TECNIPLAST

Improvement of urine retrieval and separation efficiency in a metabolic cage for single mouse

Abstract

Objectives

To enhance the performances of metabolic cages in terms of separation of urine and feces, and urine retrieval within single mouse experiments.

To minimize negative behavioral pattern, due to the stay in a stressful environment.

Methods

Food and water intake, urine and feces output and body weight were measured in B6129 mice in the different scenarios.

Moreover, an enrichment was added to the cage and a visual analysis of the animal behavior was performed.

Results

No evident difference in physiological parameters was found between the different scenarios, although a statistically relevant improvement was found in the sample collection.

In details, the new material together with the new design of the urine ring and the upper chamber showed a better efficiency in urine retrieval as well as in the separation of feces and urines.

Further, the animal behavior seemed to be positively affected by the presence of the enrichment, avoiding the undesired habit of nesting in the tiny feeder.

Conclusions

The positive output of these experiments showed how the new design and the new material significantly improved the metabolic cage performances without affecting the animals' physiological parameters and behavior.

Furthermore, such modifications limited the well-known adverse effects arising from the use of a single mouse in this kind of experiment.

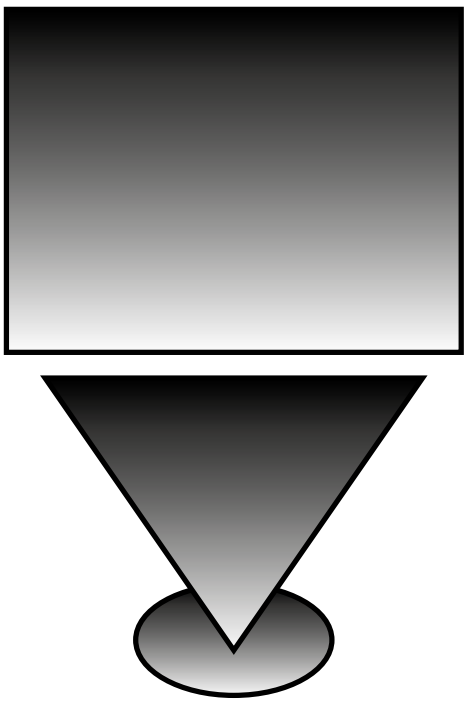
ANALYSIS

INTRODUCTION

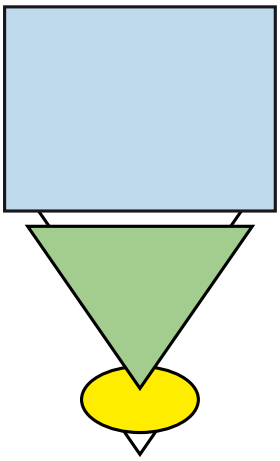
Metabolic cages have been extensively used in lab animal research to run metabolic-related studies across many disciplines, from pharmacology to nutrition through toxicology, and many others.
The aim of a metabolic cage is to permit the separation and the collection of feces and urines in order to run studies about metabolism.
A good compromise between capillarity, adhesion and slip is the real goal of these cages.
To reach this balance the shape and the treatment of all the elements of a metabolic cage are extremely important.
Despite the evolution that has occurred in the metabolic cage system, as concerns experiments on a single mouse there are still some adverse effects that affect urine separation, like evaporation. This is mainly due to the small volumes involved.
Tecniplast S.p.A has in its portfolio of metabolic cages, a specific cage for a single mouse application, therefore some modifications to overcome these issues have been developed and tested.

MATERIALS AND METHODS

Two different experiments have been carried out in order to test the best improvements for the single mouse metabolic cages.
In experiment A, we compared the two different configurations of the single mouse metabolic cage:
1) CURRENT CONFIGURATION (standard clear upper chamber + standard separation cone + standard urine ring), named "OLD CONFIGURATION"

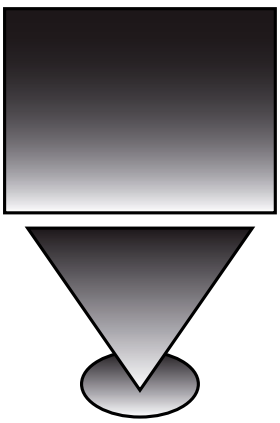


2) Modified clear upper chamber (with longer edges facing inward) + separation cone made from a different type of plastic + modified urine ring shape, named "NEW CONFIGURATION"

