A COMPARATIVE STUDY ON AMMONIA LEVEL IN IVC SEALSAFE CAGES WITH THREE DIFFERENT BEDDING MATERIALS. EFFECTS ON WATER AND FEED INTAKE, BODY GROWTH AND PRE-WEANING MORTALITY OVER A FOURTEEN DAYS PERIOD.

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Materials .

Animals: ninety 20-24 g Hsd : ICR (CD1) males and ninety 20-24 g Hsd : ICR (CD1) females mice were randomly distributed into 30 groups of 6 mice per cage. The mice were SPF in accordance with Felasa guidelines and were obtained from Harlan Italy (San Pietro al Natisone, Udine Italy). The 30 groups of six mice were housed on 3 different bedding as for the following scheme:

5 cages	30 males	alpha dri
5 cages	30 males	¾ Fasern
5 cages	30 males	Corn Cob
5 cages	30 females	alpha dri
5 cages	30 females	¾ Fasern
5 cages	30 females	Corn Cob

Fifteen monogamous pairs Hsd : ICR (CD1) with 1 to 5 days old litter were randomly distributed as follows:

5 cages	5 pairs	55 pups (11 to a litter)	on alpha dri
5 cages	5 pairs	53 pups (11 to 4 litters and 9 to 1)	on ¾ Fasern
5 cages	5 pairs	55 pups (11 to a litter)	on Corn Cob

Total number of pups 163.
Cages and rack. An IVC Slim Line with double control of the air flow (supply and exhaust) was connected to a 48, 530 cm^2 polysulphone Sealsafe cages rack. The initial set (day 0) for the air flow was 58 m^3/h (102 ACH - supply) and 47 m^3/h (90 ACH - exhaust).

Recording tools. A previously calibrated Drager Multiwarn II was used to sample and record NH_3 and CO_2. For temperature, RH and speed of air, TESTO instruments were used.

Bedding, diet. Autoclaved Alpha Dri, ¾ Fasern and Corn Cob, Harlan Teklad non autoclavable rodents maintenance and breeding diet were used during the study. Tap, untreated demineralised water was used. Three control cages were distributed on the rack, the first with alpha dri, the second with Corn Cob and the third with ¾ Fasern bedding.

Methods.

Stock animals were weighed at arrival by groups of six per sex and the mean value was recorded. Litters were standardised to 11 and cross-fostering was applied when required and possible.
The 3 different beddings were distributed at 100 g to a cage, the hoppers were filled with 350 g of diet and the bottles with 250 ml of water.
The recording schedule was the following:

ACTIVITY	DAY 0	DAY 3	DAY 5	DAY 7	DAY 10	DAY 12	DAY 14
Animal Weight	YES	NO	NO	NO	NO	NO	YES
Diet weight	YES	NO	NO	YES	NO	NO	YES
Water dosage	YES	YES	YES	YES	YES	NO	YES
Bedding weight	YES	NO	NO	NO	NO	NO	YES
Room parameters	YES	YES	YES	YES	YES	YES	YES
Trolley parameters	YES	YES	YES	YES	YES	YES	YES
Cage NH_3	NO	YES	YES	YES	YES	YES	YES
Cage CO_2	NO	YES	YES	YES	YES	YES	YES
Cage temp.(inside)	NO	NO	YES	NO	NO	NO	NO
Cage temp.(outside)	NO	NO	YES	NO	NO	NO	NO

Results.

Ammonia. **Graph 1** describe the mean values of ammonia trend for the 3 different beddings. At day 7 the ammonia value is almost negligible for both Alpha Dri and Corn Cob cages, whereas, cages with ¾ Fasern bedding reach the maximum acceptable level, 25 ppm, at that time. **Graph 2** shows the mean values recorded in the breeding cages and **Graph 3** in stock cages.

The reference study suitable for the comparison of the results was published on Contemporary Topics in 1994 by M.J Huerkamp et al. In that test Huerkamp uses only one bedding material, Corn Cob, but 3 different IVC systems. Relevant differences between Huerkamp and our study are the following:

	Stock Cages density	Stock M/cage	Stock F/cage	N° of beddings (different)	Bedding quantity	IVC systems
Huerkamp	5	NT	5	1	200 g	3
Tecniplast	6	6	6	3	100 g	1

- similar cages size -

Comparable results are available only at day 7 accordingly to Huerkamp paper and show a better result for Tecniplast cages with Corn Cob bedding, the level "0" of ammonia in Tecniplast stock cages (corn cob) equal to results obtained with Micro-Flow System Cages (Allentown Caging Equipment Co Inc.).

