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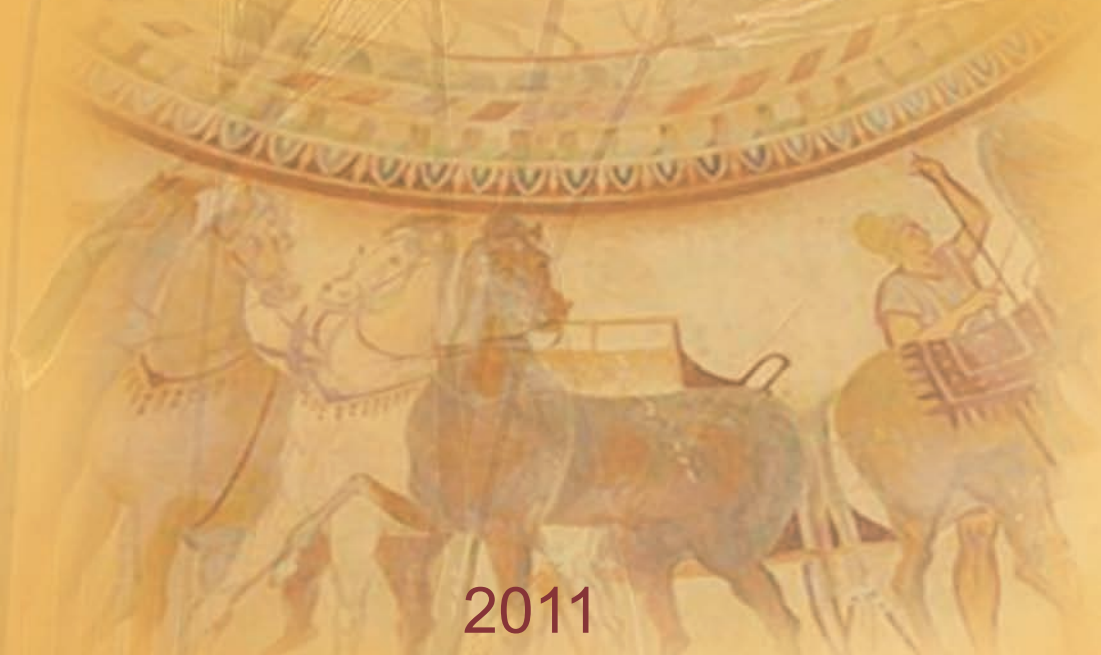

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# Development of the caecal microbiota in rabbits weaned at different age

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***Abstract.*** *The experiment was conducted to study the post-natal development of the caecal microbiota in rabbits weaned at different age. A total of 60 healthy New Zealand White rabbits of both sexes, born the same day, were used in the experiment (after controlling for the effect of litter origin and weaning weight and variability). Rabbits were weaned both at 21 days (W21 group, 30 litters) and at 35 days (W35 group, 30 litters) of age. The weaned animals were randomly housed in wire net cages measuring in well-controlled experimental facility. They received standard commercial pelleted diet without antibiotics. Feed and drinking water were available ad libitum. Results of the microbiological examination of the caecal contents indicated that rabbits weaned at 35 day had higher total bacterial count (TBC) per g of caecal content, in comparison with rabbits weaned at 21 day (P<0.001). The TBC in the caecum of earlier and later weaned rabbits after weaning increased significantly (P<0.001). The obligate anaerobic bacteria, particularly Bacteroides spp. constitute an important group of microorganisms in the rabbit caecum. The population of Bacteroides spp. increased with advancing of age. The differences between groups on days 35, 42 and 49 were statistically significant (P<0.001). Sporulating bacteria and especially Cl. perfringens was present in low variable amounts in all the caecal samples obtained from healthy animals. Caecal counts of Cl. perfringens at weaning (21 and 35 day) were very low (1.656 and 1.654 log10 CFU/g, respectively) and not affected by weaning age. To the end of the study, earlier weaned rabbits had higher caecal count of Cl. perfringens (P<0.01). Enterococcus spp. and coliforms, including E.coli are an important part of the caecal microbial population of rabbits. The caecal number of coliforms was considerably high at weaning, then decreased linearly and stabilized on low level at day 49. Our study demonstrated the absence of Lactobacillus spp. in the rabbit caecal tract. The pH of the caecal content fell linearly throughout the experiment - there are not significant differences between groups at days 21 and 49. Compared to the W21 group, rabbits in the W35 group, had a higher live body weight (P<0.001) and low mortality during the trial.*