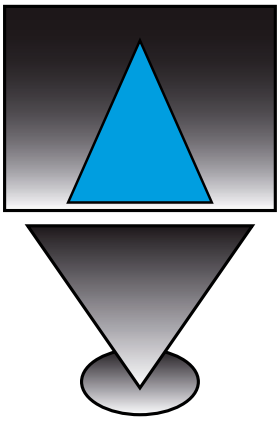


In experiment B, we set a visual analysis by comparing the current configuration of a metabolic cage for single mouse with another one in which we added an enrichment:

1) CURRENT CONFIGURATION (standard clear upper chamber +standard separation cone + standard urine ring)



2) CURRENT CONFIGURATION with enrichment (standard clear upper chamber + Plastic Insert + standard separation cone + standard urine ring)



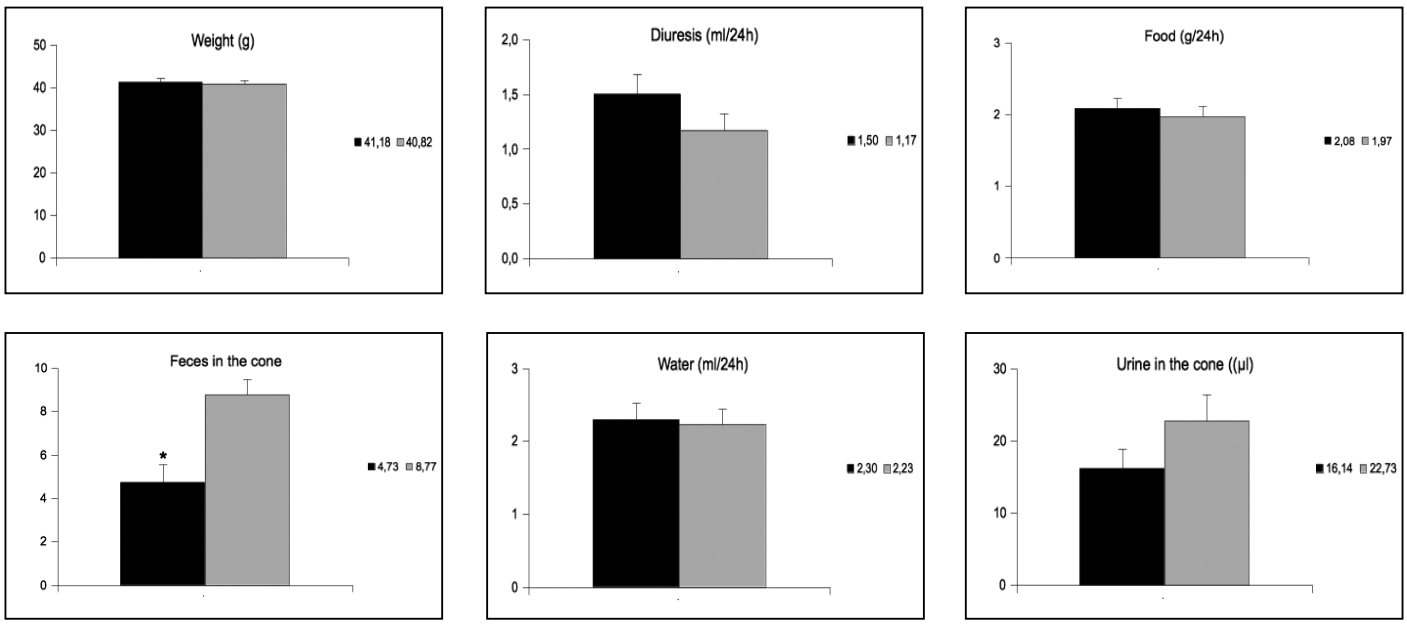
The metabolic rack with the cages were positioned in a light-controlled room kept at a temperature of about 22°C and a relative humidity of 55%, with a supply of filtered pathogen-free air, and food and water ad libitum.
At the end of each experiment that consisted of positioning the mouse in a metabolic cage for separation of feces and urine (typically within 12 to 24 hours) the following measurements were taken:
- Volume of Urine left unseparated in the cone
- Number of Feces Pellet left unseparated in the cone
Also, the following parameters have been measured for each metabolic cage on the rack, and recorded: food and water intake/24h, urine output, body weight and behaviour.

