.42 cm^2) resulting in mice with the lowest total weight gain among all the feeder designs tested. No significant difference in weight gain was noted between feeder D and feeder F.

The relationship between feeder design and weight gain in mice is complex. Feeding aperture size is not the sole factor affecting weight gain. Although feeder E had the second largest feeding aperture size, mice fed by feeder E had comparable weight gain to those fed by feeders B, C, and D which had smaller feeding apertures. Feeders B and C which are modified versions of the Tecniplast commercially available wire bar lid feeder, and feeder D, a novel floor feeder, may be more readily accessible to mice due to their large feeding surface areas (261.8 and 68 cm^2, respectively). In contrast, feeder E had a modular design that, despite its larger feeding apertures, had a smaller feeding surface area of 51.6 cm^2. Early rapid increases in weight gain appear to be associated with feeder designs that provide mice with easier access to food. Mice fed using feeders A, C, and E, which had larger feeding apertures by design, had significantly higher rates of weight gain during the first half of the experiment (weeks 1 to 3 and 4 to 6) when compared with the last 3 wk of the experiment (weeks 10 to 12). Those designs also resulted in more food wastage. Mice fed using feeders B, D, and F did not have statistically significant differences in the percentages of weight gain throughout the study but displayed a downward trend over time. The rapid decline in percent weight gain observed in mice fed using feeders A, C, and E suggests that the average percent weight gain may start to stabilize after mice are fed HFD for 6 wk. This significant change was not observed in mice fed using feeders B, D, and F. Feeders B, D, and F, where feed was not as readily accessible by design, had an average weekly percent weight gain that did not significantly differ over 12 wk. It is possible that mice fed using feeders B, D, and F would have eventually gained the equivalent weight of the mice fed using feeders A, C, and E if they had been fed HFD for a longer duration. Our study's 12-wk duration limits conclusions about overall long-term weight gain outcomes.

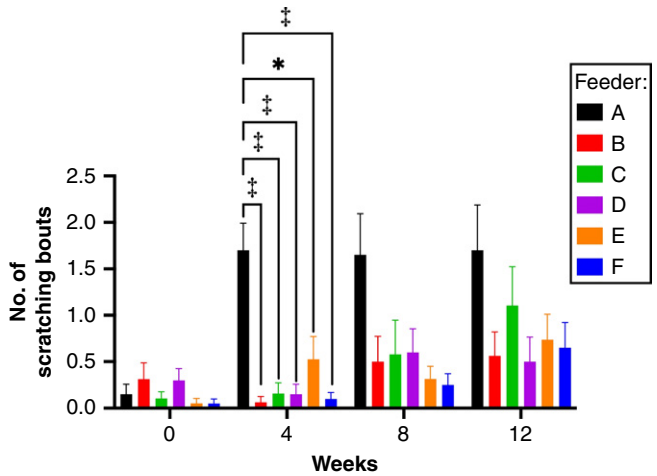

Figure 6. Average number of scratching bouts/15 min for different feeder designs at baseline, 4, 8, and 12 wk. Summary data represented as mean ± SEM. *, $P < 0.05$; ‡, $P < 0.001$.

Our findings highlight the importance of feeder design in influencing mouse weight gain by demonstrating that not only

feeding aperture size but also the overall feeding surface area of the feeder plays an important role. The variations in weight gain among the different feeder designs suggest that some designs may inadvertently function as food puzzles. Food puzzles are commonly used as enrichment for captive zoo and laboratory animals, as well as for weight loss in companion animals.[3,4,18,25] Food puzzles work by regulating food intake. Common food puzzle design often adjusts the feeding surface area, feeder configuration, and the number of feeding apertures to increase the difficulty for an animal to gain access to food.[4] For this reason, it is possible that significantly lower rates of weight gain noticed over the first 6 wk of the study may have been influenced by mice not being acclimated to the novel feeder types before the study inception.

