

## TECHNICAL NOTE: ASSESSMENT OF BEST LOCATION OF GNAWING STICKS IN GROWING RABBIT CAGES

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**Abstract:** The present work tries to determine the best location of wooden sticks for environmental enrichment, considering usage and level of contamination as variables. A total of 540 rabbits at 4 wk of age were caged in 60 groups of nine animals in conventional rabbit cages, and the effect of the allocation of wooden sticks was studied by fixing them on the floor in 30 cages and on the cage ceiling in the other 30 cages. The sticks were sampled during the fattening period (5 wk) to evaluate the contamination of the sticks, with detection of the most common faecal indicator bacteria in rabbit production (*E. coli* and *Clostridium* spp.). Likewise, quantitative enumeration analyses for *E. coli* were performed at the same sampling times for detection of both bacteria. Sticks used for environmental enrichment of the cage in this experiment were made of fir (*Picea abies*). According to the results, placing the wooden stick on the ceiling instead of the floor reduces stick consumption (6.28±1.75 vs. 13.55±1.75% of stick consumed). Moreover, microbiological analyses revealed that wooden sticks placed on the ceiling were less contaminated than those placed on the floor (2.8 and 22.8% for *E. coli*, respectively and 1.1 and 10.6% for *Clostridium* spp., respectively). In conclusion, fixing the sticks on the ceiling of the cage was revealed as a safer choice during most weeks of the growing period.

**Key Words:** animal welfare, rabbits, environmental enrichment, bacterial contamination, environmental stimulus location.

## INTRODUCTION

In nature, rabbits spend much of their time searching for food, mainly during dusk and dawn (Lockley, 1961). Food is easily accessible in commercial rabbit farming, resulting in the animals needing to spend less time searching for or eating food. Generally, the commercial meat rabbit's environment is barren, composing a wire-mesh cage equipped only with a feeder and a nipple drinker (McNitt *et al.*, 2000). Commercial meat rabbits have more unoccupied time and no way to express foraging behaviours. The boredom and frustration generated by this situation may lead the rabbits to fill their time either by resting or showing abnormal behaviours, such as biting or licking the cage, or excessive grooming (Baumans, 2005; Trocino *et al.*, 2013; Rommers *et al.*, 2014b). Therefore, one of the possibilities for improving the living conditions is to enable more species-specific behaviour by providing environmental enrichment (Love, 1994; Baumans and Van Loo, 2013; Rommers *et al.*, 2014b).

There are many possible types of environmental enrichment materials that have already been tested: edible ones such as wood (Princz *et al.*, 2008; Zucca *et al.*, 2012), hay (Berthelsen and Hansen, 1999; Maertens and Van Oeckel, 2001), straw (López *et al.*, 2004), grass, and coarse mix feeds (Leslie *et al.*, 2004), and non-edible ones such as toys, metal cans (López and Gómez, 2003), bunny sticks and blocks, nylon rods, kongs (Harris *et al.*, 2001), stainless

steel rabbit rattles on spring clips (Johnson *et al.*, 2003) and elevated platforms (Lang and Hoy, 2011). Most efforts have been focused on gnawing material or the use of an elevated platform, as studies have demonstrated that rabbits prefer edible enrichment items, and have also shown some beneficial trends in terms of welfare and even production, increasing chewing and reducing stereotypies as well as skin lesions, especially on the ears (Princz *et al.*, 2009; Rommers *et al.*, 2014a).

Rabbits also have a biological need for gnawing, which in a semi-natural environment they satisfy through gnawing on roots or branches (Stauffacher, 1992). Sticks of soft wood could be the best method of environmental enrichment for rabbits (Baumans, 2005), and no negative impact on rabbits' performance has been described (Jordan *et al.*, 2008). Although wooden sticks are very interesting environmental enrichment elements and useful to increase animal welfare, they must be tested, as they might become a contaminated and a source of infectious diseases due to hygiene problems, especially in cages that house more than one animal (Mirabito *et al.*, 2000; Dal Bosco *et al.*, 2002; Mirabito, 2003). Thus, the possible infection by fomite materials such as bedding, enrichment elements, feed and other items has to be taken into account in long-term experiments, such as fattening period, as it could be a source of pathogen contamination (Matsushita *et al.*, 1989; Kylie *et al.*, 2017).

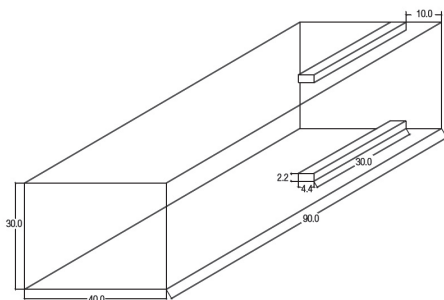
The objective of this work is to determine the best location of the environmental enrichment wooden sticks according to their use and microbiological contamination.

