THE EVOLUTION WITH AGE OF *CLOSTRIDIUM PERFRINGENS* CONCENTRATION IN SOFT FAECES IN RELATION WITH EPIZOOTIC RABBIT ENTEROPATHY SYMPTOMS

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ABSTRACT

Clostridium perfringens has been shown to play an important role in the development of the Epizootic Rabbit Enteropathy (ERE). The average count of *C. perfringens* in the caecal contents of young rabbits was found to be highly correlated with average diarrhea mortality in the fattening period, whereas high counts (>2 x 10^6 cfu/g) of *C. perfringens* were related with the appearance of the clinical signs of ERE. Accordingly, the caecal enumeration of *C. perfringens* might be used as a good indicator of ERE. Furthermore, *C. perfringens* enumeration in soft faeces was found to be highly correlated with its concentration in caecum. The present work aimed to follow the evolution with age of *C. perfringens* concentration by means of the caecotrophs excreted by rabbits and to look for a correlation between these values and the weight gain of the rabbit at each age. Thirty-eight rabbits, weaned at 31 d of age, were weighed and fitted a neck collar during four hours (from 08:00 to 12:00) at 34, 38, 41, 45 and 48 d of age. They were fed a common commercial diet, not supplemented with antibiotics, and housed individually in flat-deck cages. No significant effect of age on *C. perfringens* concentration in soft faeces was detected. Mean counts were respectively: 5.30±0.97, 5.00±0.99, 4.95±0.97, 5.25±0.99 and 5.13±0.98 log cfu/g at 34, 38, 41, 45 and 48 d. Ten rabbits among the thirty-eight used in this trial presented clinical ERE signs, such as low body weight, aqueous diarrhoea and mucus excretion. These animals had a higher (P<0.001) *C. perfringens* concentration than healthy ones: 6.50±0.69 vs. 4.97±0.95 log cfu/g. Values in diseased animals ranged from 5.23 to 8.45 log cfu/g. Regression procedures were used to relate daily weight gain (DWG, g/d) and body weight (W, g) with the *C. perfringens* concentration in the soft faeces (CPsf, log cfu/g). The regression equations obtained were: DWG=57.2 (±6.20)–2.21 (±1.19) CPsf; P=0.07 and W=1680 (±189)–65.1 (±36.1) CPsf; P=0.08. Even if the *C. perfringens* proliferation in soft faeces increased, ill animals were able to survive but ended the fattening period weighing less than the others, so that mortality is not the only economic loss in a ERE situation. In conclusion, caecotrophs can be used as a tool in evolutionary microbiological studies and the weight loss is both a ERE sign and an important economic loss.

Key words: Rabbit, ERE, *Clostridium perfringens*, Weight.

INTRODUCTION

Several works (Le Normand *et al.*, 2003; Dewrée *et al.*, 2003; Marlier *et al*., 2003; Marlier *et al*., 2006; Szalo *et al.*, 2007; Romero *et al.*, 2007a) have recently shown that the caecal proliferation of Gram-positive bacterium *Clostridium perfringens* was closely linked to the appearance of the Epizootic Rabbit Enteropathy (ERE) symptoms. Consequently, *Clostridium perfringens* is believed to be a reliable indicator of the disease even if it has not been detected up to now the aetiological agent of the disease. Likewise, Romero *et al*. (2007b) observed that *Clostridium perfringens* enumeration in soft faeces was highly correlated (r=0.885; P<0.001) with its concentration in caecum, so that caecotrophs could be useful for the prediction of *Clostridium perfringens* concentration in samples of digestive contents, avoiding the slaughter of the animals. Also, Michelland *et al.* (2007) observed that soft faeces could be used to investigate dynamic studies of caecal bacterial communities, as

caecotrophs showed a high resemblance of its microbiological composition with that of caecal contents.

High mortality rates are not the only damage caused by the ERE in intensive rabbit production. The decrease in food consumption results in lower body weights in those animals which survive to the syndrome (Licois *et al.*, 2006). Therefore, daily weight gain has been measured by researchers looking for the appearance of ERE in different studies (Szalo *et al.*, 2007; Licois, 2007).

Accordingly, the aim of this work is to follow the evolution of *Clostridium perfringens* concentration in the digestive content by the means of the caecotrophs excreted by rabbits. Moreover, daily weight gains would be calculated and related to the presence of ERE symptoms.