**Keywords:** rabbits, caecal microbiota, weaning age

**Abbreviations:** CE – caecal ecosystem, GIT – gastrointestinal tract, CFU – colony forming units, TBC – total bacterial count, CP – crude protein, BW – body weight, CW – weight of the caecal wall, CC – caecal content

## Introduction

The rabbit caecal ecosystem (CE) plays a key role in the digestive physiology, both for health and digestive efficiency, because of its size (40% of the whole tract content) and of the highly active microbiota (Gidenne, 2003; Gidenne et al., 2004; Monteils et al., 2008). In this ecosystem there are complex interactions among the biotope (i.e. the caecum) and the biocenosis (the microbiota), and its functioning still remains poorly known (Gidenne et al., 2008).

The caecum is very large, compared with the rest of the gut (Stevens and Hume, 1995) and forms a spiral that fills the abdominal cavity. Previous studies on rabbit caecal microbiota (CM) revealed that bacterial species are mainly strictly anaerobic, but they were mostly performed using classical culture-based techniques (Monteils et al., 2008). The microbial composition of rabbit CE is changed with advancing of age, particularly in association with the weaning period.

Weaning is a stressful period for rabbits when healthy caecal microflora is not yet established (Harcourt-Brown, 2002). The period around weaning is a very critical time for young rabbits as they are highly sensitive to multifactor digestive disorders (Kovacs et al., 2008). Up to 18-20 days, rabbits ingest only milk, while from this point onwards they begin to consume solid feed as well, initially in small quantities and subsequently, as their mother's milk yield diminishes in ever increasing quantities (Nizza et al., 2002). During this period, the gastrointestinal tract (GIT) of rabbits is colonized by an abundant microflora, predominantly by strictly anaerobic bacteria (Marounek et al. 2000a). The fermentative activity of caecum begins to develop (Padilha et al., 1995, 1996; Piattoni et al., 1995) and the enzymatic digestive activities show important changes (Marounek et al., 1995). The microbial and fermentative activities in the CE of young rabbits play a key role in the prevention of digestive disorders (Carabaño et al., 2006). The pre-weaning milk/feed ratio affects the microbiological and biochemical characteristics of the caecal content and also post weaning performance of rabbits (Nizza et al., 2001). On the other hand, an earlier intake of solid feed could accelerate the maturation of digestive enzymes and also reduce the risk of digestive disorders (Maertens and De Groote, 1990; Scapinello et al., 1999).

In commercial rabbit rearing, weaning of litters usually occurs from 30 to 35 days of age (Xiccato et al. 2003). An earlier weaning of young rabbits has some advantages for both kits and dams: reduces pathogen transmission by limiting contacts between litters and does and incidence of digestive disorders (Schlolaut, 1988; Gidenne and Fortun-Lamothe, 2002) specific starter diets could better cover kit nutritional requirements (Gutiérrez et al., 2002) improves the body

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condition of the doe and its health status (Pascual, 2001; Xiccato et al., 2000, 2001; Gidenne and Fortun-Lamothe, 2002) and shorter lactation periods could reduce the doe energy output for milk production and the consequent body energy deficit (Parigi-Bini and Xiccato, 1998). On the other hand, early weaning could impair the welfare of dams and litters as well as the viability of kits that are too young, even though in wild rabbits litters may be completely weaned at 23 or 24 days of age because the does, often mated soon after kindling, are going to have their successive kindling (Hudson et al., 2000).

Analysis of the literature shows that during the last decade, much research has been conducted to study the development of digestive activity around weaning (Dojana et al., 1998; Debray et al., 2001a, 2001b; Pinheiro et al., 2001; Gidenne and Fortun-Lamothe, 2002; Gidenne et al., 2004); effects of different nutritional strategies on caecal fermentation (Marounek et al., 2000b; Xiccato et al., 2003; Lavrencic, 2007); and post weaning health and performance (Xiccato et al., 2000; Hampson et al., 2001; Gidenne and Licois, 2005; Feugier et al., 2006; Alvarez et al., 2007; Gomez-Conde et al., 2007; Carabano et al., 2008). A role of caecal microbial activity has been hypothesized but not studied in details (Bennegadi-Laurent et al., 2004). Little is still known about the effects of different biotic and abiotic factors, including weaning, on the caecal microbial ecosystem (Kovacs et al., 2002, 2004, 2006, 2008).

The objective of the current experiment was to study the post-natal development of ceacal microbiota in rabbits weaned at different age.