RESULTS:

· Daily Food and Water Intake; Urine Output; Body Weight:

OLD METABOLIC CAGE						
Mouse	**Body Weight (g)**	**Food Intake (g)**	**Water Intake (ml)**	**Diuresis (ml)**	**Feces in the cone (n)**	**Urine in the cone (µl)**
1o	38	2,4	2,5	0,3	8	30
2o	40	0,9	1,5	0,3	15	60
5o	43	2,1	2,5	0,9	5	40
6o	37	1,1	2,0	1,1	10	15
3o	42	2,9	3,0	3,1	9	20
4o	38	1,7	2,0	1,5	5	10
7o	40	0,8	3,0	2,2	6	5
8o	51	1,1	2,0	1,3	7	10
1o	38	2,6	1,0	0,6	15	40
2o	39	1,7	2,0	1,1	14	5
5o	44	2,2	3,5	1,0	7	35
6o	38	1,8	1,0	0,7	12	20
3o	43	2,9	1,0	0,9	8	10
4o	39	2,1	1,0	0,8	7	10
7o	41	0,6	1,0	1,2	8	5
8o	50	2,3	3,0	1,0	12	10
1o	37	2,7	2,0	0,8	12	40
2o	38	2,3	3,5	1,4	10	5
5o	44	2,2	2,0	0,6	8	35
6o	36	1,8	1,5	0,8	6	5
3o	43	2,5	3,5	2,8	4	50
4o	39	2,6	4,5	1,4	5	40
Mean +-	**40,82**	**1,97**	**2,23**	**1,17**	**8,77**	**22,73**
SD	3,95	0,69	1,00	0,71	3,32	17,09
n	22	22	22	22	22	22
SE	**0,84**	**0,15**	**0,21**	**0,15**	**0,71**	**3,64**

NEW METABOLIC CAGE						
Mouse	**Body Weight (g)**	**Food Intake (g)**	**Water Intake (ml)**	**Diuresis (ml)**	**Feces in the cone (n)**	**Urine in the cone (µl)**
3n	43	2,7	3,5	2,2	0	25
4n	40	2,0	2,5	1,1	1	20
7n	41	1,2	3,0	2,2	2	5
8n	50	0,6	1,5	1,1	6	10
1n	37	2,8	2,5	1,0	1	40
2n	38	1,5	1,5	0,9	0	5
5n	42	2,4	1,0	0,8	4	40
6n	37	1,4	1,5	0,9	13	20
3n	42	3,2	2,5	2,1	3	10
4n	39	2,0	1,5	1,2	0	10
7n	41	0,7	2,5	4,2	3	0
8n	51	1,9	5,0	1,3	8	5
1n	37	2,5	1,0	0,6	10	15
2n	38	2,7	1,5	1,8	3	15
5n	44	2,3	2,5	0,7	2	10
6n	37	1,7	2,0	0,9	7	15
3n	43	2,6	2,5	1,7	4	15
4n	39	2,4	1,5	1,1	2	5
7n	41	1,7	5,0	3,1	11	30
8n	51	2,4	1,5	1,7	9	5
1n	37	2,3	2,5	1,6	10	45
2n	38	2,8	2,0	0,8	5	10
Mean +-	**41,18**	**2,08**	**2,30**	**1,50**	**4,73**	**16,14**
SD	4,45	0,69	1,09	0,87	3,94	12,62
n	22	22	22	22	22	22
SE	**0,95**	**0,15**	**0,23**	**0,18**	**0,84**	**2,69**



T-Student (*p<0.05 , P values were derived from unpaired t-test)

During the daily observations animal behaviour seem to be influenced by the cage enrichment since animals were often found inside it. This attitude could be a further improvement for the collection of samples avoiding the undesired habit of nesting to the feeder chamber.

COMMENT AND CONCLUSION

The new design and the new material significantly improved the metabolic cage performances without affecting the animals' physiological parameters and behavior.
A proper animal behavior analysis will be effected in further experiments.

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