



## Use of intracage ventilation systems in animal facilities

Uso de sistemas de ventilação microambiental aplicada a biotérios

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### ABSTRACT

The breeding and housing of animal species in intensive production systems require a higher control of the environmental variables inside the facilities, on behalf of an atmosphere that maintains a sanitary status and also promotes the well-being of the animals. The husbandry of laboratory animals is one of those productive systems in which the environmental control should be quite rigorous due to the maintenance of defined animals such as specific pathogen-free and gnotobiotics. For a long period, the control of ventilation and environmental factors of conventional animal facilities was achieved by using thermodynamic principles used for human comfort. However, in the last decades, new approaches to solve the environmental problem in animal rooms were studied, and the ventilation systems developed with base in forced convection of the air were the ones that presented the best results until the present time. Thus, this review presents, in an historical perspective, the evolution of alternative devices for caging of laboratory animals and their effects on the animals.

**Key words:** animal facilities, housing, caging, ventilation, rodent, environment.

### RESUMO

A criação e manutenção de espécies animais em sistemas intensivos de produção requerem um maior controle das variáveis ambientais dentro das instalações, em prol de um ambiente que mantenha um padrão de sanidade na colônia e promova o bem-estar dos animais. A produção de espécies de animais de laboratório é um desses sistemas produtivos em que o controle do ambiente deve ser bastante rigoroso devido a manutenção de linhagens sanitariamente definidas como animais livres de patógenos específicos ou gnotobióticos. Por um longo período, os princípios de ambiência utilizados em biotérios não passavam de uma mera aplicação dos princípios termodinâmicos para conforto humano. Entretanto, nas últimas décadas, novas abordagens para o equacionamento do problema ambiental em salas de animais foram estudadas, sendo que os sistemas de ventilação desenvolvidos com base no princípio da convecção forçada do ar foram os que apresentaram os melhores resultados até o presente momento. Dessa forma, esta revisão apresenta, em uma perspectiva histórica, a evolução dessa alternativa para alojamento de animais de laboratório além de abordar os avanços na área de ambiência em biotérios e seus efeitos sobre os animais.

**Descritores:** Biotério, alojamento, ambiência, ventilação, roedor, ambiente.

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### I. INTRODUCTION

Since last century, the animal experimentation has been contributing to the evolution of the biological sciences and the study of new theories based on *in vivo* assays with animal models. Thus, with the development of the scientific research there was the requirement to supply and to house animals with the quality demanded by the researchers. In this way, the animals were standardized in the genetic and sanitary status, appearing thus new strains and the concern with the sanitary status of the animals. At the same time, there was the requirement of standardization of the environment where the animals are bred and maintained; therefore, it is known that certain experimental results are susceptible to alterations for variations in the environmental factors [10].

For a long time, it was considered that the control of the environmental conditions in animal rooms (macroenvironment) it would be enough for the control of the environmental variables inside of the animal cages (microenvironment). In this manner, the concepts recommended for human comfort were applied to animal rooms [46]. These systems are known as VGD system (General Diluting Ventilation system) and although, unquestionably efficient for the man, the use of VGD settings in animal facilities demonstrated to be inadequate.

The microenvironment possesses environmental characteristics different to those of the macroenvironment, mainly in function of the mechanisms of thermal changes and liberation of gases by the animals that are processed inside the cage [1,2]. As a result of the several metabolic processes, products are detected as water vapor, carbon dioxide (CO<sub>2</sub>), particles in suspension and ammonia (NH<sub>3</sub>), being necessary the removal of those pollutants on behalf of the maintenance of favorable conditions for breeding of laboratory animals. Besides the removal of the

polluted air, it is necessary the supply of new, clean air in adequate amount and quality [6,30,48,60].

The process of air exhaustion or supply, conditioned or not, by natural or mechanical means, from/ for a closed enclosure, with the purpose of removing pollutants or to maintain comfort conditions is denominated ventilation [41]. The ventilation is divided in two great principles: the ventilation for process (VLE) and the ventilation for comfort (VGD). The VLE consists, generally, in removing the industrial pollutants, while VGD is based on temperature control and reduction of the carbon dioxide tension (CO<sub>2</sub>) in the areas destined to the human use [3,61].