Our data demonstrate a correlation between mouse hair coat grease scores and the amount of HFD wastage created by feeder design. Mice fed using the commercially available wire bar lid feeder (feeder A) exhibited the highest hair coat grease scores. Hair clumping and skin exposure were evident in 100% of the mice fed using feeder A during at least one time point in the study (Figure 2). The higher hair coat grease scores (average score of 3.75) indicate that continuous exposure to HFD wastage on the cage floor had a significant impact on the mouse's ability to groom and maintain a clean hair coat. Despite twice weekly cage changes, the mice fed using feeder A maintained elevated hair coat grease scores when compared with all other feeder types where cages were changed only once a week. This highlights the persistent challenge of maintaining mouse hygiene when mice are fed from a feeder design that does not adequately contain soft-pelleted HFD. By contrast, mice with the lowest hair coat grease scores, fed using feeders D and F, had the least HFD wastage. The feeder design of both feeders was highly efficient at containing the soft-pelleted HFD and resulted in average hair coat grease scores of 1.2 and 1, respectively. These findings demonstrate that reduced contact with HFD waste reduces mouse hair coat grease accumulation.

The standard rodent model of pruritus measures hindlimb scratching frequency. Frequent scratching is considered a behavioral response to pruritic stimuli.[19] Scratching frequency is an important indicator of murine well-being and was examined in our study. Scratching frequency, before starting HFD, was similar for mice across all feeder types indicating that baseline behaviors were comparable. Mice fed using the commercially available wire bar lid feeder (feeder A) displayed a significantly higher scratching frequency when compared with mice fed using all other feeder types at the 4-wk time point. In addition, mice fed using feeder A had an increased scratching frequency at the 4-, 8-, and 12-wk time points when compared with baseline. Interestingly mice fed using feeder A had significantly higher hair coat grease scores when compared with mice fed using all other feeder types. These findings demonstrate a correlation between hindlimb scratching frequency and hair coat grease accumulation. We observed that mice fed using feeder A experienced pruritus throughout the duration of the study. While mice fed using other feeder types displayed an upward trend in scratching frequency across study time points, scratching frequencies were not significantly different from baseline.

Overall, data were analyzed from 113 of the original 120 mice. A total of 7 mice were excluded from the study due to health concerns. Five mice assigned to the feeder B experimental group were euthanized due to fighting. Two mice, assigned to feeder C and feeder E experimental groups were euthanized due to UD. Self-inflicted trauma, due to hindlimb scratching can lead to progression of UD, a condition characterized by pruritic open-skin lesions that can detrimentally impact animal well-being.[6,7,16] UD has been identified as a common condition of C57BL/6 mice as well as mice fed a HFD.[11,12,17] In the context of our study, when both risk factors were present, strain predisposition and HFD consumption, mice were considered at high risk for the development of UD. Given the low number of UD affected animals in our study population (2/115), we were unable to draw any meaningful conclusions as to whether the type of feeder used had any effect on UD incidence. Another limitation of this study was that it was only conducted over a short duration (12 wk) during which mouse body weights did not reach a plateau. Extending the study length may provide additional insight into differences in the rate of weight gain and the total weight gain between cohorts. Additional studies investigating whether an increase in hair coat grease scoring in combination with a higher frequency of scratching behaviors affects UD prevalence could provide additional support for refinement of feeding strategies as a way to improve animal welfare. This study was only performed using male C57BL/6NCrl mice and did not investigate sex or strain differences. Finally, only one type of soft-pelleted HFD was tested. Future studies should seek to evaluate the impact of novel feeder designs when mice are fed other HFDs, including those with differing fat content.

In conclusion, feeder design, in particular feeding aperture size and feeding surface area, significantly influence the amount of HFD dispensed and wasted, the amount of weight gained, the amount of hair coat grease accumulation, and the frequency at which mice exhibited scratching behavior when fed a soft-pelleted HFD. Careful consideration of feeder design is crucial to achieving desired research and welfare outcomes. Inappropriate feeder selection, or the use of more than one feeder type, may introduce scientific variables when rapid weight gain is needed to generate a DIO model. When appropriate, we implemented feeder C for feeding HFD to male C57BL/6NCrl mice because this feeder design resulted in the second highest rate of mouse weight gain without significantly increasing hair coat grease scores or scratching frequency. Feeder C's smaller feeding apertures resulted in a significant reduction in HFD wastage netting a cost savings of $395 per cage over a 12-wk period.

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Conflict of Interest

The authors have no conflicts of interest to declare. Feeders evaluated in this study were provided to the authors at no cost for an independent review. Tecniplast, Process Control Solutions, and Lab Products did not provide funding support and did not participate in the design of this study.

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