MATERIALS AND METHODS

Animals and experimental design

Thirty-eight mixed-sex rabbits from ten litters of New Zealand x Californian does (originating from strains genetically improved at the Universidad Politécnica of Valencia, Spain) were chosen at random for this study. Weaned at 31 days of age, all rabbits were fitted a neck collar and weighed at 34, 38, 41, 45 and 48 days of age. Collars were made on transparent plastic (33.0 g and 330 mm of diameter on average) and were put from 8:00 a.m. to 12:00 a.m. These collars allowed to collect caecotrophs in which *Clostridium perfringens* enumeration was determined the same day and according to the standard ISO 7937 (1997). The cultural medium used was agar tryptose sulphite added with antibiotic D-cycloserine. Later on, the plates were incubated during 18 hours at 37ºC.

Rabbits were kept under controlled environmental conditions (room temperature between 16 and 24ºC with a light/dark cycle of 12 hours) and housed individually in flat-deck cages measuring 60 cm x 25 cm x 33 cm. This trial was carried out at the Universidad Politécnica of Madrid facilities according to the principles of the Spanish Royal Decree 1201/2005. Animals were fed the same diet (Tables 1 and 2) and had *ad libitum* access to the feed and water throughout the whole experimental period. The experimental diet was formulated according to the nutrient recommendations of De Blas and Mateos (1998). Neither feed nor drinking water was medicated with antibiotics. A coccidiostat (robenidine) was given in the feed.

Table 1: Ingredient composition of the experimental diet (%)

Wheat bran	15.0
Barley	6.00
Alfalfa meal, 17% CP	28.1
Sunflower meal, 30% CP	19.7
Beet pulp	15.0
Wheat straw	10.0
Soybean oil	2.10
Sodium chloride	0.40
Calcium carbonate	1.40
Monocalcium phosphate	0.57
L-lysine	0.15
Sodium bicarbonate	0.20
Sepiolite	0.88
Mineral and vitamin premix[1]	0.50

[1] Premix provided by Trouw Nutrition España S.A. (Madrid, Spain): mineral and vitamin composition (mg/kg diet): Mg, 290; Na, 329; S, 275; Co, 0.7; Cu, 10; Fe, 76; Mn, 20; Zn, 59.2; I, 1.25; Choline, 250; Riboflavin, 2; Niacin, 20; Vitamin B_6, 1; Vitamin K, 1; Vitamin E, 20 IU/kg; Thiamine, 1; Vitamin A, 8375 IU/kg,, Vitamin D_3, 750 IU/kg, Robenidine, 60

Chemical analyses

Chemical analyses were performed at the Poultry and Rabbit Research Center of Nutreco using the procedures of the Association of Official Analytical Chemists (2000) for dry matter (930.15), ash

(923.03), Dumas N (968.06), ether extract (920.39), crude fibre (978.10), sugars (974.06) and starch (996.11). Contents of NDF, ADF and acid-detergent lignin were determined according to the sequential method of Van Soest *et al.* (1991).

Table 2: Chemical composition and nutritive value of experimental diet (% as fed)

Dry matter	90.8
Crude protein	14.7
Ether extract	4.00
Ash	9.90
Starch	6.70
Crude fibre	19.9
NDF	42.5
ADF	24.9
ADL	5.50
Sugars	0.90
Soluble fibre[1]	12.1
Digestible energy (MJ/kg)[2]	8.67

[1]Estimated as (100 – moisture – ash – CP – ether extract – NDF – starch – sugars)
[2]Value estimated according to FEDNA (2003)

Statistical analysis

Data were analyzed using a MIXED procedure according to an auto-regressive model to analyse repeated measures including the effect of the age, the litter and their interactions as main factors. A Levene´s test showed lack of homogeneity of variance; accordingly, values were transformed to a logarithmic scale. Regression procedures (Statistical Analysis Systems Institute, 1991) were used to relate *Clostridium perfringens* colonies enumeration in soft faeces with the body weight and daily weight gain of each rabbit.