The comparison of results between the Tecniplast and the Huerkamp tests is very difficult because no accurate details (Table results) are given in the paper. A figure, in the paper, shows the trend of ammonia in stock cages up to 14 days. Two systems, MP (Lab. Products, Inc.) and MM (Maxi-Mister, Thoren Caging Systems.) seem to reach an approximate mean ammonia level of 20 ppm, whereas MF (Micro-Flow System Cages, Allentown Caging Equipment Co Inc.) stays probably below 10 ppm. In our study the mean ammonia value detected in stock females cages with 50% less corn cob bedding was 1,6 ppm, well below the results obtained in the Huerkamp reference study .

No clear comments are given in relation with ammonia level at 14 days for the monogamous pairs with litter in Huerkamp study however in figure 2 of the paper the range of ammonia level for the 3 systems under test was approximately between >20 (Allentown) and +/- 60 ppm (Lab.Products) whereas, in our test, we recorded a mean ammonia value of 20,2 ppm in corn cob breeding cages.

The level of ammonia recorded from the negative plenum of the rack before the filters versus room ammonia level is shown on **Graph 4.** There was no detectable ammonia in the room during the 14 days of test period.

Room environmental parameters. Room temperature an RH was recorded at approx. 1,5 m from the floor in the centre of the room every time before the recording of racks and cages parameters. Results are shown on **Graph 5.**

Cages air changes. The calculated number of air changes per hour (supply and exhaust ac/h are shown on **Graph 6** the mean values of air changes and air speed were the following:

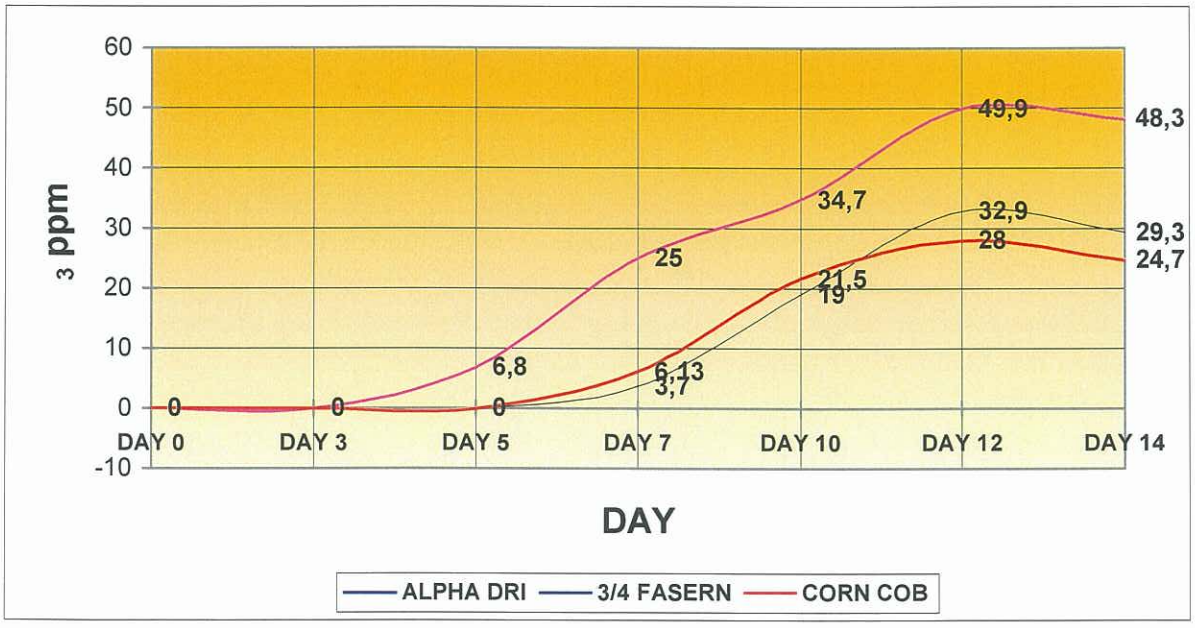
	DAY 0	DAY 3	DAY 5	DAY 7	DAY 10	DAY 12	DAY 14	MEAN
Supply ACH	102	96	95	100	100	107	101	***100***
Exhaust ACH	90	91	77	85	85	76	84	***84***
Plenum Supply Air speed	1,84	1,72	1,70	1,81	1,81	1,93	1,82	***1,8***
Plenum Exhaust Air speed	1,64	1,63	1,38	1,52	1,53	1,36	1,5	***1,5***

Mice body weight and pre-weaning mortality. There was no mortality during the test period, both stock and breeding pairs with all their pups (163) were alive at the end of the study. No differences were found between mean of body weight for both males and females housed on different bedding Furthermore, the mean body weight of stock animals was in full compliance with that expected for the standard Harlan Italy growth chart.

