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Housing influences tissue cytokine levels and the fecal bacterial community structure in rats

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ABSTRACT

Immune measures and the fecal bacterial community were examined in female Biobreeding rats housed in wire bottom cages (wire) or in solid bottom cages containing hardwood chips (bedding). Housing did not affect food intake, weight gain, fecal output or fibre content, serum liver enzymes, or spleen and mesenteric lymph node immune cell populations. Bedding-housed rat feces were enriched in phylotypes aligning within the phylum Firmicutes (families Lactobacillaceae and Erysipelotrichaceae) and had a 2-fold lower content of phylotypes aligning within the phylum Bacteroidetes. Feces from bedding-housed rats also contained significantly more acetic acid and less propionic, isobutyric, valeric and isovaleric acids than those housed on wire. Bedding-housed rats had significantly higher splenic concentrations of interleukin-4 (P < 0.001). These results demonstrate that bedding can indirectly influence systemic and mucosal immune measures, potentially adding additional complexities and confounding results to nutrition studies investigating the health effects of dietary fibres.

1. Introduction

Environmental factors, including housing, contribute to the complexity of interpreting gut microbiota-mediated effects in rodent studies (Gill & Finlay, 2011). For example, changes in the dominant lineages of the fecal microbiota were observed when mice were moved to different locations within the same facility (Friswell et al., 2010) and differences have been noted in the same strain bred in different centres (Hufeldt, Nielsen, Vognesen, Midtvedt, & Hansen, 2010). In addition, strain-dependent alterations in environmental enrichment have been linked to differences in immunological parameters in mice (Marashi, Barnekow, Ossendorf, & Sachser, 2003). Environmental considerations are important when designing immunological studies since the gut bacterial community continuously interacts with the immune system both directly and indirectly through microbial-derived antigens and metabolic products (Slack et al., 2009).

Gut commensal microbes also participate in the induction of immune tolerance in the intestine. For example, segmented filamentous bacteria and polysaccharide A of Bacteroides fragilis have been shown to induce Treg populations (Hooper & Macpherson, 2010). Metabolic products such as propionate are involved in maintenance of a regulatory immune milieu in the colon of specific pathogen free mice (C. Smith, Rocha, & Paster, 2006) and SCFA influence immune activity at the systemic level (Maslowska et al., 2009), as do microbial components able to act as TLR or NOD ligands (Balmer et al., 2014). Factors influencing bacterial community composition may, therefore, affect immune outcomes at mucosal and systemic levels.

Housing and type of bedding are important in studies measuring the impact of gut microbiota changes but their effects on host immune status have not been fully addressed. The Canadian Council of Animal Care strongly suggests the use of plastic cages and bedding for rodents (Canadian Council on Animal Care, 1993). Recommendations also include pair housing (when possible) and replacing wire cages with solid platforms or bedding (National Research Council (NRC), 2011) to alleviate stress, which appears to affect cecal microbial diversity in mice (Bangsgaard Bendtsen et al., 2012). Ingestion of bedding material is a concern (Le Leu, Conlon, Bird, & Clarke, 2015) as is coprophagy (Levine & Saltzman, 1999) in studies examining immune and gut community changes associated with dietary fibre consumption since these can affect nutritional measures. Here, we have examined the effect of two different types of housing (woodchip bedding vs wire-bottomed cages) on various mucosal and systemic immune parameters, the fecal bacterial community structure, and fecal metabolic activity. This study was undertaken to address animal care committee criticisms concerning...
the use of wire bottomed cages when studying the functional properties of fermentable materials. We found that the use of bedding adds additional complexities to nutritional studies by altering bacterial community composition and immune parameters in the absence of dietary change.

