# EFFECT OF DIFFERENT WEANING AGE (DAYS 21, 28 AND 35) ON CAECAL MICROFLORA AND FERMENTATION IN RABBITS

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## ABSTRACT

One-day-old Pannon White rabbits of average birth weight were distributed into litters of eight, and these litters were randomly divided into three groups (21-22 litters/group) according to the weaning age: 21 (G21), 28 (G28) and 35 (G35) days. Milk consumption, feed intake and body weight were measured weekly. On day 14, 22, 29, 36 and 42 subsequent to birth 6 healthy animals from each group were examined for some digestive physiological parameters: pH and volatile fatty acid content (VFA) of the caecal content, and composition of the caecal microflora. There was no significant difference between groups in body weight till the 28$^{th}$ day of age. At day 35 and 42 d, G35 rabbits had a significantly higher body weight compared to rabbits of the other two groups. Milk consumption increased from day 21 to day 28. Thereafter G35 rabbits had lower milk consumption, presumably due to the decrease in the mothers milk production and the increase in the solid feed consumption. There was a sudden increase in the solid feed consumption after weaning. In group G35 the feed intake was higher already on day 28, related to the decreasing milk production of the doe. Anaerobic bacteria growing on the Schaedler agar were in a high amount ($10^8$/g) already on the 14$^{th}$ day of age. On day 42 their number was significantly higher in G35 and G28 than in G21 rabbits. Counts of coliforms and bacteria growing in the presence of air were in accordance with the literature and decreased with age and decreasing milk intake. Concentration of total VFA was significantly higher in G21 than in G28 and G35. The percentage of acetic acid (C2) was above 70% after weaning in G21. In the other two groups rabbits consumed more milk and less solid feed, so the concentration of C2 was significantly lower. Because of the early solid feed consumption of G21, the concentration of propionic acid (C3) was significantly lower, while that of butyric acid (C4) was significantly higher on day 29 than in G28. The highest C4 content was found in G21 during the whole experimental period. In G35 milk consumption and the simultaneous increasing solid feed intake resulted in better growth. The composition of the caecal microflora was mainly influenced by age, effect of weaning was less pronounced. On the other hand milk and/or feed consumption, i.e. age of weaning, had major effect on the VFA composition. In summary, early weaning (age 21 d) did not result in detrimental changes in the digestive physiological parameters examined, but resulted in lower production in rabbits.

**Key words:** Caecal microflora, Fermentation, Weaning age, Rabbit.

## INTRODUCTION

In rabbit production weaning period is the most crucial part, as at that time rabbits are highly sensitive to multifactorial digestive disorders. The high mortality and morbidity has a great economic impact on rabbit meat production. In order to reduce losses, antibiotics have been added to the diet, which have been banned recently because of human health and animal welfare aspects. Early weaning of rabbits can be advantageous from nutritional and animal health point of view. In commercial farms dams and kits get the same diet before weaning which is inadequate to cover the nutritional requirements of the lactating does and to promote the development of the growing animals (Gidenne and Forthun-Lamothe, 2002). In the prevention of digestive disorders the composition of the microflora and fermentation pattern in the caecum play a key role in rabbits (Carabaño *et al*., 2006).

The aim of the present study was to examine the effect of weaning age and feeding with antibiotic-free diet on certain physiological parameters of the caecal function in rabbits.

## MATERIALS AND METHODS

Pannon White does and their progeny were used in the experiment. The one-day-old pups of average birth weight were distributed into litters of eight, and these litters were randomly divided into three groups (21-22 litters/group) according to the weaning age: rabbits weaned at the age of 21 (G21), 28 (G28) and 35 (G35) days. A total of 512 rabbits were used in the experiment. Three days prior to kindling and up to weaning, the does were fed a non-medicated basal diet containing 9.7 MJ DE/kg, 16.0% crude protein, 4.2% crude fat and 31.6% NDF. Young rabbits were allowed to consume the same diets besides their mother's milk before weaning. Milk consumption of the litter was measured from 14 days of age till weaning. Solid feed consumption of the litter and body weight (BW) of the kits was measured weekly.

On the day 14, 22, 29, 36 and 42 subsequent to birth 6 healthy animals from each group were sacrified using $CO_2$ gas at 14:00 h. The digestive tract was removed immediately and the caecum was separated. According to the literature (Gouet and Fonty, 1973) the caecal microflora in rabbits consists of simple, non-sporulated, strictly anaerobic, Gram-negative *Bacteroides.* For the microbiological examinations of these strictly anaerobic bacteria, they were cultured on Schaedler's agar (Sharlan Chemie, Barcelona, Spain), the selectivity of which was increased by the addition of esculin (Merck, Darmstadt, Germany), neomycin (Merck, Darmstadt, Germany) and Fe-ammonium citrate (Sharlan Chemie, Barcelona, Spain). Samples were taken, diluted and thereafter incubated in strictly anaerobic conditions at 37°C for 96 hours. Coliforms were cultured on a Chromocult differentiation medium (Merck, Darmstadt, Germany). The samples were incubated at 37°C, under aerobic conditions, for 24 hours. Total aerobic germs count was determined on blood agar after incubation at 37°C, under aerobic conditions, for 48 hours. After the incubation time had elapsed, the colonies were counted with a Titriplaque colony counter (LMIM, Esztergom, Hungary). The colony counts were expressed in $\log_{10}$ colony forming units (CFU) related to 1 g of sample.

