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## Cage Bedding affects Microbiota and Metabolism in Mice

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## Abstract

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The growing problem with the irreproducibility of the results of metabolic research urged us to analyse potential critical factors contributing to the disparities. Microbiota composition constitutes crucial variable in animal research. Gut flora changes depending on multiple factors including fibre intake. Mice derive fibre from diet but, especially under fasted conditions, also from bedding ingestion. Our project aims at assessing the impact of cage bedding on several aspects of metabolic research. Mice were divided into an overnight (ON) fasted, caloric restricted (CR) and *ad libitum* groups. In order to evaluate the effects of different beddings, mice were housed in cages with the most commonly used beddings: wooden, corncob or cellulose. Additionally, ON-fasted mice were kept without bedding or on metal grid preventing coprophagy. Mice bedding consumption and faeces production were measured. Oral glucose tolerance test (OGTT) was performed. Metabolites composition and microbial diversity were analysed in caecum content. Mice organ weight and gene expression measurements were conducted. Mice show preference towards wooden bedding and consume it in highest amount followed by corncob and cellulose beddings. As a result of the bedding ingestion, caecum weight increases in the CR compared to ad libitum fed mice. CR leads to a reduced faeces production and it is the lowest in the CR wood and CR cellulose groups. Housing on metal grid results in greater body weight loss and decreases stomach and caecum size. ON-fasted mice housed without bedding or on grids show lower glucose levels when submitted to OGTT. CR influences the caecal bacterial diversity as well as the caecal metabolic profile. In the CR group, mice on the corncob bedding show a distinct caecal microbial composition and caecal metabolic profile compared to wooden and cellulose groups. CR is associated with increased abundance of Marinifilaceaefrom the phylum Bacteroidales, and Erysipelotrichaceae from the phylum Firmicutes and with a decreased abundance of Lachnospiraceaefrom the phylum Firmicutes. The main metabolites effected by CR are organic acids, fructose, sucrose and glutamine. The type of bedding exerts the strongest effect on valeric acid, lysine, threonine and organic acids. CR- and ON-fasting-associated weight loss, bedding consumption and fibre accumulation in the caecum depends on the bedding type. CR mice housed on wooden and cellulose beddings show similarities and are distinguishable from CR corncob group. Thus, depending on the composition and consumed amount, bedding intake affects numerous parameters and shapes the microbiome.

## **Conflict of Interest**

There is no conflict of interest.