2. Materials and methods

2.1. Animals

A total of 24 female BioBreeding control rats (BBC; inbred line originally derived from Wistar rats obtained from Health Canada Animal Resources Division), ages 28–42 d were randomly assigned to one of two groups: wire bottomed cages with metal plates covering ¼ of the floor space (12 rats: wire) or plastic suspended housing with maple wood chips (12 rats:bedding; P.W.I. Industries, Saint Hyacinthe QC). Female rats were selected for this study to reduce the possibility of immune effects not directly related to housing. Female rats are superior to males in two respects: the intestinal tract of female rats is naturally less permeable than that of males (Homm et al., 2005; Shastri, McCarrville, Kalmokoff, Brooks, & Green-Johnson, 2015), and female rats have been reported to be more resilient to stress-induced physiological effects (Rakoff-Nahoum, Paglino, Esami-Varzaneh, Edberg, & Medzhitov, 2004).

The number of animals/housing group was determined from previous measurements in our lab showing that a 30% CV was common for many of the immune parameters measured. Power calculations indicated that 6 animals/group were required to determine a physiological significant difference of about 1 ng/g (power of 80% with a type 1 error rate of 0.05). A total of 2 animals were required to measure all parameters of interest. Thus, 6 × 2 = 12 animals/group were required for the study. Rats were transferred from the breeding room to the housing room (same facility) at the start of the experiment and housed individually for 63–67 d with free access to an AIN93G purified diet (Reeves, Nielsen, & Fahey, 1993) containing 5% w/w alphacel. Rats were subjected to two groups: wire bottomed cages with metal plates covering ¼ of the floor space (12 rats: wire) or plastic suspended housing with maple wood chips (12 rats:bedding; P.W.I. Industries, Saint Hyacinthe QC). Female rats were selected for this study to reduce the possibility of immune effects not directly related to housing. Female rats are superior to males in two respects: the intestinal tract of female rats is naturally less permeable than that of males (Homm et al., 2005; Shastri, McCarrville, Kalmokoff, Brooks, & Green-Johnson, 2015), and female rats have been reported to be more resilient to stress-induced physiological effects (Rakoff-Nahoum, Paglino, Esami-Varzaneh, Edberg, & Medzhitov, 2004).

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Health Canada maintains a specific pathogen free facility with weekly testing for lymphomacogenicinfection bias virus, mouse adenovirus FL/K87, hantavirus, Encephalitozoon cuniculi, and cilia-associated respiratory bacillus. Other yearly tests included bacterial cultures of nasal aspirate and cecum cultures as well as tests for endoparasites (including helminthes and protozoa) and ectoparasites. Tests repeated every three months included: sendai virus, pneumonia virus of mice, rat coronavirus, Kilham rat virus, Toolan’s H-1 virus, rat parvovirus, rat minute virus, parvovirus generic assay, reovirus, rat theilovirus, and Mycoplasma pulmonis. All animals were negative.

2.2. Automated biochemical analyses

Serum samples (N = 6/housing group) were analysed for blood urea nitrogen (Talke & Schubert, 1965). Liver enzymes were measured using an ABX Pentra 400Automated clinical chemistry analyzer using ABX Pentra test kits (Burlington, Ontario, Canada). Specific reactions pathways as follows: alanine amino transferase (ALT) using L-alanine and 2-oxoglutarate as substrate coupled to lactic dehydrogenase and following the disappearance of NADH; alkaline phosphatase using p-Nitrophenylphosphate as substrate; aspartate amino transferase (AST) using 2-oxoglutarate plus L-aspartate as substrate coupled to malate dehydrogenase and following NADH disappearance; and lactate dehydrogenase using pyruvate as substrate following the disappearance of NADH.