## Material and methods

*Animals, Housing and Diet*

The experiment was performed at the Section of Animal Physiology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria, between March and May 2009. The rabbits were taken from a commercial farm of the Agricultural Research Institute, located around Stara Zagora. New Zealand White does and their progeny were used in the experiment. All animals were healthy, i.e. no diarrhoea or losses of live weight were observed. The animals were kept in standard wire net cages for reproducing does. At one end of the cage there was a nest box, with a creep-hole that could be closed. The nest box contained a nest tray. The does were fed a standard medication-free pelleted diet. Feed and drinking water were available *ad libitum*. From 14 day of age, the young rabbits had free access to their mothers feed. The temperature of the room was 20-22°C, humidity about 65%, and the daily lighting period was 14 h.

A total of 60 healthy New Zealand White rabbits of both sexes, born the same day at the commercial farm, were used in the experiment (after controlling for the effect of litter origin and weaning weight and variability). Young rabbits were weaned either at 21 days (W21 group, n=30) or at 35 days (W35 group, n=30) of age. The weaned animals were randomly housed in standard cages (800 x 600 x 400 mm) (5 animals per cage) in a well-controlled experimental facility. All cages were equipped with feeding hoppers and drinking nipples. Before the experiment the facility was cleaned and disinfected. The ambient temperature was maintained between 20 and 22ºC and relative humidity about 65%. The daily lighting period was 14 h. The weaned rabbits from both groups had access to the same standard commercial pelleted diet, without antibiotics. The ingredients and chemical composition of the diet are listed in Table 1. Throughout the experimental period feed and water were provided without restriction (*ad libitum*). The health status of the young rabbits before and after weaning was strictly controlled, both to ensure animal welfare and to avoid bias in the results. Mortality was checked daily, while morbidity was assessed weekly through an individual control of all clinical signs of digestive troubles, such as transitory diarrhoea, presence of mucus in excreta, abnormal behaviour, etc., over the whole period of experiment. No signs of disease were registered during the experimental period. All care and experimental procedures involving animals followed the guidelines defined in the Guide to the Care and Use of Laboratory Animals (1996) and the trials were agreed by the Animal Use and Care Advisory Committee of the Faculty of Veterinary Medicine, Trakia University, Bulgaria.

**Table 1.** Ingredients and chemical composition of the diet

| Ingredients | % |
|---|---|
| Alfalfa meal | 20.00 |
| Corn | 5.00 |
| Wheat | 5.00 |
| Barley | 23.00 |
| Oats | 4.00 |
| Soybean meal (44% CP) | 3.50 |
| Extracted sunflower meal | 16.00 |
| Wheat bran | 19.00 |
| Sugar-beet pulp | 2.00 |
| Vitamin-mineral premix* | 0.50 |
| Limestone | 1.00 |
| Dicalcium phosphate | 0.14 |
| Sodium chloride | 0.30 |
| Acidifier (Biotronic®SE) | 0.25 |
| Dry binder (Kembind®) | 0.30 |
| Antioxidant (Endox®) | 0.01 |
| Chemical composition** | |
| Dry mater,% | 88.00 |
| Digestible energy, kcal/kg | 2 590 |
| Crude Protein,% | 18.00 |
| Crude Fat,% | 2.70 |
| Crude fibre,% | 13.00 |
| Ash,% | 5.00 |
| Lysine,% | 0.77 |
| Methionine + Cystine | 0.65 |
| Ca | 1.00 |
| P | 0.50 |

* Vitamine-mineral premix per kg: Vitamins: A 10 000 IU; $D_3$ 1 000 IU; E 50 mg; $B_1$ 5 mg; $B_2$ 14 mg; D-panthotenic acid 25 mg; $B_6$ 10 mg; $B_{12}$ 0.04 mg; PP 80 mg; H 0.3 mg; Folic acid 2 mg; K 8 mg; Choline 1 300 mg; Minerals: Fe 160 mg; I 2.5 mg; Co 0,6 mg; Cu 40 mg; Mn 120 mg; Zn 120 mg; Se 0,2 mg.
** AOAC (2000)

*Sampling procedures*

Five healthy rabbits, selected randomly from each group, were examined: Group A on days 21, 28, 35, 42, 49 and Group B on 35, 42 and 49 after birth. The feed was removed at 19.00 h on the day before sampling, but water was still available *ad libitum*. The animals were euthanized (between 9:00 and 10:00 a.m.) by an overdose of carbon dioxide and bled. After opening the abdominal cavity the digestive tract was removed and the caecum was separated by tying

off the two extremities with nylon string to prevent movement of the digesta and immediately transported to the laboratory of microbiology in a pre-warmed (39°C) container. The caecum was weighted before and after emptying to determine the weight of its content. The samples were taken from the caecal content with sterile precautions and subjected to microbiological analysis. The manipulations were done under the constant flushing of the oxygen free $CO_2$ gas to assure anaerobic conditions and the temperature was kept at 39°C.