## MATERIAL AND METHODS

During this study, 540 four-week-old rabbits were caged in 60 groups of 9 animals in conventional wire-mesh meat rabbit cages, 40×90×30 cm (W, L, H) in only one tier, which means 40 kg/m<sup>2</sup> at the end of the experiment. Enrichment of the cages with wooden sticks was performed by fixing the sticks just under the ceiling in 30 cages and on the floor in the other half of the cages (Figure 1). All the cages had 4 females and 5 males, chosen randomly, as the commercial conditions were being reproduced.

Sticks used for the cage environmental enrichment in this experiment were made of fir (*Picea abies*) and their dimensions were 4.4×2.2×30 cm. The material was selected according to previous publications, which had proposed *Picea abies* as suitable for gnawing sticks in rabbits (Princz *et al.*, 2007; Jordan *et al.*, 2008). All sticks were weighed at the beginning and end of the experiment and, in order to correct the degree of moisture, sticks were dried at 100°C for 24 h before each weighing. Then, the weight loss percentage was obtained, assuming it as the level of consumption.

The sticks were sampled during the fattening period to evaluate the faecal contamination of the stick throughout rearing with the most common faecal indicator bacteria in rabbit production (*E. coli* and *Clostridium* spp.). Sticks were sampled at the start of the experiment (wk0) to assess the initial microbiological status of the sticks, and then were sampled at weekly intervals (1-5 wk of fattening period). Sampling was performed by rubbing a sterile swab along the stick surface. To standardise the sampling, the sticks were divided into 5 consecutive equal sections to ensure that at least 2 cm<sup>2</sup> from each stick face were sampled every week. Coinciding with the sampling day, physical conditions of the animals were evaluated (skin lesions).



**Figure 1:** Scheme of the rabbits' cage and the two locations (fixed on the ceiling or on the floor) of the gnawing sticks. Dimensions in cm.

### Microbiology analysis

A total of 360 samples were taken and analysed at the beginning (wk0) and during the fattening period (wk1-wk5) for the isolation of *E. coli* and *Clostridium* spp. All samples were transported to the laboratory, refrigerated,

and analysed within 24 h. The swab was inoculated into 9 mL of buffered peptone water (BPW, Scharlab®) and homogenised. For *E. coli* detection, 1 mL of the homogenised sample was transferred into a tube with 9 mL of BGBL (Brilliant Green Bile Lactose Broth, Oxoid®) with a Durham bell and incubated at 45°C for 24-48 h. The sample was considered as positive growth if the Durham bell had gathered gas proceeding from the lactic fermentation in presence of bile salts. If positive, 0.1 mL of the corresponding dilutions were deposited (by duplicate) on the surface of Levine agar plates (Oxoid®) and distributed quick and uniformly. Then, Levine plates were incubated at 45°C for 48 h. After the incubation period, plates presenting grey-brown colonies of metallic sheen were considered positive. For *E. coli* enumeration, 10-fold dilutions in phosphate-buffered saline were prepared from the homogenised BPW sample. Then, 0.1 mL of each dilution was deposited (by duplicate) onto Levine agar plates and distributed quick and uniformly. Plates were incubated at 45°C for 48 h. After incubation, all the bacterial colonies were enumerated using the colony counter and further identification of *E. coli* was carried out on the basis of morphology, cultural characteristics and biochemical tests (API-20 test, Biomerieux®).

For *Clostridium* spp. isolation, 1 mL of the homogenised sample was inoculated on SPS agar tube and covered with 1 mL of liquid Vaseline to induce anaerobiosis (Sulfite Polymyxin Sulfadizine agar, Oxoid®). All tubes were then incubated at 45°C for 48 h. After the incubation period, tubes presenting black pepper-like colonies were considered positive.

### Statistical analysis

Statistical analyses were carried out using Statgraphics Centurion v. XV (Statpoint Technologies, Inc. Warrenton, Virginia, USA) and the significance level was set to 0.05.

Consumption percentages of the sticks were assessed using ANOVA, where treatment (floor or ceiling) was included as explanatory factor. As consumption percentages did not behave normally, a logarithmic transformation was performed. Microbiological analysis results were subjected to chi-squared tests (for absence/presence tests) and ANOVA (for *E. coli* enumeration).

## RESULTS

Results showed that stick consumption was significantly higher ( $P=0.0048$ ) on the floor ( $13.55\pm 1.75\%$ ) than in the ceiling sticks ( $6.28\pm 1.75\%$ ).

Out of the total 360 analysed samples, 180 were obtained from sticks allocated to the floor and 180 from sticks allocated to fixing under the cage ceiling.