2.3. Short chain and branched chain fatty acid analysis

Fecal SCFA and branched chain fatty acids were measured after acidification using sulfuric acid (Weaver, Krause, Miller, & Wolin, 1992) and analysed by gas chromatography on an Agilent 6890 gas chromatograph (Agilent Technologies Canada Inc., Mississauga ON). An internal standard (2-ethylbutyric acid) was added to the samples to correct for loss during extraction and the samples were filtered through a 0.45 μm syringe filter. The column was a 60 m × 0.25 mm, I.D. 0.25 μm film thickness Nukol column (Supelco-Sigma-Aldrich, Mississauga ON), run at 0.8 mL/min with helium carrier gas and the temperature was increased from 100 to 200 °C at 8 °C/min. Total run time was 30 min. The injector was washed in between runs by injecting acetone followed by 10 injections of water. A mixture of 2-ethylbutyric acid, sulphuric acid and volatile standard mix (Sigma-Aldrich, Mississauga ON) was injected every 10th run to ensure system calibration. Data was analysed using MSD Chemstation software (Agilent Technologies Canada Inc., ON, Canada).

2.4. Flow cytometry

Mucosal (mesenteric lymph node) and systemic (spleen) samples were analysed using a FACS Calibur flow cytometer (BD Biosciences, ON) and standard gating techniques (Shingadia, O’Gorman, Rowley, & Shulman, 2001). The following antibodies were used for cell surface staining: anti-CD45 (PE-Cy5, Clone Ox-1) (Leukocyte Common Antigen), anti-CD3 (APC, Clone 1F4) (total T cell), anti-CD4 (PE, Clone Ox-35) (T helper), anti-CD25 (FITC, Clone Ox-39), anti-CD8a (FITC, Clone Ox-8) (cytotoxic T cell), anti-CD45RA (FITC, Clone Ox-33), (B cell), and anti-CD161a (PE, Clone 10/78) (Natural Killer cell). Cells were also permeabilized and intracellularly stained with anti-FOX3 (PE, Clone FJK-16s) to detect regulatory T cell populations. Macrophage activation was analysed using anti-CD68 (Alexa Fluor 488, Ed1 clone) (Onodera et al., 1997). Fluorochrome-matched isotype controls were used to assess non-specific binding. Anti-CD68 was purchased from AbD Serotec (NC, USA). All other antibodies were obtained from BD Biosciences (ON, Canada).

2.5. Tissue preparation & cytokine analysis by ELISA

Tissues were collected at necropsy, snap frozen and stored at −80 °C. The ileum, colon, cecum, and spleen were homogenized (Hoentjen et al., 2005) and then centrifuged for 30 min at 16,100g. Supernatants were stored at −80 °C and later analysed for the presence of cytokines: interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), transforming growth factor-β1 (TGF-β1) and cytokine-induced neutrophil chemoattractant-1 (CINC-1). ELISAs (Duoset sandwich kits; R & D Systems, MN, USA) were performed in 96 well plates (Greiner Bio-One, NC, USA) and were read at a wavelength of 450 nm on a Synergy HTTR microplate reader (BioTek, VT, USA).

2.6. Fecal community analysis

Fresh, manually expressed feces were collected 1–2 days prior to the initiation of the balance phase and immediately stored at −20 °C. Fecal material from individual rats (1.0 g/rat) was pooled (N = 6/housing) to minimize inter-rat fecal community variability (Kalmokoff, Franklin, Petronella, Green, & Brooks, 2015). Fecal community DNA was prepared (Kalmokoff et al., 2015) and duplicate near full length 16S rRNA gene libraries were constructed from two independently derived community DNA samples for each housing condition. Ribosomal genes were...
amplified using the primers F44/R1543 and cloned following 12 cycles of amplification (Kalmokoff et al., 2013). One hundred clones were randomly selected from each duplicate clone library (2 × 100 clones/housing) and sequenced. Near full length 16S rRNA clone sequences were initially aligned against the Silva database (Quast et al., 2013) and sequences demonstrating < 3% DNA sequence divergence binned into the dominant phyotype. The data was checked for chimeric sequences using subroutines available in Mothur (Schloss et al., 2009). Final binned sequences were assigned to the phylogeny using the seqmatch function available at the Ribosome Data Project (Cole et al., 2009).