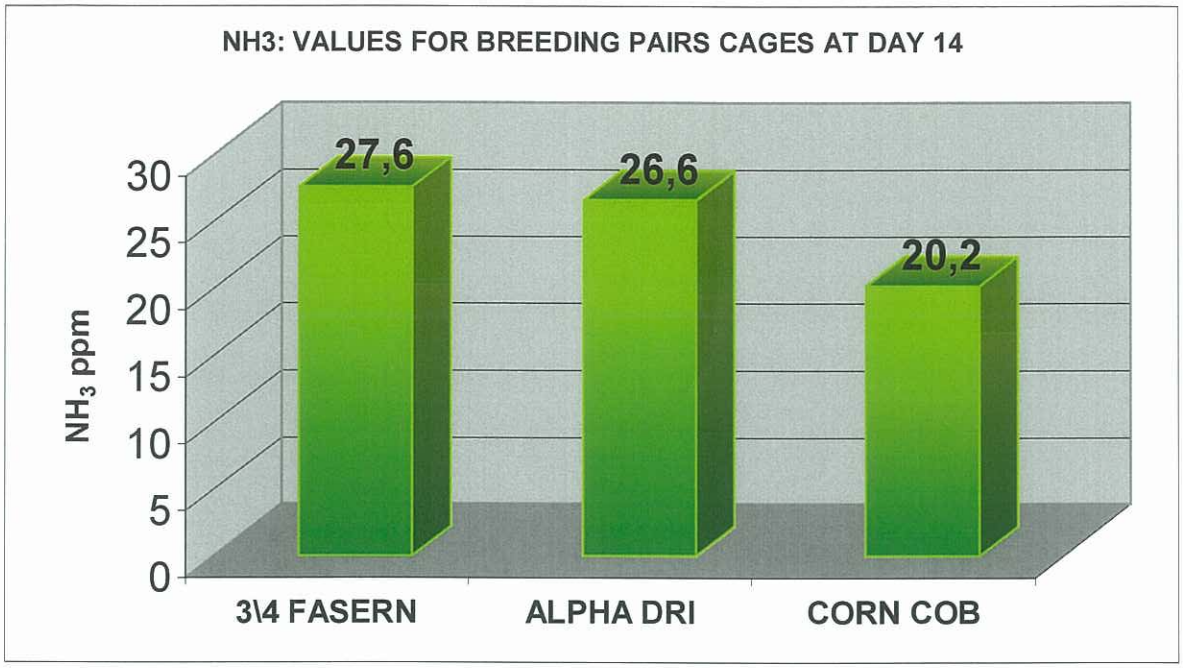
Discussion.

Influence of bedding material on ammonia growth within cages seems to be unquestionably confirmed in this study. Wood beddings do not seem to be a particularly suitable material for breeding and stock mice, even so, ventilation efficiency plays a major role. Tecniplast IVC Sealsafe system has shown its superior capability to smooth the interference of "ammonia friendly bedding" in extreme conditions: higher animal density and lower bedding content. Uniformity of ventilation between cages is important to guarantee a more homogeneous and lower level distribution of ammonia. Any feature interfering especially with the exhaust air capability of the system should be removed.
Opposite to Huerkamp findings, in our study, breeding cages have shown better performances in terms of ammonia growth versus stock cages.

