Variability of caecal parameters in rabbits

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Introduction

The caecum is the major site of fibre digestion in rabbits. Caecal fermentation converts organic substrates to volatile fatty acids (VFA), gases and compounds incorporated into bacterial cells. The VFA are absorbed, providing an important source of energy for the host (Parker, 1976). The caecum is also supposed to have an important role in the etiology of digestive disturbances. Various nutritional and ontogenic factors affecting the caecal fermentation have been reviewed by Gidenne (1996). According to our knowledge, there is no study on animal-to-animal variation of caecal parameters in rabbits. Age-dependent variation of caecal traits in rabbits has been reported by Piattoni et al. (1995). The animal variability should be taken into account when evaluating results of in vitro experiments and various in vivo measurements. The aim of this study was to assess variability of caecal parameters in three groups of rabbits of different origin.

Material and methods

Rabbits of the first group (29 animals) were Hyla 2000 broilers from the Research Institute of Animal Production. Rabbits were fed a granulated concentrate feed similar to the feed of the first group. No details on the feed composition were available.

Three groups of rabbits of different origin (29, 27 and 28 animals; 3 or 4–6 months of age) were slaughtered, their caecal contents analyzed and used for inoculation of in vitro cultures. Whereas the caecal pH, dry matter percentages and acetate molar proportions in caecal volatile fatty acids (VFA) were relatively stable, molar proportions of other VFA varied considerably. In vitro incubations, caecal parameters varied somewhat less than in vivo. Methane production varied much more than total VFA production. No non-methanogenic rabbit, however, was found. The hydrogen recovery correlated significantly with the methane production and, in two out of three groups of rabbits, also with the propionate molar percentage. The caecal pH was inversely related to VFA concentration.

Key words: rabbit, caecum, metabolism, variability.

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The caecal pH and dry matter were determined immediately. Other analyses were performed using conserved samples. Samples of the headspace gas were taken at the end of the incubation and analysed by gas-liquid chromatography (FID) at the room temperature on a column of the Chromosorb WAW with −15% SP 1220 and 1% H3PO4 (Supelco, USA). Total VFA were estimated by titration, after steam distillation. Their molar composition was determined employing the gas chromatograph and the same column at 140 °C. Metabolic hydrogen balance was calculated according to Demeyer (1991) as: 

$$2H_{\text{rec}} = 100 \left(2P + 2B + 4M\right)/(2A + P + 4B),$$

where A, P, B, M represent molar production of acetate, propionate, butyrate and methane, respectively. Such calculation compares the amounts of metabolic hydrogen produced and recovered in reduced end products. The statistical treatment of data was performed using the GraphPAD Software, version 1.14. One-way analysis of variance was used to evaluate differences among groups. Variability of data was expressed as coefficient of variation (CV), i.e. as percentage of standard deviation from the mean.

**Results and discussion**

Results are summarized in Table 1 (in vivo measurements) and Table 2 (in vitro fermentation pattern). The caecal contents of rabbits slaughtered 2 h after the last feed intake (group 1) had lower pH and higher VFA concentration and dry matter percentage than caecal contents of rabbits slaughtered after 14–16 h fasting. In fasted rabbits (groups 2 and 3), molar proportion of caecal acetate was higher and that of propionate and butyrate lower than in non-fasted rabbits. Similar finding was reported earlier by Piattoni et al. (1997). Whereas the pH, dry matter contents and acetate percentages were relatively stable (CVs from 2.9 to 10.6%), molar proportions of other VFA varied considerably (CVs from 15.4 to 100%). Caecal parameters tended to be more stable in non-fasted rabbits (group 1) than in rabbits of other groups.

**Table 1**

| Parameter       | Rabbit group |
|-----------------|--------------|
|                 | 1            | 2            | 3            |
| pH              | 5.58 ± 0.16  | 6.26 ± 0.19  | 6.34 ± 0.30  |
| (2.9%)          | (3.0%)       | (4.7%)       |
| Total VFA (µmol/ml) | 101.1 ± 20.5 | 75.0 ± 0.19  | 74.9 ± 0.30  |
| (20.3%)         | (24.9%)      | (19.8%)      |
| Acetate (%)     | 71.4 ± 2.5   | 92.1 ± 6.2   | 84.7 ± 3.3   |
| (3.5%)          | (6.7%)       | (3.9%)       |
| Propionate (%)  | 10.4 ± 1.6   | 2.7 ± 2.4    | 6.0 ± 1.8    |
| (15.4%)         | (88.9%)      | (30.0%)      |
| Butyrate (%)    | 16.6 ± 2.5   | 3.9 ± 3.8    | 7.6 ± 2.8    |
| (15.1%)         | (97.4%)      | (36.8%)      |
| Other VFA (%)   | 1.6 ± 1.6    | 1.3 ± 1.2    | 1.7 ± 0.9    |
| (100%)          | (92.3%)      | (52.9%)      |
| DM (%)          | 23.1 ± 1.8   | 21.7 ± 2.3   | 19.4 ± 1.5   |
| (7.8%)          | (10.6%)      | (7.7%)       |

Means ± standard deviations. Coefficients of variation are given in parentheses.

abc Values in the same row with different letters differ significantly (P < 0.001).