Changes in community structure determined from the analysis of 16S RNA clone libraries were confirmed using two additional analyses. First, differences in 16S rRNA gene content (as a percentage of total 16S rRNA gene copy number) within the phylum Bacteroidetes and the genus Lactobacillus (Castillo et al., 2006; Rinttila, Kassinen, Malinen, Krogius, & Palva, 2004) were quantified using quantitative PCR (qPCR). Total 16S rRNA gene content was determined using the universal primer set HDA1/HDA2 (Walter et al., 2000). Second, individual rat fecal pellets (N = 8/housing condition) were placed into separate pre-weighed 50 mL screw-cap plastic tubes containing 1.0 g of sterile glass beads, weighed, and transferred into an anaerobic hood. Following the addition of 10 mL anaerobic 0.1% peptone water, the pellets were mixed to obtain homogenous slurry, and subjected to a 10-fold dilution series. Dilutions were plated on L-10 anaerobic medium to obtain the total anaerobic count (Caldwell & Bryant, 1966) and de Man-Rogosa-Sharpe medium (De Man, Rogosa, & Sharpe, 1960) for total lactobacilli. Plates were incubated in an atmosphere of H2/CO2/N2 (10:20:70) at 38 °C for 48 h, and the dilution plate having 30–300 colonies counted, with final counts expressed in terms of percentage of the total anaerobic count.

2.7. Statistical analysis

Immunological data were analysed after log transformation by two-way ANOVA with bedding and tissue as factors (Statistica; Statsoft, OK, USA). Fecal SCFA, food and energy consumption, feed efficiency, serum biochemical parameters, and Lactobacilli plating counts were analysed using the two-tailed Student’s t-test (equal variance). The results are presented as mean ± standard error (SE). Results were considered significant when P < 0.05.

3. Results

No housing-related differences in food consumption or weight gain were observed (Table 1). Similar feed efficiencies suggested similar energy expenditure between groups. Fecal output and fecal fibre content over the 2 week balance phase were also not affected by housing. While dry weight fecal outputs were not significantly different, bedding material was present in the insoluble fraction derived from feces indicating that the rats housed on bedding ingested this material (Fig.1).

No differences in the levels of serum enzymes normally associated with liver pathology were observed. Blood urea nitrogen values were also similar between housing groups (Table 1). The proportion of fecal SCFA corresponding to acetic acid was significantly higher in bedding-housed rats. On the other hand, the proportion of propionic, isobutyric, valeric and isovaleric acids was significantly lower (Fig. 2). No differences in butyric acid were observed.

3.1. Immune parameters

Housing environment had no effect on immune cell population percentages (B cells, T cell subsets, macrophages and natural killer cells) in spleen and mesenteric lymph nodes (data not shown). Housing environment also had no effect on the levels of regulatory cytokines at the ileum (TGF-β1 and IL-10). However, bedding-housed rat ileal IL-6 levels (pro-inflammatory) tended to be higher when compared to wire-housed rats (P = 0.052; Table 1). No changes in cytokine levels were detected in the colon or cecum. At the systemic level, splenic CINC-1 levels were unchanged but splenic IL-4 concentrations were higher in rats housed on bedding versus those on wire (P < 0.001; Table 2).