The application of VGD in animal facilities began in the 1940's, with the use of a system in which the ventilation occurred through a mixture of external and internal air, recirculated after previous filtration [46]. For many years, the VGD system was used in animal facilities. The VGD system should maintain the temperature as uniform as possible, the relative humidity around 50%, with an air renewal of 18 changes per hour, without recirculation, and insufflation by the ceiling and exhaustion at the floor level. In spite of the limitations of VGD, the animal facilities projected with it undoubtedly present better quality than facilities without any ventilation devices.

Nevertheless, the use of VGD system for laboratory animals presents several limitations. The most important is that even increasing a maximum ventilation rate for a room it does not yield the same effect inside the cages, its efficiency is decreased and the microenvironment presents the challenges of high temperature, humidity and pollutants concentration due to decreased air speed inside the cages [47,79].

The pollutants formed inside the cages reach the macroenvironment in virtue of the airflow distribution pattern. Some works describe a significant difference of the air speed among the airflow directions existent inside and outside the boxes, favoring

the accumulation of substances inside the cages [47, 79]. The airflows are also responsible for the removal of the corporal heat of the animals, because that process occurs mainly through the free convection. The convection is one of the ways of heat transmission, caused by the relative movement of the particles of the fluid, due to the difference of pressure generated by the difference of temperature and consequent difference of density of the air mass [18,24,25]. Basically, the cages possess low thermal conductivity because they are manufactured with plastic material (polypropylene and polycarbonate) and, consequently, increase the difference of temperature between micro and macroenvironment, making the temperature higher in the microenvironment, facilitating naturally the convection process (free convection) [4,15, 21,75].

## II. INTRACAGE VENTILATION SYSTEMS

### 1. Antecedents

A more specific approach for animal facilities has began to be studied, based on the forced convection. The forced convection is the phenomenon due to the intensification of that process produced by the agitation of the air mass, in this case, by fans that promote the passage of an unidirectional airflow inside the boxes [24,26,27,70,74].

In the late 1950's, it was built the first cage using the forced convection principle in order to maintain animals free from contaminations. That system had cylindrical format and it was elaborated with stainless steel covered by a filtering membrane that assured the health of the animals [33].

In 1970, the first step of a new technology for animal facilities ventilation was proposed, consisting of a rack that was composed by a *plenum* chamber responsible by promoting an airflow upon the cages using the same air of the room [35]. A similar device was capable to prevent the contamination of gnotobiotic animals maintained in cages without filter tops [73]. The first registration of a cabinet for animals with equipments for thermal control of the air occurred in 1972 for the housing of specific pathogen-free chickens [75].

The reduction in the bacterial counting inside a rack for maintenance of rats was obtained with use of a vertical descendant curtain of air at 0.9 m/s [42,73]. The low counting of bacterial colonies were

also verified inside a cabinet for maintenance of guinea pigs, however, unlike the previous, that equipment was the first to operate with internal pressure higher than the external pressure [55].

In 1973, it was described a system in which the air was thrown at the room by the ceiling and removed through openings on the wall, behind the shelves, that coincided with the superior level of the cages. In such system, the exhausted air was really removed from the room after contact with the boxes, decreasing the possibility of return to the room [1,20, 72,77]. The same system served as a model for the development of another one where the exhaustion was performed by a plenum chamber of negative pressure ("full-wall") whose structure provided also the separation of the animal rooms [25]. The use of a chamber ventilated by a vertical descendant airflow demonstrated a directly proportional relationship between the filtered air speed and the maintenance of the sanitary status of axenic mice [43,57].

