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# Dietary level of fibre and age at weaning affect the proliferation of *Clostridium perfringens* in the caecum, the incidence of Epizootic Rabbit Enteropathy and the performance of fattening rabbits

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ABSTRACT

An experiment was conducted to investigate the effects of dietary fibre content and weaning age on *Clostridium perfringens* proliferation in the caecum and fattening mortality in growing rabbits farmed in a facility having Epizootic Rabbit Enteropathy. The experiment consisted in a 2 × 2 factorial arrangement with two weaning ages (28 days vs. 42 days) and two levels of dietary neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash (aNDFom; 330 g/kg vs. 425 g/kg). Controls were made during two consecutive experimental periods that differed in hygienic environmental conditions by modifying the intensity of cleaning and disinfection in the farm previous to the trial. An interaction (P<0.001) was detected among the independent variables studied on *Cl. perfringens* enumeration in the caecal contents, as minimal values for this trait were obtained in non-medicated animals reared in a clean environment, and especially when they were weaned at a later age and fed the diet with the lower fibre content. The treatments studied also led to a variation in fattening mortality (from 4.7% to 34.0%), which was highly and positively correlated (P<0.001) to the average *Cl. perfringens* caecal counts in each combination of treatments. The results of the current study indicate

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that high counts of *Cl. perfringens* in the caecum can be used as an indicator of Epizootic Rabbit Enteropathy, and suggest that strategies designed to control its proliferation in the caecum might help to limit fattening mortality in rabbit fed diets not-medicated with antibiotics.



## 1. Introduction

Since its emergence in early 1997, the condition referred to as Epizootic Rabbit Enteropathy (ERE) has caused an increase in the fattening mortality rate in European intensive farms up to average values of 15% (Licois et al., 2006), and hence has been an important cause of economic loss. Even if the aetiology remains unknown and poorly understood, several works have pointed out that proliferation of spore-forming bacterium *Clostridium perfringens* could be a consequence of ERE and may be associated to mortality caused by this disease (Marlier et al., 2006; Dewrée et al., 2007; Szalo et al., 2007). Currently, the harmful effects of ERE have broadly been controlled by adding some antibiotics to the feed (Duperray et al., 2003; Boisot et al., 2003b; Bostvironnois and Morel, 2003; Chamorro et al., 2007b). However, the use of antibiotics raises human food safety concerns, which emphasizes the need for the design of alternatives to control ERE.

As ERE is a digestive disorder, appropriate nutrition in the post-weaning period might have a significant role as a prevention factor. Insoluble fibre (NDF) supply decreases caecal retention time (Gidenne et al., 1998), thereby reducing caecal microbial growth (García et al., 2000). On the other hand, an excess of NDF in the diet also has a detrimental effect on mucosal integrity of jejunum at 45 days of age (Álvarez et al., 2007). A delay of age at weaning might also be a useful strategy in order to improve intestinal health in fattening rabbits, as milk intake seems to confer a transitory protection against gut pathogens, such as enteropathogenic *E. coli* O103 strain (Gallois et al., 2007). However, neither the effect of NDF supply nor that of weaning age on *Cl. perfringens* enumeration in digestive contents and mortality rate due to ERE have been studied so far in young rabbits.

The aim of this study was to evaluate in two consecutive fattening periods the effect of two different concentrations of dietary neutral detergent fibre (aNDFom; 330 and 425 g/kg), and two weaning ages (28 days vs. 42 days) on the enumeration of *Cl. perfringens* colonies in gut contents at 0 or 14 days after weaning and on the mortality throughout the fattening period.

## 2. Material and methods

### 2.1. Animals and housing

A total of 80 New Zealand × Californian rabbit does (originating from strains genetically improved at the Universidad Politécnica of Valencia, Spain) were used. Half of them were artificially inseminated 4 days after parturition and their litters were weaned at 28 days of age (early weaning, EW), whereas the other half was inseminated 14 days post-partum and weaned at 42 days (late weaning, LW). Data were obtained in two consecutive parturitions, in which animals stayed in the same reproductive rhythm. Average litter size at weaning was 8.9 rabbits.

Trials were conducted in a farm sited at the University experimental facilities. Rabbit does and litters up to weaning were kept in the same building. A cycle of 16 h of light and 8 h of dark was used throughout the experiment. Heating and forced ventilation systems allowed the building temperature to be maintained between 16 and 24 °C. Weaned rabbits were housed in a separate building. They were kept under controlled environmental conditions (room temperature between 16 and 24 °C; 12 h of light per day) and housed in pairs in wire flat-deck cages measuring 600 × 250 × 330 mm$^3$. Before the first experimental period, faeces were removed from the pits. Afterwards, cages, walls, the ceiling and the floor were cleaned using high pressure water containing a detergent (RM 806 ASF, Alfred Kärcher GmbH & Co. KG). Once dry, walls were whitewashed and finally buildings were disinfected spraying a disinfectant active against Gram(+) and Gram(−) bacteria, spores, virus, fungi and micoplasms (SANIVIR