

ノトバイオート鶏の盲腸内におけるEimeria tenella感染によるpH,酸化還元電位(ORP)および揮発性脂肪酸濃度(VFA)の変化

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Influence of *Eimeria tenella* Infection on the Cecal pH, Oxidation-reduction Potential and Concentration of Volatile Fatty Acids in Gnotobiotic Chicken

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Important factors for the growth of intestinal microorganisms or protecting host animals from incoming exogenous pathogens are pH level, oxidation-reduction potential (ORP), and concentrations of volatile fatty acids (VFA) in the intestine [4, 9, 10]. Baba *et al.* examined these factors in cecal contents of conventional chickens and reported that high concentrations of VFA inhibited salmonella infection and decreased level of VFA caused by *E. tenella* infection resulted in increased infection of salmonella [2]. These three factors in the intestine are undoubtedly influenced by a shift in populations in intestinal microorganisms or other factors that may disturb ecological equilibrium in the intestine. There seemed to be composite effects of multiple intestinal organisms on these values. Studies in conventional chickens are not suited in some instances for detecting factors that influence ecological system in the intestine.

Populations of bacteria in the cecal contents or cecal mucosa of gnotobiotic chickens are influenced, as in conventional birds, by *E. tenella* infection [6, 7]. Therefore, the present study was aimed to see the changes of pH, ORP, and VFA values in the cecal contents of germ-free or monoflora chickens infected with *E. tenella*.

Preparations of germ-free, gnotobiotic chickens and diets were reported [4-8]. The composition of the basal feed was reported previously [1]. One kilogram of basal feed was packaged in polyethylene bags and sterilized by exposing to 5M rad of gamma radiation. Feed and autoclaved water were available *ad libitum* during the experiments.

The *E. tenella* strain was originally supplied by the National Institute of Animal Health, Tsukuba. Sporulated oocysts were prepared from the feces of donor chickens 6 to 8 days after oral inoculation. Sporulated oocysts were sterilized with 0.5% peracetic acid solution.

Five species of bacteria; *Lactobacillus acidophilus*, *Bacteroides vulgatus*, *Bifidobacterium thermophilum*, and *Clostridium perfringens*; were supplied from the Institute of Physical and Chemical Research in Saitama. *Escherichia coli* 0-150 for 0 antigen was used [5-7].

Measurements of pH and ORP were reported previously [2]. For a measurement of VFA concentration, cecal contents were weighed and 9 volumes (w/v) of autoclaved distilled water were added. It was shaken thoroughly until homogenous mixture was obtained (tube #1). One ml of the mixture was withdrawn and diluted similarly with 9 volumes of autoclaved distilled water (tube #2). The contents of tube #2 were filtrated with a membrane filter (0.45 μm pore size, Sartorius Membranfilter GmbH, Göttingen, West Germany). The filtrated samples were used for the analysis of VFA. The analysis was carried out by a gas chromatograph (type GC-3BF, Shimazu Seisakusho Inc., Kyoto) with glass column, 3 mm in diameter and 2.1 m in length, charged with Unisole F-200 30/60 (Gaskuro Kogyo Inc., Tokyo) at 120°C and flow rate of N₂ at 100 ml/min [2, 3].

The present experiment consisted of two sets of 6 groups; five groups of monoflora chickens inoculated with one of the five microorganisms and a germ-free control group. One set of 6 groups was infected with *E. tenella* and another was not infected. All chickens at 2 days old except for the germ-free group, were once inoculated orally one ml of overnight cultured broth (Gifu anaerobic medium broth, Nissui Pharmaceutical Co., Tokyo) of the respective bacterium. Each chicken of *E. tenella* infected groups received a single oral dose of 5×10^4 sporulated oocysts at 4 days old. Four to nine chickens from each group were killed at 5, 7, and 9 days after *E. tenella* infection. At the necropsy, one cecal pouch was isolated for pH and ORP measurements, and the other pouch was used to measure VFA concentration.

Statistical analysis was conducted using Stu-

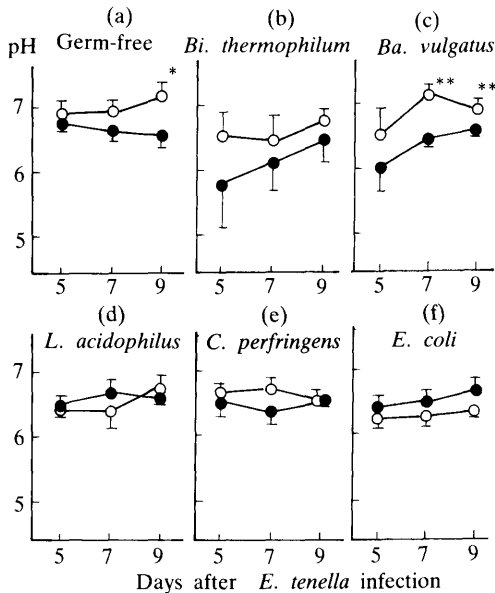


Fig. 1. The changes of pH value of cecal contents from gnotobiotic chickens infected with *E. tenella* (●) or uninfected controls (○). Significant difference between the groups on the corresponding day, *: $P < 0.05$, **: $P < 0.01$. Bar: Standard deviation.

dent's *t*-test.