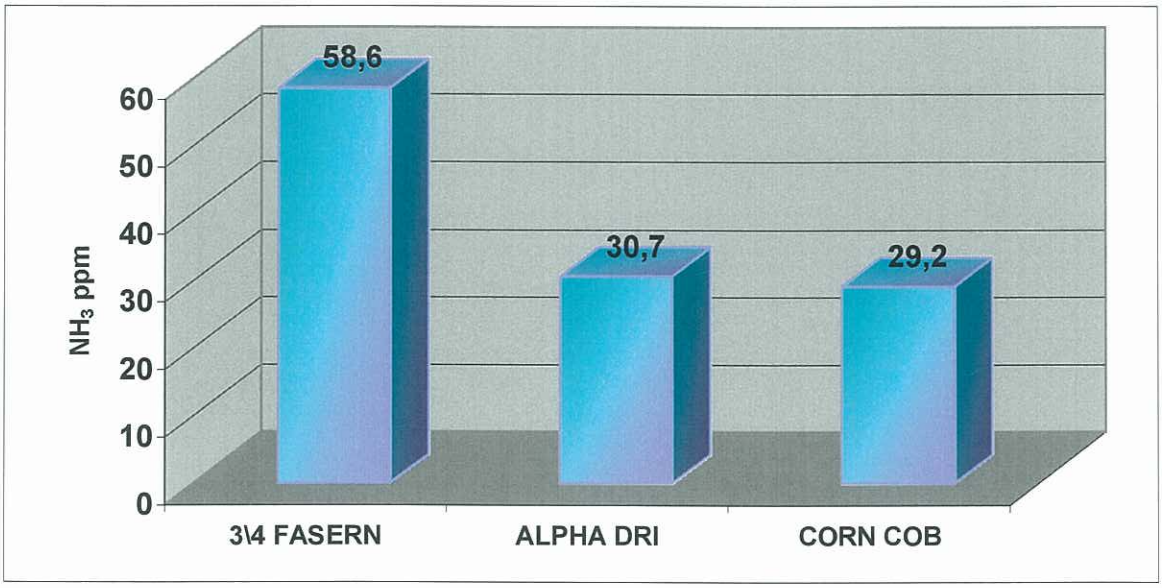
When the 25 ppm ammonia level is overtaken an effect was detected in terms of slight increased water intake (statistically not significant), but no effects on feed intake and final body weight was seen. The lower relative and absolute Alpha Dri bedding content at the end of the study is probably in relation with an higher exposed surface to the air flow for this bedding and final lower humidity level. This could be investigated in a future study.