*Microbiological analyses*

Immediately after sampling serial 10-fold dilutions were prepared with sterile pre-reduced anaerobic dilution solution, containing (g/l): ($K_2HPO_4$ x $3H_2O$ - 4,105; $KH_2PO_4$ - 1.636; Cysteine hydrochloride - 0.5; Gelatin - 2.0; Resasurin - 1.0), according to procedure described by Holdeman and Moore (1977). The serially diluted caecal content were used for determination of the total bacterial count (TBC); anaerobic (*Bacteroides*, *Clostridium, Lactobacillus* spp.); facultative anaerobic organisms (*Enterococcus* spp., and Coliform counts). The different dilutions ($10^{8-9-10}$ for TBC; $10^{6-7-8}$ for bacteroides; $10^{3-4-5}$ for enterococci; $10^{2-3-4}$ for clostridia, lactobacilli and coliforms) were smeared onto the surface of the selective culture media, previously reduced by placing under anaerobic condition (Gas Pak® anaerobic system) for 18 to 24 h prior to use. The obligate anaerobe *Bacteroides spp.* were cultured on Schaedler's Agar (BBL®, USA), the selectivity of which was increased by the addition of esculin, neomycin and Fe-ammonium citrate (Merck, Germany) (Kovacs et al., 2004, 2006); *Lactobacillus* spp. were cultured on MRS Agar (BBL®, USA); *Enterococcus* spp. on M-Enterococcus Agar (BBL®, USA); Coliforms, including *E. coli* on Mac Conkey Agar (BBL®, USA); *Clostridium* spp. – Clostridium Selective Agar (Merck, Germany). The plates were incubated anaerobically in Gas Pak® anaerobic system at 37°C for 96 h (TBC, *Bacteroides* and *Clostridium* spp.); at 37°C for 48 h (*Lactobacillus* and *Enterococcus* spp.), and aerobically at 37°C for 24 h (coliforms). *Cl. perfringens* enumeration was performed according to the Standard ISO 7937 (2004). This technique analyses all the toxin types of *Cl. perfringens*. The cultural medium used was Agar tryptose sulphite added with antibiotic d-cycloserine (Byrne et al., 2008). Agar plates were incubated at 37°C in Gas Pak® anaerobic system with carbon dioxide generating envelopes. *Cl. perfringens* reduced sulphite to sulphide, and in the presence of iron, black colonies developed. These colonies were observed and counted.

After the incubation time had elapsed, the bacterial colonies were counted with an automatic colony counter. The total colony counts per g of undiluted caecal content from each medium were obtained as the weighed mean from two or three highest duplicate dilutions that showed growth. The bacterial counts were expressed as the average of five parallel replications of each sample in $Log_{10}$ colony forming units (CFU) per g caecal content ($Log_{10}$ CFU/g ± SEM).

*Chemical analyses*

After the caecal samples were taken for microbiological analyses the pH of the caecal digesta was measured immediately with a glass electrode pH meter. The chemical composition of the diet was analyzed according to standard methods of Association of Official Analytical Chemists (AOAC, 2000). Energy concentration of the diet was measured by adiabatic calorimeter.

*Statistical analysis*

All bacterial populations estimates were calculated and reported on a per g (wet weight) basis of caecal content. The mean and standard deviation of the bacterial counts were calculated using logarithmic values ($Log_{10}$ CFU/g). Statistical analysis of the results was performed by one-way analysis of variance (ANOVA). The results are quoted as means ± SEM.