The influences of *E. tenella* infection on the tested values of the germ-free and the monoflora chickens are shown in Fig. 1-3.

Significant decrease of pH levels in *Ba. vulgatus* gnotobiotic chickens infected with *E. tenella* (Fig. 1c) was not directly related to the volume of VFA produced (Fig. 3c). Conversely, significant increase in VFA production caused by *E. tenella* in *L. acidophilus* gnotobiotic chickens (Fig. 3d) did not bring about lowering pH levels (Fig. 1d). These findings can hardly be explained only by the bacterial species involved. Other factors, however, ought to be investigated.

E. tenella infection resulted in reduction of ORP levels (Fig. 2). Significant reductions of ORP were seen in *E. tenella* infected gnotobiotic chickens with *Bi. thermophilum* (5, 7, and 9 days after *E. tenella* infection), *Ba. vulgatus* (7 days), *L. acidophilus* (7 days), and *E. coli* (7 and 9 days). *E. tenella* alone reduced ORP levels very slightly (Fig. 2a). These findings indicate that reduction of ORP levels was caused by a combination of either one of these bacteria and *E. tenella*.

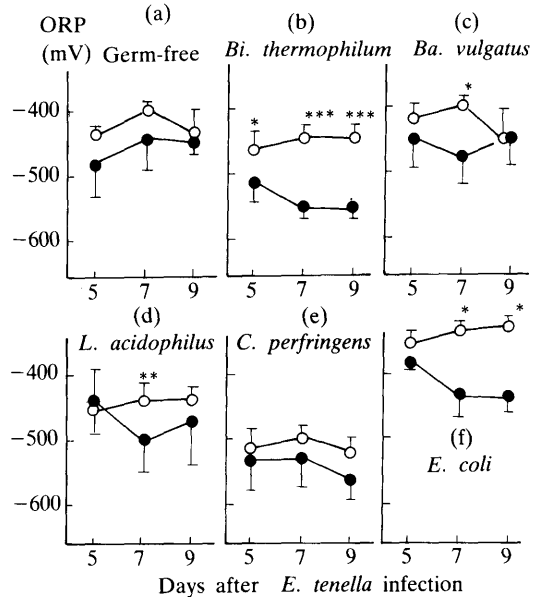


Fig. 2. The changes of oxidation-reduction potential (ORP) of cecal contents from gnotobiotic chickens infected with *E. tenella* (●) or uninfected controls (○). Significant difference between the groups on the corresponding day, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. Bar: Standard deviation.

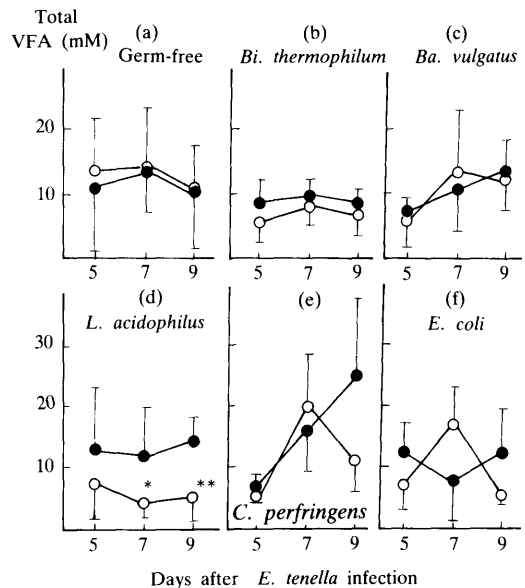


Fig. 3. The changes of total concentrations of volatile fatty acids (VFA) of cecal contents from gnotobiotic chickens infected with *E. tenella* (●) or uninfected control (○). Significant difference between the groups on the corresponding day, *: $P < 0.05$, **: $P < 0.01$. Bar: Standard deviation.

It is of a great interest to note that introduction of aerobic organism such as *E. coli* brought about increased levels in ORP values, an aerobic condition (Fig. 2f), while inoculation of *C. perfringens* caused decreased levels of ORP, an anaerobic condition (Fig. 2e), when compared with germ-free chickens (Fig. 2a).

Concentrations of VFA in gnotobiotic chickens and those infected with *E. tenella* in this study (Fig. 3) were lower than those of conventional chickens [2, 3]. This indicates that the VFA produced by each of 5 microbes in gnotobiotic chickens are very limited.

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要 約

ノトバイオート鶏の盲腸内における *Eimeria tenella* 感染による pH, 酸化還元電位 (ORP) および揮発性脂肪酸濃度 (VFA) の変化 (短報) : 深田恒夫・明貝俊彦・馬場栄一郎・荒川 皓 (大阪府立大学農学部家畜内科学教室)——*E. tenella* 感染時のノトバイオート鶏盲腸内 VFA 値の変動は盲腸内の pH 値の変化に影響を及ぼさなかった。*E. tenella* 感染群の ORP 値は非感染群のそれに比べて減少する傾向がみられ, 特定のノトバイオート鶏においては, 有意に減少した。