Table 1

|                           | Wire       | Bedding    |
|---------------------------|------------|------------|
| **Physiological measures for rats housed on wire or on bedding.** |            |            |
|                           | N Value    | N Value    |
| **Food, body weight:**    |            |            |
| Total food consumption (g) | 12 872 ± 31 | 10 881 ± 48 |
| Daily food consumption (g/d)| 12 19.8 ± 0.7 | 10 20.7 ± 1.1 |
| Total energy consumption (kcal) | 12 3447 ± 124 | 10 3482 ± 191 |
| Daily energy consumption (kcal/d) | 12 78.3 ± 2.8 | 10 81.7 ± 4.3 |
| Total body weight gain (g) | 12 85.1 ± 3.5 | 12 85.9 ± 3.3 |
| Daily body weight gain (g/d) | 12 1.93 ± 0.08 | 12 2.01 ± 0.08 |
| Energy efficiency (g BW/kcal consumed) | 12 25.0 ± 1.3 | 10 24.5 ± 1.0 |
| **Feces**                 |            |            |
| Fecal output (g dry weight/d) | 12 1.16 ± 0.02 | 12 1.23 ± 0.02 |
| Fecal fibre (%) (g dry weight) | 12 57.5 ± 0.8 | 12 58.2 ± 0.6 |
| **Serum biochemical parameters** |       |            |
| Lactate dehydrogenase (IU/L) | 6 116 ± 16 | 6 137 ± 35 |
| Blood urea nitrogen (mg/dL) | 6 23.0 ± 0.6 | 6 22.3 ± 1.1 |
| Alkaline phosphatase (IU/L) | 6 201 ± 9 | 6 176 ± 15 |
| Aspartate aminotransferase (IU/L) | 6 58.5 ± 1.3 | 6 59.5 ± 2.0 |
| Alanine aminotransferase (IU/L) | 6 30.7 ± 2.1 | 6 29.8 ± 1.2 |

All P values were not significant (two-tailed t-test); values represent means ± SE for indicated N.

Fig. 1. Insoluble fecal fraction from rats housed on wire (A) or bedding (B). This material consists predominantly of wood cellulose added to the AIN93G diet as a source of insoluble dietary fibre.

Fig. 2. Percentage of total SCFA corresponding to acetic, propionic, butyric, isobutyric, valeric and isovaleric acids in feces from rats housed on wire (black filled rectangles) or bedding (white filled rectangles). *Significantly different from wire housed rats. Values represent mean ± SE (N = 12).
3.2. Fecal bacteria community

In total, 114 phylotypes were identified in feces, a level of community richness similar to previous fecal surveys of BBc rats (Abrous et al., 2009; Shastri et al., 2015). The fecal community of bedding-housed rats was enriched in clones aligning within the phylum Firmicutes, but depleted in clones aligning within the phylum Bacteroidetes compared to the community of wire-housed rats (14% versus 26% of total community respectively; Fig. 3A). The altered community structure resulted from differences in the content of clones aligning within the families Lactobacillaceae (14% versus 2% of total community in bedding versus wire housed respectively), Erysipelotrichiaceae (14% versus 6%), and Bacteroidaceae (9% versus 1% Fig. 3B).

Lactobacilli gene copy numbers, determined by qPCR, represented 22% and 3% of the total 16S rRNA gene copy number in rats on bedding or wire, respectively. Lactobacilli plate counts were also significantly different between bedding and wire housed animals (18 ± 11% versus 5 ± 4% of the total anaerobic plate count respectively, P = 0.014 two-tailed t-test). qPCR estimates for Bacteroidetes 16S rRNA gene content were higher than those found in the 16S rRNA clone libraries but followed a similar trend (29% versus 44% for bedding versus wire, respectively). We previously found that 16S rRNA gene based community analysis of samples from the rat gut tend to underestimate Bacteroidetes (Kalmokoff et al., 2015).

4. Discussion

In laboratory animals, the gut microbial community is sensitive to changes in a variety of environmental parameters, including diet (Abrous et al., 2009), the nutrition source provided during weaning (O’Sullivan et al., 2013), and conditions and husbandry practices used in the animal facility (Ivanov et al., 2009; Ma et al., 2012). A recent report also showed that bedding can influence the gut bacterial community and its metabolic activity (Le Leu et al., 2015). Similar to that study, we found that changes in housing affected fecal community diversity and metabolic activity. We also found limited changes in host systemic and mucosal immune parameters.