## Results and discussion

The caecal bacterial counts of rabbits weaned at 21 (W 21 group) and 35 day (W 35 group) is shown in Table 2. Results of the microbiological examination of the caecal contents indicated that at the days of weaning total bacterial counts (TBC) are 8.768 and 9.422 $log_{10}$ CFU/g, respectively. Rabbits weaned at 35 day had higher TBC per g of caecal content in comparison with rabbits weaned at 21 day ($P<0.001$). The TBC in the caecum of earlier and later weaned rabbits after weaning increased significantly ($P<0.001$). At the end of the experimental period (49 day) TBC in the caecal contents is 10.934 and 10.942 $log_{10}$ CFU/g, respectively. The differences between groups at the end of the trial, however, were never statistically significant. At this age the anaerobic bacterial flora in the

**Table 2.** Concentrations of different bacterial populations on caecal contents of rabbits ($log_{10}$ CFU/g)

| Group/Day | Total Bacterial Count | *Bacteroides* spp. | *Clostridium* spp. | *Clostridium perfringens* | *Enterococcus* spp. | *Coliforms* | *Lactobacillus* spp. |
|---|---|---|---|---|---|---|---|
| W21 Group* | | | | | | | |
| 21 | $8.768 \pm 0.012^{a}$ | $7.298 \pm 0.015^{a}$ | $3.354 \pm 0.037^{a}$ | $1.656 \pm 0.101^{a}$ | $6.494 \pm 0.037^{a}$ | $5.582 \pm 0.067^{a}$ | <1 |
| 28 | $9.564 \pm 0.022^{b}$ | $8.250 \pm 0.025^{b}$ | $3.732 \pm 0.033^{b}$ | $2.202 \pm 0.047^{b}$ | $5.768 \pm 0.221^{b}$ | $4.472 \pm 0.018^{b}$ | <1 |
| 35 | $10.836 \pm 0.021^{c}$ | $9.436 \pm 0.033^{c}$ | $3.964 \pm 0.018^{c}$ | $2.402 \pm 0.024^{c}$ | $4.674 \pm 0.023^{c}$ | $3.656 \pm 0.022^{c}$ | <1 |
| 42 | $10.918 \pm 0.008^{d}$ | $9.756 \pm 0.016^{d}$ | $4.364 \pm 0.043^{d}$ | $2.526 \pm 0.020^{d}$ | $3.762 \pm 0.053^{d}$ | $3.194 \pm 0.025^{d}$ | <1 |
| 49 | $10.934 \pm 0.005^{d}$ | $9.962 \pm 0.011^{de}$ | $5.446 \pm 0.035^{de}$ | $2.794 \pm 0.071^{de}$ | $3.242 \pm 0.045^{de}$ | $1.958 \pm 0.037^{de}$ | <1 |
| W35 Group** | | | | | | | |
| 35 | $9.422 \pm 0.018^{e}$ | $8.164 \pm 0.015^{e}$ | $3.500 \pm 0.063^{e}$ | $1.644 \pm 0.094^{a}$ | $4.674 \pm 0.023^{c}$ | $4.314 \pm 0.033^{e}$ | <1 |
| 42 | $10.624 \pm 0.014^{f}$ | $9.524 \pm 0.017^{f}$ | $4.218 \pm 0.081^{f}$ | $1.994 \pm 0.032^{f}$ | $3.462 \pm 0.047^{f}$ | $3.020 \pm 0.018^{f}$ | <1 |
| 49 | $10.942 \pm 0.013^{d}$ | $9.856 \pm 0.012^{d}$ | $5.448 \pm 0.046^{de}$ | $2.408 \pm 0.015^{dc}$ | $3.086 \pm 0.030^{dc}$ | $1.865 \pm 0.022^{dc}$ | <1 |

* Weaned at 21st day; ** Weaned at 35th day ; Significant differences in the same column and between groups: $^{a, b, c, d, e, f, de, dc}$ ($P<0.001$);

CE of the weaned rabbits develops and stabilizes, which is important for digestive health and productivity (Carabano et al., 2006). Several studies demonstrated also that strictly anaerobic bacteria are the main constituent of the CE in rabbits (Gouet and Fonty, 1979; Bónai et al., 2008; Monteils et al., 2008). Padilha et al. (1995) reported that the anaerobic bacterial flora in the caecum develops significantly from 3 weeks of age, as the feed intake of the young rabbits shifts from milk to dry feed.

Results of the microbiological examination of the caecal content indicate that the obligate anaerobic, Gram-negative, non-sporulating bacteria, particularly *Bacteroides* spp. constitute an important group of microorganisms in the rabbit caecum (Table 2). They were largely dominant compared with the other bacterial populations in the CE of young rabbits not only on the day of weaning, but later. During the experimental period the caecal population of *Bacteroides* spp. increased with advancing of age. The differences between the two groups on the day 35, 42 and 49 were statistically significant (P<0.001). To the end of the trial the caecal number of *Bacteroides* spp. was significantly higher in early weaned rabbits (P<0.001). Several studies also suggest that *Bacteroides* spp. is the main bacterial species in the rabbit's intestinal tract (Yanabe et al., 1999; Kovács et al., 2006). Some Bacteroides spp. play an important role on the immune response of the gut associated lymphoid tissue (Bry et al., 1996; Brubaker et al., 1999; Rhee et al., 2004; Mazmanian et al., 2005), and more knowledge on these species could be important to implement the intestinal health of rabbits.