RESULTS AND DISCUSSION

During this trial, no rabbit died so that all of them were weighed at each time. No significant effect of age on growth traits was detected. Mean weights were 706 ± 153 (SD), 874 ± 153, 1021 ± 152, 1205 ± 152 and 1348 ± 152 g. As a result, average daily weight gains were 42.9 ± 21.3, 45.2 ± 12.1, 45.9 ± 9.60 and 45.8 ± 6.94 g/d for the 34-38 d, 38-41 d, 41-45 d and 45-48 d period respectively. Caecotrophs were respectively excreted by 100%, 97%, 100%, 95% and 97% of the animals, being the average of *Clostridium perfringens* counts 5.30 ± 0.97, 5.00 ± 0.99, 4.95 ± 0.97, 5.25 ± 0.99 and 5.13 ± 0.98 log cfu/g at 34, 38, 41, 45 and 48 days of age.

Ten among the thirty-eight rabbits presented clinical ERE symptoms: low body weight, aqueous diarrhoea and mucus excretion. Besides, in all these cases, excepting one animal which did not excrete soft faeces, *Clostridium perfringens* concentration in caecotrophs was higher than in healthy animals (6.50 ± 0.69 vs. 4.97 ± 0.95 log cfu/g; $P<0.001$), values in diseased animals ranging from 5.23 to 8.45 log cfu/g. This observation agrees with a previous study (Romero *et al.*, 2007b). The other three animals which did not produce soft faeces were apparently healthy. Animals that never presented ERE symptoms had bacterial counts always below 5.95 log cfu/g.

When dealing with the whole experimental period (34-48 days), it was found (see Figure 1) that the daily weight gain (DWG, g/d) and the body weight (W, g) were linearly related to the number of colony forming units of *Clostridium perfringens* found in soft faeces (CPsf, log cfu/g). The regression equations obtained were:

DWG = 57.2 (± 6.20) – 2.21 (± 1.19) CPsf ; r = -0.30, RSD = 6.71, P = 0.07
W = 1680 (± 189) – 65.1 (± 36.1) CPsf ; r = -0.29, RSD = 204, P = 0.08

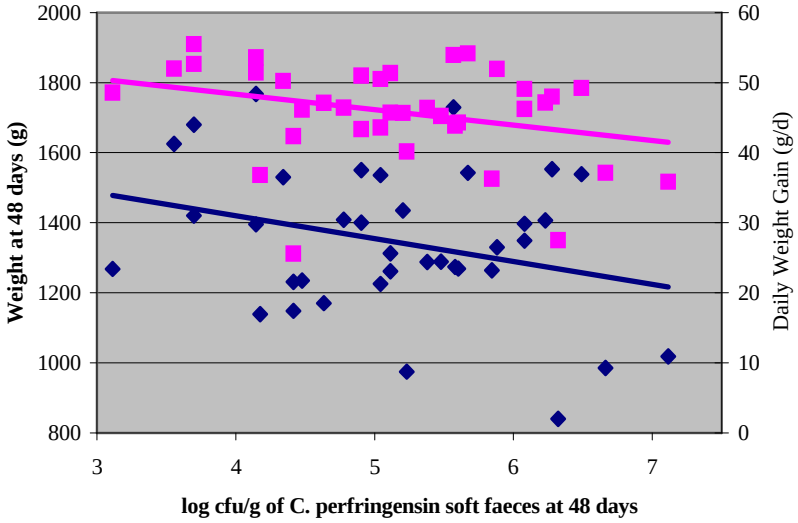

Figure 1: Regression between weight (g) and daily weight gain (g/d) with counts of Clostridium perfringens (log cfu/g) in samples of soft faeces obtained from the same animals in the 34-48 days period

The influence of *Clostridium perfringens* concentration in the caecotrophs on growth traits at earlier ages (34 to 45 days) was significant (P<0.06) after rabbits reached 41 days of age (Table 3).

Table 3: Significance of the effect of Clostridium perfringens counts in soft faeces on DWG and W of rabbits at different ages

Age (days)	DWG		W	
	Linear	Quadratic	Linear	Quadratic
34	NS	NS	NS	NS
38	NS	NS	NS	NS
41	0.02	0.04	0.06	0.05
45	NS	0.06	0.03	NS

CONCLUSIONS

Clostridium perfringens concentration in caecotrophs might be a good indicator of the prevalence of the ERE because of its high correlation with the appearance of ERE symptoms, including loss of weight.

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