Housing rats on bedding altered fecal community structure by increasing Firmicutes content. This change resulted primarily from the enrichment of phylotypes aligning within the families Lactobacillaceae (Lactobacillus) and Erysipelotrichiaceae at the expense of those aligning within the family Bacteroidaceae (Bacteroides). These general trends in the 16S rRNA-based analysis were confirmed by qPCR and anaerobic plating. Lactobacilli have an absolute requirement for peptidyl-nitrogen (De Man et al., 1960; Kandler & Weiss, 1986), whereas genera within the family Bacteroidaceae use ammonia as a source of nitrogen for growth (C. Smith et al., 2006). Thus, increases in the content of Lactobacillus likely reflect increases in the availability of peptidyl-nitrogen sources in the gut (Kalmokoff et al., 2015). We have observed similar phylum-level and species shifts in the feces of male rats fed various diets (Kalmokoff et al., 2013; Kalmokoff et al., 2015; Shastri et al., 2015).

In common with a previous study (Le Leu et al., 2015), we also observed a housing-related change in fecal SCFA output. In bedding-housed rats, the shift towards acetate at the expense of propionate was consistent with the decrease in the family Bacteroidaceae, as propionate production is common across this taxa (C. Smith et al., 2006). Furthermore, branched chain fatty acid concentrations (produced by the fermentation of valine, isovaline and lysine) were also significantly lower in feces from bedding housed rats compared to those housed on wire, which likely reflects an increased use of gut peptidyl-nitrogen to support continued growth rather than as an energy source (i.e., protein fermentation (E. A. Smith & Macfarlane, 1998)). Changes in gut community metabolic activity may also result from the fermentation of the bedding material used to house rats (Le Leu et al., 2015). However, it is unlikely that this material affected SCFA profiles for the following reasons: (i) fecal output was not affected by housing indicating that rats ingested only a very small amount of bedding material; (ii) woodchips (primarily cellulose) are fermented to a limited extent (Davies, Brown, & Livesey, 1991); and (iii) the cellulose content of the AIN93G
diet (5%) is in great excess to any amount of ingested bedding. While changes in the fecal community of rats housed on bedding may be explained on the basis of increased peptidyl-nitrogen in the gut, the primary question is the source of this material. Since both groups were fed only the AIN93G diet and showed no difference in total food consumption, we suggest that the most likely source for the additional dietary protein would be from feces. In fact, coprophagy has long been known to be a concern in bedding housed rats, altering the cultivable fecal community (Fitzgerald, Gustafsson, & McDaniel, 1964) and, depending on the diet, improving weight gain by providing an additional (microbial) source of dietary protein (Midvindt & Gustafsson, 1981; Torrallardona, Harris, & Fuller, 1996). Increasing dietary protein content via increased coprophagy could explain both the shift in community structure and changes to the overall community metabolic activity observed in rats housed on bedding. The changes in fecal community diversity are also consistent with observations in previous rodent studies. For example, anal cupping of rats to prevent coprophagy significantly reduced cultivable fecal Lactobacilli (Fitzgerald et al., 1964). Similarly, disrupting the normal behaviour of mice (i.e., coprophagy) by physical relocation resulted in a short term transition in the fecal community structure to one where phytophages homologous with Lactobacilli decreased, while those homologous with Flavobacterium (Phylum Bacteroidetes) and aligning within the order Clostridiales increased (Ma et al., 2012). These changes are similar to those observed here in the fecal community of wire housed rats.