About 3 g of caecal chyme was homogenized with 4.5 ml metaphosphoric acid (4.16%), then centrifuged at 10,000 x g for 10 min and filtered. The concentration of volatile fatty acids was measured by gas chromatography (Shimadzu GC 2010, Japan). 2-ethyl-butyrate (FLUKA Chemie GmbH, Buchs, Switzerland) was used as the internal standard. Parameters: Nukol 30 m x 0.25 mm x 0.25 μm capillar column (Supelco, Bellefonte, PA, USA), FID detector, 1:50 Split ratio, 1 μl injected volume, helium 0.84 ml/min. Parameters of the detector: air 400 ml/min, hydrogen 47 ml/min, temperature: injector 250°C, detector 250°C, column 150°C).

Statistical analysis of the data obtained was carried out by the SPSS statistical software package using the version 10.0. Effect of treatment, age and their interaction was analyzed by the analysis of variance. The significance of between group differences was tested by the Tukey post-hoc test.

## RESULTS AND DISCUSSION

There was no significant difference between groups in BW till the 28th day of age (Table 1). At day 35 G35 rabbits had a significantly higher BW compared to the other two groups, this difference still remained on day 42. Milk consumption increased from days 21 to 28. Thereafter in rabbits weaned on day 35 had lower milk consumption presumably due to the decrease in the mothers milk production and the increase in the solid feed consumption.

There was a sudden increase in the solid feed consumption after weaning. In group G35 feed intake was higher already on day 28, related to the decreasing milk production of the doe.

**Table 1**: Litter weight, milk and feed consumption (mean ± SE)

| Group | Age (days) 14 | 21 | 28 | 35 | 42 |
|---|---|---|---|---|---|
| | | | Litter weight (g) | | |
| G21 | 273±7[a] | 381±9[b] | 558±13[c] | 837±16[dA] | 1072±14[eA] |
| G28 | 247±14[a] | 343±21[b] | 559±24[c] | 850±35[dA] | 1068±45[eA] |
| G35 | 262±10[a] | 381±13[b] | 597±16[c] | 940±21[dB] | 1175±52[eB] |
| | | | Milk consumption (g) | | |
| G21 | | 210±12 | | | |
| G28 | | 192±14[a] | 263±16[b] | | |
| G35 | | 181±18[a] | 267±12[b] | 177±21[a] | |
| | | | Feed consumption (g) | | |
| G21 | | 7.7±2.7[a] | 242±65[bA] | 589±24[c] | 624±32[c] |
| G28 | | 11.9±4.3[a] | 137±31[bB] | 553±46[c] | 661±41[d] |
| G35 | | 10.1±4.2[a] | 140±35[bB] | 507±44[c] | 697±39[d] |

[a,b,c,d,e] significant differences in the same row, or [A,B] in the same column (P<0.05)

The pH of the caecal content was around 7 before weaning, and then decreased to 6.3-6.7 by 42 days of age (data not shown).

Anaerobic bacteria growing on the Schaedler agar were present at high amount ($10^8$/g) already on the 14th day of age (Table 2). On day 42 their number was significantly higher in G35 and G28 than in early weaned rabbits (G21). The decrease from 22 to 29 days in G21 could be due to the increasing intensity of the caecotrophy (Smith, 1965).

The amount of coliforms and bacteria growing in the presence of air were in accordance with the literature, they were decreasing with the age and paralelled the reduction in milk intake (Bornside and Cohn, 1965; Gouet and Fonty, 1979; Kovács *et al.*, 2004). There were no significant differences between groups (Table 2).