All samples collected from sticks at the beginning of the experiment (wk0) were negative for *E. coli* and *Clostridium* spp. detection. According to the samples collected throughout the fattening period, 12.5 and 5.8% were positive for *E. coli* and *Clostridium* spp. respectively. Wooden sticks were more contaminated with *E. coli* if they were located on the floor (22.8%) than on the ceiling (2.2%) ( $P<0.001$ ). Likewise, sticks were more contaminated with *Clostridium* spp. if they were located on the floor (10.6%) than on the ceiling (1.1%) ( $P<0.001$ ). The results are weekly summarised in Table 1. In addition, when the enumeration of *E. coli* was analysed, statistically significant differences were found among samples collected from the sticks located on the floor or ceiling ( $P<0.001$ ). Samples collected from floor sticks were over 3.000 Log<sub>10</sub> colony forming units (CFU)/cm<sup>2</sup> from week 1 until the end of the fattening period (Table 2). As also shown in Table 2, the counts from ceiling sticks were negative until week 5. In this specific week, no statistical differences in counts were found from sticks located on the ceiling or the floor (3.513 and 3.468 Log<sub>10</sub> CFU/cm<sup>2</sup>, respectively).

According to the most contaminated sticks (floor sticks), no statistical differences were found between the bacterial

**Table 1:** *E. coli* and *Clostridium* spp. detection per week and treatment (number of positive samples).

Week	Positive samples			
	<i>E. coli</i>		<i>Clostridium</i> spp.	
	Ceiling	Floor	Ceiling	Floor
0	0	0	0	0
1	0	5	1	4
2	0	16	0	4
3	0	8	0	6
4	0	7	1	4
5	4	5	0	1
Total	4	41	2	19

**Table 2:** *E. coli* enumeration per week and treatment (Total count log<sub>10</sub> colony forming units/cm<sup>2</sup>).

Week	Ceiling	SD	Floor	SD
0	0	-	0	-
1	0	-	3.422	0.639
2	0	-	4.187	1.206
3	0	-	3.844	1.0749
4	0	-	3.837	1.149
5	3.513	0.521	3.468	0.556

SD: standard deviation.

those that do not (Jones, 2002). However, it should also be considered that the elements used for environmental enrichment may occasionally be of unknown origin, and are introduced in the productive environment with barely any control. Moreover, these objects could be safe at origin but in a farm environment could be contaminated by rabbit enteric microorganisms such as *E. coli*, *Clostridium* or *Lawsonia intracelularis*. In consequence, they can represent an important focus of growth and accumulation of pathogenic microorganisms (Kylie *et al.*, 2017). It is considered that if the environment of a farm has a low hygienic quality, then the sanitary status of an animal may be endangered. For this reason, pathogens such as *E. coli* have been widely analysed as an indicator of hygiene quality in surfaces and water worldwide.

Enteric disorders in rabbit kits from birth to the end of the fattening process are the main cause of mortality, and thus the main factor behind economic losses in rabbit production in European countries (Nouaille and Alberti, 2006; Rosell and De La Fuente, 2009; El-Helw *et al.*, 2014). Thus, it is important to reduce the possible appearance and accumulation of microorganisms that enhance enteritis or diarrhoea on environmental enrichment elements given to fattening rabbits, as microbial growth can persist on them even after they have been sanitised (Bayne *et al.*, 1993, Kylie *et al.*, 2017).

All these implications make the placement of the environmental enrichment elements a key point to take into account if animal welfare is to be improved.

In this study, a wooden stick for gnawing and 2 placements were tested. Regarding the animals' placement preference, they clearly used the sticks located on the floor more than the ones on the ceiling. This preference might be caused by a simple accessibility issue, given that the stick is more readily accessible while on the floor rather than on the ceiling. On the contrary, the microbiological study showed that the optimal placement of the wooden stick was the ceiling rather than the floor, as shown by the percentage of positive samples of faecal indicators. This might indicate that an intermediate solution such as placing the sticks fixed in the lateral wall could be a possibility. In this study, it was observed that there is no influence between the contamination of the sticks and the animals' health (Kermauner *et al.*, 2004; Jordan *et al.*, 2008). However, it is important to highlight that the infectious nature of enteric disease of rabbits combined with poor biosecurity practices may contribute to disease transmission, so for this reason it is necessary to avoid possible infection by materials such as enrichment elements (Kylie *et al.*, 2017)

## CONCLUSION

In conclusion, fixing the wooden sticks to the cage floor allows the best enrichment of the animals' environment (which can translate as better productive and welfare parameters). However, fixing the sticks on the ceiling of the cage was revealed as a safer choice during most of the weeks in the growing period. This allows enrichment of the animals' environment and avoids contamination of the sticks by pathogenic microorganisms that might cause the animals severe illnesses during the fattening period, and thus entail severe economic losses.

contamination (detection/enumeration) and the moment of sampling (week 1 to 5). Finally, no skin lesions were observed on the animals throughout the fattening period.

## DISCUSSION

Both for society and according to current standards, it has become increasingly important that animals in production should live in an environment that ensures their welfare (Blokhuis *et al.*, 2003; Duncan, 2005). It has been proven that animals living in favourable environments during their production cycle have higher productive results than

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