The equipments developed until then promoted the change of air of the interior of the cages through diffusion at the superior level of the cage covers [44]. However, when the use of filter diffused for control of infections in animal facilities was spread, the differences between macro and microenvironment increased. The evaluation of three types of filters for box covers, taking in account environmental variables, has demonstrated that cages with polycarbonate filters tops presented higher carbon dioxide concentrations when compared to those with top filters constituted of polyester [17,37]. The relative humidity of the air was higher in the boxes equipped with filters tops, independently of the type of used filter. However, significant differences were not observed in the temperature and ammonia concentrations of the microenvironment, for all types of used filters. However, these results were significantly higher than those observed in the macroenvironment [17,38].

The alternative to reduce the levels of pollutant inside the cages was the adoption of a system of individual ventilation for each cage (called intracage ventilation system) as previously proposed [33].

### 2. Effects on Animal Health

Aiming the improvement of the microenvironment, ventilation systems with positive pressure inside the cages were developed and they demonstrated ability to control more efficiently temperature,

humidity relative of the air, levels of ammonia, carbon dioxide and allergens [5,22,23,29,31,32,34,38,39,56,58,76]

The ventilation systems with positive pressure (PIV systems - Positive Intracage Ventilation) described until now are characterized by the insufflation of conditioned air inside the cages with air speed ranging from 0.09 m/s to 0.5 m/s [4,34,71] and exhaustion through a plenum chamber of negative pressure. With speed of air of 0.09 m/s, the levels of ammonia remained around 20 ppm in rat cages (biomass of 500 grams by box) along a period of 13 days without bedding changes in a PIV system [78]. In a subsequent study, it was observed that the same speed was enough to control the CO<sub>2</sub> and oxygen (O<sub>2</sub>) concentrations inside the rat boxes [28].

A commercially PIV system available was analyzed and demonstrated to attend the exigency of England's "Home Office Code of Practice", being capable to maintain the animals free from contaminants and other stressful factors, making possible a less variable environment and, therefore, minimizing possible interferences on the experiments, besides protecting the researcher [16].

The formation of thirteen pollutants, including ammonia and CO<sub>2</sub>, in eight different bedding types was measured in PIV cages with air speed of 0.3 m/s. The corn cob bedding was the one that favored less the production of ammonia, increasing the interval of bedding changes frequency and thus reducing the stress of the animals caused by handling the boxes [36,49,51]

In three PIV systems the levels of pollutants were compared with those found in conventional boxes. As results, significant differences in the temperature and in the levels of CO<sub>2</sub> were not observed, but ammonia levels and relative humidity of the air were higher in the conventional cages when compared to the three types of PIV cages. As for the intensity and the frequency of the sounds, the three PIV systems produced more sounds, up to 250 Hertz (Hz), than the macroenvironment. However, those systems generated less high frequency sounds than the macroenvironment that are less tolerated by the animals. Such results indicate that the noises produced by the PIV systems in the range of frequency measured (31.5 Hz up to 16000 Hz), probably exert little effect on the animals [50].

Although an air renewal rate in the macroenvironment about five air changes per hour is known

to be enough to control levels of ammonia and CO<sub>2</sub> inside the boxes [52,53], individually ventilated caging systems from several manufacturers are available with a variety of air renewal rates. Thus, optimal ventilation rates for PIV systems have not been determined yet. Rates differ among systems and they may vary according to the cage design as well. In spite of different configurations, it is not known yet which is the best distribution of air inside the cage, the most appropriate speed, or which air renewal rate per hour is more appropriate to provide a better housing for the animals [71]. Although increased airflows may have advantage in reducing pollutants such as ammonia, carbon dioxide and humidity values, they may have potentially detrimental effects upon the animals (chilling). Those data can establish a new concept regarding to cage density and conditions in these PIV systems [53].

The temperature variation, relative humidity of the air, levels of ammonia and CO<sub>2</sub> in function of the bedding change frequency were studied in PIV cages with mice. With a ventilation rate of 30 air changes/hour, cages with four adult mice reached ammonia levels of 10 ppm in the seventh day without bedding changes. However, a box containing a couple and their offspring need 100 air changes/hour to maintain the same levels of ammonia in the same period [51,58].