**Table 2**

| Parameter       | Rabbit group |
|-----------------|--------------|
|                 | 1            | 2            | 3            |
| Total VFA (mmol/flask) | 11.42 ± 1.26 | 11.44 ± 1.25 | 10.32 ± 1.09 |
| (11.0%)         | (10.9%)      | (10.6%)      |
| Acetate (%)     | 72.6 ± 4.9   | 64.4 ± 3.8   | 65.6 ± 3.0   |
| (6.8%)          | (5.9%)       | (4.6%)       |
| Propionate (%)  | 8.8 ± 1.5    | 13.1 ± 3.7   | 13.0 ± 2.4   |
| (17.1%)         | (22.8%)      | (18.5%)      |
| Butyrate (%)    | 17.0 ± 3.7   | 20.1 ± 2.8   | 19.0 ± 3.2   |
| (21.8%)         | (13.9%)      | (16.8%)      |
| Other VFA (%)   | 1.6 ± 1.6    | 2.4 ± 1.1    | 2.4 ± 0.9    |
| (100%)          | (4.6%)       | (37.5%)      |
| Methane (µmol/flask) | 952 ± 338    | 1027 ± 507   | 813 ± 555    |
| (35.5%)         | (49.4%)      | (68.3%)      |
| 2H recovery (%) | 37.1 ± 6.5   | 45.8 ± 10.4  | 42.5 ± 10.1  |
| (17.5%)         | (22.7%)      | (23.8%)      |

Means ± standard deviations. Coefficients of variation are given in parentheses.

abc Values in the same row with different letters differ significantly (P < 0.001)
In in vitro experiments, caecal parameters varied somewhat less than in vivo. Methane production varied more than VFA production (CVs 35.5 – 68.3% and 10.6 – 11.0%, respectively). No non-methanogenic rabbit among 84 animals was found. Piattoni et al. (1997) observed that in in vitro incubations the effect of fasting was not pronounced when substrate was added to cultures. In this study, caecal microbes from non-fasted rabbits (group 1) produced significantly more acetate and less propionate than caecal microorganisms of fasted rabbits (groups 2 and 3). It should be, however, taken into account that diets were not the same in the three groups.

Statistical analysis revealed that the 2H recovery correlated significantly (P < 0.01) with the methane production. Correlation coefficients were 0.78, 0.68 and 0.96 in the group 1, 2 and 3, respectively. In groups 1 and 3, the 2H recovery correlated also with the propionate percentage (correlation coefficients, 0.46 and 0.45, respectively). The caecal pH and VFA concentration values were inversely related Correlation coefficients were -0.61, -0.46 and -0.10 in groups 1, 2 and 3, respectively. The latter coefficient was not statistically significant. Similar relationship between pH and VFA observed Jensen (1977) in the rumen fluid and Piattoni et al. (1995) in rabbit caecal contents. No significant correlation between production of methane and that of other metabolites was found. In the rumen, an inverse relationship between production of methane and propionate exists (Van Nevel et al., 1974).

Conclusions

Article is dedicated to research of peculiarities of microbial metabolism in caecum of rabbits of different nutrients, especially carbohydrates, in connection with age and action of many exogenous factors, which have goal-directed influence on common metabolism in growth organism, anabolic processes are intensified and the productivity of animals is increased.

It was determined, that microbial metabolism in caecum is at high level and it basic peculiarities is increased production of volatile fatty acids (VFA), the content of acetate (C2) of which consists of 80 mol%, propionate (C3) is formed not enough (< 5 mol%).The intensity of metabolism and the correlation of last products of enzyme depends on the age of rabbits. Methanogenesis begins its functioning from 8-week of age. Thanks to transition on plant feeds, the production of C2 is increased and forming of C3 is decreased twice.

Not only scientific and cognitive information on quantitative evaluation of end products of microbial fermentation in the caecum is important for regulating metabolism in the direction of enhancing nutrient assimilation and improving the productive features of rabbits, but also knowledge about the individual molar redistribution of low-molecular-weight carboxylic acids derived from carbohydrates and other sources of carbon, nitrogen and energy under certain conditions of nutrition.

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