Sporulating bacteria (*Clostridium* spp.) were about 1000 times less numerous than the *Bacteroides*, which are considered to belong to the sub-dominant flora. The total number of *Clostridium* spp. in the caecal content of early weaned rabbits increased from 3.354 (21 day) to 3.964 (35 day) and to 5.446 $\log_{10}$ CFU/g (49 day) (P<0.001). Similar results were obtained in the second experimental group where *Clostridium* count increased from 3.50 (35 day) to 5.448 $\log_{10}$ CFU/g (49 day, P<0.001). At the end of the trial (42 and 49 day) there were not significant differences in caecal concentrations of *Clostridium* spp. between groups (Table 2). Data from the current study show that *Cl. perfringens* was present in low variable amounts in all the caecal samples obtained from healthy animals. Caecal counts of *Cl. perfringens* at weaning (21 and 35 day) were very low (1.656 and 1.654 $\log_{10}$ cfu/g, respectively) and not affected by weaning age. According to Maertens et al. (2006) rabbit milk contains antimicrobial compounds, such as medium chain fatty acids (caprylic and capric acids), that have been proved to have a significant bactericide effect *(in vitro)* against *Cl. perfringens* (Skrivanova et al., 2005).

The results from the current study indicate that after weaning caecal counts of *Cl. perfringens* were increased (P<0.001), but rabbits weaned at 35 day had lower *Cl. perfringens* counts in comparison with rabbits weaned at 21 day (P<0.001). To the end of the study earlier weaned rabbits had significantly higher caecal concentration of *Cl. perfringens* (P<0.01) (Table 2). According to Chamorro et al. (2007), early substitution of solid feed for milk in early weaned rabbits decreased the digestibility of nutrients and increased the flow of nutrients reaching the ileum. An increase in the flow of ileal protein has been correlated with a higher frequency of detection of *Cl. perfringens* in the ileal contents. On the other hand, in early weaned rabbits intestinal digestion and absorption of starch is not as efficient as in adults and residual amounts of carbohydrate may reach the caecum to act as a substrate for bacterial fermentation. *Clostridium* spp. and other pathogenic bacteria can proliferate in the caecum of young rabbits and can cause intestinal inflammation, enteritis, diarrhoea and rapid death due to the effects of enterotoxins (Harcourt-Brown, 2002). Marlier et al. (2006) and Szalo et al. (2007) suggest a correspondence between high *Cl. perfringens* counts (>$2\times10^6$ CFU/g) and the incidence of epizootic rabbit enteropathy symptoms. According to Hatheway (1990), Songer (1996) and EFSA (2005) a high concentration of *Cl. perfringens* (>$10^6$ CFU/g) in the caecal content is a predisposing factor for the release of toxins that causes clostridial enterotoxaemia.

Facultative anaerobic, Gram-positive bacteria belonging to the genera *Enterococcus* are an important part of the caecal microbial population of rabbits (Table 2). At the day of weaning the caecal count of *Enterococcus* spp. was higher in early weaned rabbits in comparison with rabbits weaned later (6.494 and 4.674 $\log_{10}$ CFU/g), respectively. After weaning *Enterococcus* count in the caecal contents of groups A and B decreased considerably to the end of the trial to 3.242 and 3.086, respectively. The differences between the groups during the experimental period are statistically significant (P<0.01).

Our study demonstrated that the coliforms count, including *E. coli* in the caecum of young rabbits, weaned at different age, is very low. The caecal number of coliforms was considerably high at weaning of the rabbits, then decreased linearly and stabilized on low level at day 49. The decrease in the level of coliforms between weaning and 49 days of age is in agreement with the results previously reported in healthy weaned rabbits (Gouet and Fonty, 1979; Padilha et al., 1999; Kovács et al., 2004a, 2008; Bónai et al., 2008). Our study demonstrated also significant differences between groups at 35 and 42 day when the caecal count of coliforms were higher in earlier weaned rabbits (P<0.01). The higher number of coliforms in the caecum of earlier weaned rabbits to 42 day is considered to be a high risk from the animal health point of view (Bónai et al., 2008). Coliforms and especially *E. coli* are important opportunist pathogens and can be a major cause of enteritis and losses in rabbit farms. Under some circumstances pathogenic and toxigenic strains of the organism proliferate and cause diarrhea (Harcourt-Brown, 2002). Probably, early weaning (21 day) of rabbits makes them more conductive to coliform infections in comparison with later weaning (35 day).