The limiting factor of PIV systems commercially available is the expensive acquisition and operation cost, since air insufflated inside of the system must be previously conditioned [16].

### **3. Microenvironmental Ventilation Systems (VMA systems)**

With base on the same process, an equipment was developed using just ventilation inside the boxes of the animals, with exhaustion of the air outside of the room (system VMA) [45,54]. Such system operated through plenum chambers of insufflation and of exhaustion dispensing secondary ducts of connection between the boxes and main ducts, as in the systems previously described and neither needed the air conditioning [7,11,12,14,62,68].

The advantages of this system are not limited to economical costs of operation, but mainly it possesses sanitary advantages since the animals evaluated until the moment showed increased productivity, less respiratory lesions and rheological proper-

ties similar to those animals maintained in systems with air conditioning [40,63-66]. Regarding to the reproductive performance, both mice and rats maintained in the VMA system presented reproductive indices higher than the animals maintained in a conventional ventilation system [8,13,69]. Swiss mice maintained in VMA system were accompanied during four gestations and they presented shorter intervals between parturitions starting from the second delivery when compared with animals housed in the conventional system. The number of born and weaned animals was also higher from the second litter in the breeders maintained in the VMA system [69]. Similar trial using Wistar rats demonstrated that couples housed under the VMA system, with air speed between 0.03 and 0.80 m/s, did not present alteration in fertility or life span indices. However, the offsprings were significantly larger and more uniform at the birth and at the weaning there was less mortality of neonates [13].

The reduction of the levels of ammonia was also observed in all air speed ranges used in the VMA system, with the consequent reduction of respiratory lesions induced by the concentration of ammonia in the atmosphere [7,59]. Histopathological analyses have been demonstrating to smaller incidences of lung lesions in animals maintained in the system [59], attributed probably to the low ammonia concentrations inside the cages in VMA system by increasing evaporation of bedding humidity provided by air flow [9,67]. As result, the offsprings of the mice and rats maintained in the VMA system presented smaller incidences of lung lesions in a broad range of air speed, especially those settled between 0.19 and 0.80 m/s [13, 69]. Similar data were obtained in adult Wistar rats housed for 180 days in VMA system with different intervals of bedding changes [8]. The group maintained with bedding change frequency of 7 days presented lower incidence of lung lesions (congestion, peribronchitis and interstitial pneumonia) when compared to all bedding change intervals group [8]. The epithelial integrity was also studied by transepithelial membrane

potential (DDP) on the nostrils, epiglottis and trachea of rats kept under VMA systems and submitted to different intervals of bedding change. DDP evaluation presented significant differences in the results from trachea and epiglottis in the group housed with bedding changes of 7 days when compared to the others groups. This group showed minor lesions, presumably by lower ammonia concentrations in caging conditions [8]. Thus, the use of the VMA system is may possibly increase the bedding change frequency, reducing the operational cost of the animal facilities without prejudice for the sanitary quality of the animals kept in animal facilities [8].

The promotion of an appropriate environmental condition is also proven by the studies of rheological properties of the mucus of animals maintained in VMA system. In spite of being submitted to speeds of air superior to the animals maintained in conventional system, parameters as *in vitro* mucus transportability, contact angle and rigidity of the mucus do not differ from material originated from animals maintained in systems with air conditioning [19,65].

### III. CONCLUSION

Although there are different conceptions among the systems that use unidirectional airflow inside the cages (PIV systems and VMA system), the results obtained up to now demonstrate that a technology type provides an improvement in the quality of the air, making possible the increase of the interval of bedding change without any prejudice for the sanitary status of the animals. The VMA system differs of the PIV system for the fact of not using the conditioning of air, becoming more accessible to the researcher and reducing the operational cost at the same time that the animals maintained in that system possess similar characteristics to the animals maintained in PIV systems [8].

Although the accomplished studies approach just outbred rats and mice, it is important to evaluate the use of the VMA system for other strains and species of laboratory animals.

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