Housing also affected host mucosal and systemic immune parameters but the effect was minor. At the systemic level, spenic IL-4 concentrations were higher in bedding-housed rats relative to wire-housed rats. Both dietary and gut bacterial components can influence systemic immune measures at the spleen (Neyrinck, Mouzon, & Delzenne, 2007). The ileum is more permeable than the colon and translocated bacteria and bacterial components from the ileum have previously been shown to affect host immune parameters in distal locations (Schulz & Pabst, 2013). Although ascertaining whether gut-derived factors had translocated across the epithelium into the circulation was outside of the scope of the current study, effects at the systemic level would be in keeping with the possibility of bedding-associated damage and IL-6-mediated repair at the ileum. Tissue injury stimulates intestinal epithelial cells to produce IL-6 (Rakoff-Nahoum et al., 2004) and the rodent gut mucosa has been shown to be sensitive to abrasion by fibrous substrates such as wheat bran (Perrin et al., 2001). One possible explanation is that the ingested wood chip material resulted in minor abrasive injuries during transit through the gut. The gut of female rats is more resistant to inflammation-induced injury (Homma et al., 2005) and β-estradiol ordinarily regulates levels of pro-inflammatory cytokines (including IL-6) in the intestinal tissue of female rats (Kilicdag et al., 2016) suggesting that the bedding-associated increase in ileal IL-6 levels reflected epithelial repair rather than inflammation.

On the other hand, fecal acetate levels also increased in bedding-housed rats. SCFAs can influence the immune system within (Smith et al., 2013) and external to the gut (Tan et al., 2016), and acetate has been shown to modulate immune activity and inflammatory responses at the systemic level after absorption (Maslowski et al., 2009). Acetate absorption can occur anywhere along the gut, so ingestion and absorption of microbial-derived fermentation products represents a reasonable explanation for the observed increases in splenic IL-4 levels. Studies have shown that mice housed on hardwood chips (vs. iso- PAD cotton bedding) had increased numbers of Peyer’s patches and increased virus-specific IgA production by Peyer’s patches and mesenteric lymph nodes (Sanford, Clark, Talham, Sidelsky, & Coffin, 2002). Although the authors did not report on cytokine levels, their results suggest a compromised barrier function in animals housed on wood bedding, which agrees with our observation of a local mucosal and systemic immune effect. The type of wood is also important. For example, softwood bedding induced liver drug-metabolising enzymes as well as reduced time spent asleep in rats and mice, and this was reversed by housing on hardwood bedding (Vesell, 1967). In agreement with these observations, we observed no effect of hardwood bedding on liver enzyme levels. Hardwood chip housing can be beneficial; it is superior in reducing ammonia volatiles when animals are housed in static air-flow cages (the cages used for bedding in this study) (Smith, Stockwell, Schweitzer, Langley, & Smith, 2004). Cotton bedding and paper can be problematic if total fecal output is to be measured, as we have observed considerable ingestion of these types of materials in other experiments.

Wire grid housing is known to induce stress in rodents (Giral, Garcia-Olmo, & Kramer, 2011) and this has been reported to alter certain behavioural and immune parameters as well as cecal community diversity in mice (Bangsgaard Bendtsen et al., 2012). In our study, a partial solid floor was provided for the wire-housed rats as well as environmental enrichment. These routine measures are carried out to reduce the stress of housing on bare wire housing. Since we did not determine circulating corticosterone levels, we cannot rule out the possibility that stress may also be contributing to the observed changes in the fecal community and immune parameters.

5. Conclusion

Rodent models remain an important tool for investigating and substantiating mechanisms by which functional foods or food components may affect host health. While the use of bedding is important for the welfare of laboratory animals, ingestion of bedding is known to impact various nutrition determinations. Here we have shown that the inclusion of bedding indirectly influences gut community structure as well as mucosal and systemic immune parameters, most likely by facilitating coprophagy. While alternative approaches such as rectal cups or constrained tube enclosures can be applied to prevent this, wire bottom cages likely remain the most humane and practical approach for nutritional studies where this may be of concern. It is apparent that several considerations must be taken into account when deciding on appropriate housing conditions and when interpreting results from experiments using differently housed rodents.

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Conflict of interest statement

This work was undertaken with financial support from A-base funding from Agriculture and Agri-Food Canada (MK) and Health Canada (JG, FM, SB) as well as a grant from the National Science and Engineering Research Council (PS, JG-J). We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced the outcome.

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