Reports on the distribution of lactobacilli in the rabbit digestive tract are limited and those that are available have been inconsistent in results. Straw (1988) reported that cell population densities of lactobacilli in the rabbit gut were $10^6$ per g of content. The results of the present experiment demonstrated the absence of *Lactobacillus* spp. in the rabbit caecal tract (Table 2). In most previous studies *Lactobacillus* spp. was also generally absent in the digestive tract of healthy rabbits (Gouet and Fonty, 1979; Penney et al., 1986; Straw (1988), Ducha et al., 1990; Harcourt-Brown, 2002). According to Pérez de Rozas et al. (2008**)** *Lactobacillus* spp. is not a habitual component of the microbiota of rabbits. Yu and Tsen (1993) also investigated the distribution of lactobacilli in the GIT of rabbits, but the results showed that they are present only in a few of the experimental rabbits and in small numbers. They studied the factors that might be responsible for such effects. These studies included investigation of the effect of gastric juice and bile salts on the viability of lactobacilli, assay of the survival rates of lactobacilli in the GIT of the rabbits and measurement of the adhesive capability of lactobacilli to the rabbit intestinal epithelial cells. Results showed that, although some lactobacilli were resistant to the rather low pH levels of rabbit gastric juice, lack of adhesive capability may prevent

them from colonizing in the intestinal tract.

There was considerable development of the caecum after weaning of the rabbits (Table 3). Age affected the empty caecum and the caecal contents. All parameters (CW and CC) significantly increased by the end of the trial (49 day) (P<0.001). There were also significant differences between the two groups. Rabbits weaned at 35 day had significantly higher CW and CC at 35, 42 and 49 day in comparison with rabbits, weaned at 21 day (P<0.01).

The pH of the caecal content fell linearly (P<0.01) throughout the experiment (Table 3), from 6.78 on day 21 to 5.64 on day 49 (W 21 group) and from 6.78 on day 21 to 5.57 on day 49 (W 35 group). There are not significant differences between groups at the beginning (21 day) and at the end (49 day) of the trial. On the other hand, during the experimental period the early weaned rabbits (W 21 group) had significantly lower caecal pH only on day 28 and 35 (P<0.01) in comparison with later weaned rabbits (W 35 group). The consistent decrease in pH between days 21 and 49 related to post weaning development of caecal microbial activity, fiber intake, and volatile fatty acid concentration in the caecum (Garcia et al., 2002; Bennegadi-Laurent et al., 2004).

**Table 3.** Zootechnical and caecal weight traits of the rabbits weaned at different age

| Trait | Age (day) | | | | |
|---|---|---|---|---|---|
| | 21 | 28 | 35 | 42 | 49 |
| W 21 Group | | | | | |
| BW (g) | 354$^{a}$ (30) | 456$^{b}$ (22) | 570$^{c}$ (64) | 954$^{d}$ (102) | 1240$^{e}$ (100) |
| CW (g) | 3.7$^{a}$ (0.33) | 6.9$^{b}$ (0.36) | 10.4$^{c}$ (0.34) | 16.4$^{d}$ (0,36) | 21.1$^{e}$ (0.51) |
| CC (g) | 6.5$^{a}$ (0.40) | 18.5$^{b}$ (0.98) | 24.6$^{c}$ (1.54) | 29.7$^{d}$ (2.87) | 37.7$^{e}$ (2.55) |
| pH | 6.78$^{a}$ (0.13) | 5.62$^{b}$ (0.63) | 5.61$^{b}$ (0.36) | 5.61$^{b}$ (0.31) | 5.64$^{b}$ (0.19) |
| W 35 Group | | | | | |
| BW (g) | 353$^{a}$ (35) | 860$^{c}$ (16) | 1206$^{f}$ (52) | 1410$^{g}$ (155) | 1780$^{h}$ (134) |
| CW (g) | 3.7$^{a}$ (0.36) | 7.1$^{b}$ (0.23) | 14.3$^{f}$ (0.32) | 19.7$^{g}$ (0.43) | 26.8$^{h}$ (0,44) |
| CC (g) | 6.4$^{a}$ (0.25) | 19.0$^{b}$ (0.95) | 29.2$^{f}$ (1.25) | 36.1$^{g}$ (1.54) | 45.4$^{h}$ (2.74) |
| pH | 6.78$^{a}$ (0.13) | 6.48$^{d}$ (0.60) | 6.54$^{d}$ (0.40) | 5.85$^{b}$ (0.41) | 5.57$^{b}$ (0.19) |