**Table 2**: The composition of the caecal microflora expressed in CFU $\log_{10}$/g chymus (mean ± SE)

| Group | Age (days) 14 | 22 | 29 | 36 | 42 |
|---|---|---|---|---|---|
| | | | anaerobic bacteria growing on the Schaedler agar | | |
| G21 | 8.3±0.3[ab] | 9.5±0.3[a] | 8.1±0.2[ab] | 8.2±0.1[ab] | 7.8±0.2[bA] |
| G28 | | | 7.6±0.7 | 8.3±0.3 | 8.1±0.1[B] |
| G35 | | | | 7.9±0.3 | 8.3±0.1[B] |
| | | | coliforms | | |
| G21 | 6.5±0.9[a] | 3.2±0.2[b] | 3.0±0.0[b] | 3.4±0.3[b] | 3.2±0.2[b] |
| G28 | | | 3.0±0.0 | 3.4±0.4 | 3.0±0.0 |
| G35 | | | | 3.4±0.3 | 3.6±0.6 |
| | | | total aerobic germs | | |
| G21 | 6.9±0.5[a] | 5.5±0.5[b] | 4.6±0.1[b] | 4.8±0.1[b] | 4.6±0.1[b] |
| G28 | | | 4.4±0.1 | 4.8±0.2 | 4.5±0.1 |
| G35 | | | | 4.4±0.1 | 4.7±0.4 |

[a,b,c,d,e] significant differences in the same row, or [A,B] in the same column (P<0.05)

Concentration of total VFA was significantly higher in G21 than in G28 and G35 during the whole experimental period (Table 3). The lowest VFA concentrations were found in G35 rabbits, presumably due to the lower solid feed intake till day 35. There was a decrease in VFA concentration on day 42, attributable to a temporary decrease in the feed intake. It was observed in all groups, and could be attributed to the sudden increase in the temperature. The percentage of acetic acid (C2) was above 70% after weaning in G21. In the other groups rabbits consumed more milk and less solid feed, so the concentration of C2 was significantly lower. With correspondence to the literature (Gidenne, 1996; Kovács *et al.*, 2002) the percentage of propionic acid was higher than that of butyric acid before weaning, but thereafter it changed, so the ratio of the two fatty acids (C3/C4) decreased from >1.0 to <1.0. Because of the early solid feed consumption of G21, the concentration of C3 was significantly lower, while that of C4 significantly higher on day 29 than in G28. The highest C4 content was measured in G21 during the whole experimental period.

**Table 3**: Volatile fatty acid content of the caecal chyme (mean±SE) (% of total fatty acid content)

| Group | Age (days) | | | | |
|---|---|---|---|---|---|
| | 14 | 22 | 29 | 36 | 42 |
| | | | Total VFA (mmol/l) | | |
| G21 | 43.9±10.4[a] | 58.7±17.2[ac] | 100.0±28.2[bA] | 93.3±17.9[bcA] | 76.6±6.4[abA] |
| G28 | | | 53.2±12.3[B] | 71.9±13.2[B] | 61.5±10.5[B] |
| G35 | | | | 38.2±7.8[C] | 48.3±4.5[C] |
| | | | Acetic acid (%) | | |
| G21 | 24.8±4.9[a] | 49.9±10.4[ac] | 74.7±10.0[bA] | 73.9±7.2[bcA] | 59.1±1.4[abA] |
| G28 | | | 41.5±5.6[B] | 57.4±6.7[A] | 50.3±5.0[A] |
| G35 | | | | 27.6±2.1[B] | 20.7±1.3[B] |
| | | | Propionic acid (%) | | |
| G21 | 5.5±2.3 | 4.6±0.8 | 5.9±0.3[B] | 6.0±0.6 | 5.7±0.5 |
| G28 | | | 7.4±1.2[A] | 5.1±0.6 | 4.2±0.7 |
| G35 | | | | 3.5±0.3 | 2.3±0.3 |
| | | | Butyric acid (%) | | |
| G21 | 4.7±1.9[a] | 10.0±2.8[ab] | 10.8±1.3[bA] | 12.0±0.8[ab] | 10.6±0.7[abA] |
| G28 | | | 7.4±1.7[B] | 8.8±1.3 | 6.1±1.1[B] |
| G35 | | | | 6.5±0.9 | 4.6±0.6[B] |
| | | | $C_3/C_4$ | | |
| G21 | 1.41±0.41[a] | 0.62±0.15[b] | 0.54±0.07[bA] | 0.51±0.06[bA] | 0.53±0.03[bA] |
| G28 | | | 1.0±0.05[B] | 0.60±0.06[B] | 0.70±0.10[B] |
| G35 | | | | 0.58±0.08[B] | 0.54±0.06[A] |

[a,b,c,d,e] significant differences in the same row, or [A,B] in the same column (P<0.05)

## CONCLUSIONS

The age of weaning significantly influenced the production parameters in rabbits. In G35 milk consumption and the simultaneous increase in solid feed intake resulted in better production. The composition of the caecal microflora was mainly influenced by age, effect of weaning was less pronounced. On the other hand milk and/or feed consumption, i.e. age of weaning had major effect on the composition of VFA. In summary, early weaning (at the age of 21 d) did not cause detrimental changes of the digestive physiological parameters examined, but resulted in lower production in rabbits.

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