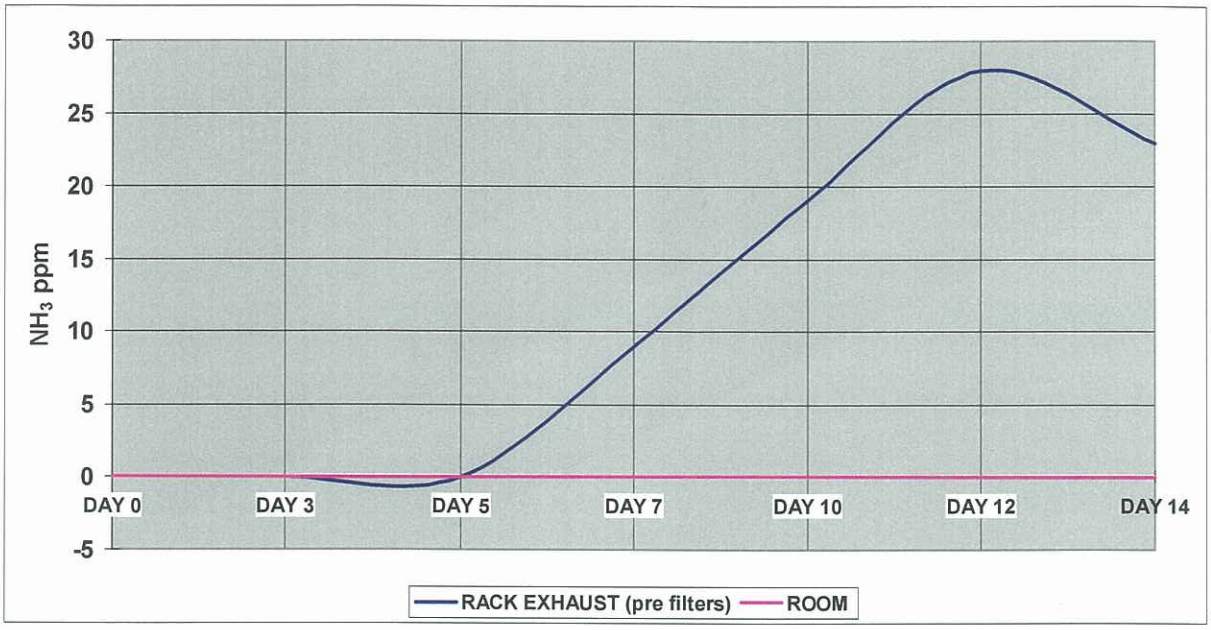
Graph.1



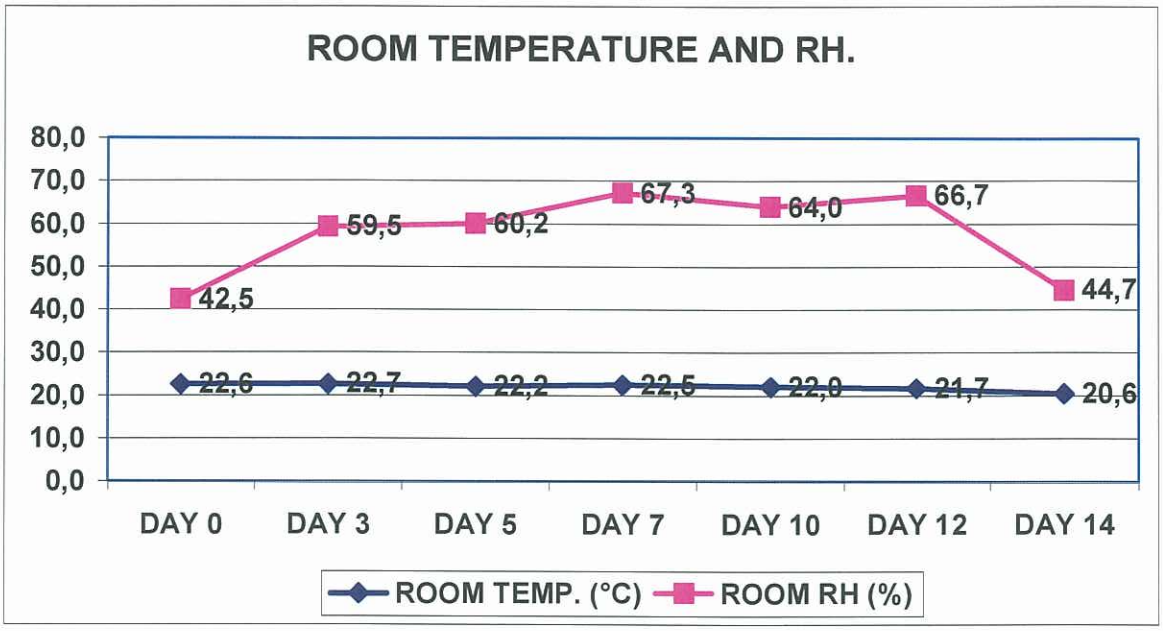
Graph. 2



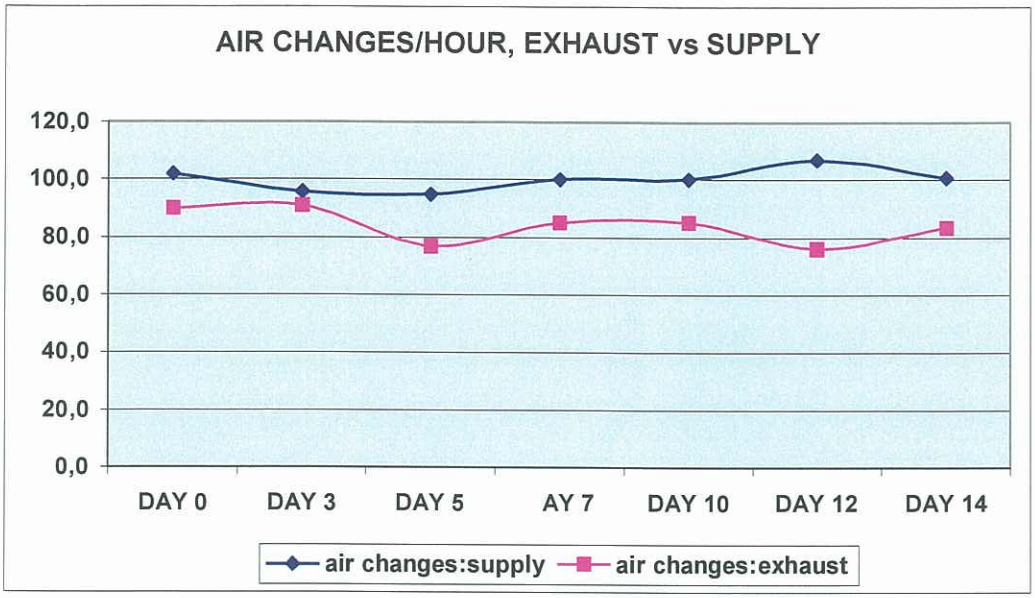
Graph.3



Graph.4



Graph.5


AIR CHANGES/HOUR, EXHAUST vs SUPPLY
120,0
100,0
80,0
60,0
40,0
20,0
0,0
DAY 0
DAY 3
DAY 5
AY 7
DAY 10
DAY 12
DAY 14
air changes:supply
air changes:exhaust

Graph 6