Data are mean (n=5) ± SEM (standard error of the mean).
Mean values in a same row with different superscripts were significantly different (P<0.001)
Mean values in a same column with different superscripts were significantly different (P<0.01)

The live body weight (BW) of the young rabbits had a typical evolution (Table 3). At weaning (21 and 35 day) the BW of the rabbits were respectively 354±30 (W21 group) and 1206±52g (W35 group). These results were in agreement with McNitt and Moody (1988) and Scapinello *et al.* (1999), who reported a higher weaning weight when the pups consumed more milk during lactation. During the experimental period BW of the early weaned rabbits increased 1.6 times, between 21 and 35 day, and 1.47 times between days 35 and 49. Considerable differences between groups were also observed at the end of the experiment (49 day). Compared to the W21 group, rabbits in the W35 group, had a significantly higher BW (1240±100 *versus* 1780±134g) (P<0.001).

During the trial post-weaning mortality was observed only in early weaned rabbits (W21 group), between days 21 and 28, and amounts in total to nearly 10%. The major part of these losses is doe to diarrhea, whose etiology is multifactorial (Harcourt-Brown, 2002). On the other hand, weaning is a stressful period for young rabbits when healthy caecal microbiota is not yet established. The period around weaning is a very critical time for young rabbits as they are highly sensitive to multifactor digestive disorders (Kovacs et al., 2008). Up to 18-20 days, rabbits ingest only milk, while from this point onwards they begin to consume solid feed as well, initially in small quantities and subsequently, as their mother's milk yield diminishes in ever increasing quantities (Nizza et al., 2002). These results confirm the observations of Padilha et al. (1995) showing clearly that the change from milk to solid feed is much more important than the weaning is from an exclusive milk intake to an exclusive solid feed intake. During this period, the GIT of rabbits is colonized by an abundant microflora, predominantly by strictly anaerobic bacteria (Marounek et al. 2000a). The fermentative activity of caecum begins to develop (Padilha et al., 1995, 1996; Piattoni et al., 1995) and the enzymatic digestive activities show important changes (Marounek et al., 1995). The microbial and fermentative activities in the CE of young rabbits play a key role in the prevention of digestive disorders (Carabaño et al., 2006). The pre-weaning milk/feed ratio affects the microbiological and biochemical characteristics of the caecal content and also post weaning mortality and performance of rabbits (Nizza et al., 2001).

## Conclusion

Post-weaning developments and composition of the caecal microbiota in rabbits are influenced mainly by age and nutritional factors. The strictly anaerobic bacteria, particularly *Bacteroides* spp. were the main constituent of the caecal microbial ecosystem in the

weaned rabbits. *Clostridium* spp. was sub-dominant flora. Caecal counts of *Cl. perfringens* at weaning (21 and 35 day) were very low and not affected by weaning age. After weaning, to the end of the study, later weaned rabbits had lower *Cl. perfringens* counts in comparison with early weaned rabbits. *Enterococcus* spp. and coliforms were also an important part of the caecal microbial population of rabbits. The higher number of coliforms and especially *E. coli* in the caecum of earlier weaned rabbits is considered to be a high risk from the animal health point of view. *Lactobacillus* spp. is not a habitual component of the caecal microbiota of healthy rabbits weaned at different ages. Early weaning of rabbits did not cause detrimental changes of the caecal microbiota, but resulted in higher post weaning mortality and lower growth performance. Later weaning had positive effects related to post-natal development of the caecal microbial ecosystem, morbidity, mortality and live body weight of growing rabbits. However, further studies are necessary to acquire more information about this topic. The development and application of new molecular methods will provide a more extensive view of the caecal microbiota.

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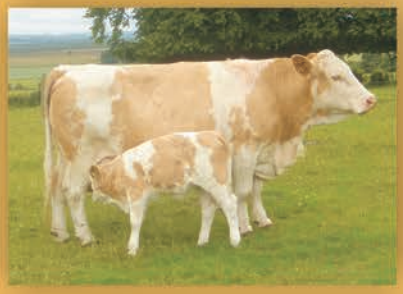

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