Diarrhea is often life threatening to piglets, and it can cause major financial loss in the pig industry [14]. Pathogenic bacteria and viruses cause diarrhea, which can also be caused by large amounts of undigested nutrients entering the large intestine due to dyspepsia [12] or, in the case of humans, due to short-bowel syndrome [2, 10]. These types of diarrhea are fermentative [15]. Furthermore, diarrhea can be caused by antibiotic treatment [8, 10]. The mechanisms involved in must not be the same for the different types of diarrhea, but there is little information available on the subject. We have observed significant differences in the organic acid profiles of feces from diarrheal pigs with these three types of diarrhea. Some of the information presented here will be important for the development of rapid diagnoses for the diarrhea, which should lead to the selection of appropriate therapeutic measures.

Sixty-five crossbred (Landrace × Large white × Duroc) commercial pigs of both sexes weighing approximately 10 kg each were used for an experiment with dyspeptic diarrhea. The animals were healthy and had not received any antibiotics for at least two weeks prior to the study. They were housed at Kyodoken Institute, Kyoto, with concrete floors. The animals were fed with a commercial diet for early growing piglets (Koromeal GS; Nippon Formula Feed Co., Yokohama) once a day at 9 a.m. (0.6 g ERFX/day injected intramuscularly). The remaining two pigs were used as control. All feces were collected (not pooled) for five days and stored at –20°C until analyses of organic acid concentration and moisture. Forty-six diarrheic or loose feces (moisture content in excess of 69%) were collected from AAD-induced piglets during the five days. Twenty-seven fecal samples considered normal (moisture content less than 69%) were collected from the control piglets. All animals used in the three experiments were handled in accordance with the guidelines established by the Kyoto Prefectural University Experimental Animal Committee for studies with laboratory animals.

The feces of three experimental piglets (dyspeptic diarrhea, 10 samples; AAD, 10 samples; colibacillosis, 11 samples) were examined for the presence of pathogenic bacteria as indicated by the Japanese standard methods described in

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Organic Acid Profiles in Feces of Pigs with Pathogenic or Non-Pathogenic Diarrhea

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ABSTRACT. A chemical characteristic of the feces of diarrheal piglets permits differentiation among piglets receiving antibiotic treatment and those with colibacillosis or dyspepsia. A high concentration of lactic or succinic acid was observed in the diarrheic feces of piglets receiving antibiotic treatments and those with dyspepsia; however, no lactic or succinic acids were detected in piglets with colibacillosis. There was, however, little difference in the total concentration of organic acids among the three types of diarrheal illnesses. A quantitative analysis of lactic and succinic acids in diarrheic feces might provide a means for rapidly differentiating between colibacillosis and non-pathogenic diarrheas in piglets.

KEY WORDS: diarrhea, fecal organic acid, swine.

the bacteriology book titled *Shin Rinsyo Kensagishiki Kouza vol. 11* [13]. Normal feces were not subjected to this bacteriological examination. The fresh feces were mixed with a bacteria-free swab and smeared on three types of agar plates. For the identification of pathogenic *E. coli*, samples were smeared on DHL agar plates (Nissui, Tokyo) and streaked. *E. coli*-like colonies (pink in color) were picked up after 37°C for an 18–24 hr incubation and transferred to Heart Infusion agar plates (Difco, Detroit, MI), then further incubated at 37°C for 18–24 hr. Hemolytic colonies consisting of rod-shaped bacteria that were stained Gram-negative were isolated and transferred to Minca agar plates [7] for K88 antigen determination. The K88 antigen was determined by a kit of K88 antibody (Dokusogenitsu Daitchoukin Senmou Koukessei, Seiken, Tokyo) after incubation at 37°C for 18–24 hr. These colonies were further identified as *E. coli* by Api 20E (Nihon bioMerieux, Tokyo). For identification of *Salmonella* spp., samples were smeared on SS agar plates (Nissui, Tokyo) and streaked. Black-purple-colored colonies were picked up after incubation at 37°C for 18–24 hr, transferred to Heart Infusion agar plates, and incubated at 37°C for another 18–24 hr. Non-wandering colonies were picked up for identification by Api 20E and *Salmonella* anti-antibody (Salmonella Men-ekkessei, Seiken, Tokyo). For identification of *C. perfringens*, samples were smeared on 5% (v/v) egg-yolk added to CW agar plates supplemented with lecithinase (Nissui, Tokyo) and streaked. Colonies positive for lecithinase consisted of rod-shaped bacteria that were stained Gram-positive. They were picked up after anaerobic incubation at 37°C for 18–24 hr, and identified by Api 20A (Nihon bioMerieux, Tokyo).

Fecal moisture and organic acid contents were measured. Moisture contents were determined by lyophilization as described elsewhere [9]. Organic acid (short-chain fatty acids, lactic, and succinic acids) contents were analyzed by ion-exclusion HPLC as described elsewhere [21].

Fecal samples were collected from 54 piglets that unexpectedly defecated loose or diarrheic feces. At the time, it was difficult to judge whether the diarrhea was caused by infection of enteropathogens or simple dyspeptic diarrhea, because the living conditions of these pigs, such as feed consumption, were maintained as usual from the initiation of diarrhea. However, the diarrhea appeared to be caused by factors other than infection by pathogenic bacteria because bacteriological examinations of the fecal samples did not reveal any infection by enteropathogens such as *E. coli*, *Salmonella* spp., and *C. perfringens*. Accordingly, we believe that the diarrhea was dyspeptic in nature. Succinic acid was not detected in the feces, but lactic acid (average 10 mmol/kg) was (Fig. 1-A, B). When fecal lactic acid concentration was plotted against fecal-moisture content (Fig. 1-A), the accumulation of this acid in feces was shown when the moisture content was at least 69%. The lactic acid in the feces was accumulated particularly between 69 and 82% of moisture content.

Seventy-three fecal samples were obtained from the control piglets as well as those induced to show AAD. The moisture content of the feces varied from 56 to 94%. The bacteriological examination on fecal samples from each piglet did not indicate any infection of enteropathogens. In AAD piglets, succinic acid (average 25 mmol/kg) and lactic acid (average 15 mmol/kg) were detected in feces (Fig. 1-A, B). Succinic and lactic acid concentrations in feces were plotted against fecal-moisture contents (Fig. 1-A, B). Accumulation of these acids in feces started at 79 and 83% moisture contents, respectively.

The fecal moisture contents of piglets with colibacillosis diarrhea ranged from 84 to 94%. Hemolytic and K88-antigen (-) *E. coli* was detected in all these samples. *Salmonella* spp. and *C. perfringens* were not detected. Since enteropathogenic *E. coli* was detected in these piglets, ampicillin (Vicillin-sol, Meiji Seika, Tokyo) was injected intramuscularly into the piglets. The treatment cured the diarrhea. Lactic and succinic acids were not detected in the feces of the piglets with colibacillosis diarrhea (Fig. 1-A, B).

Lactic and succinic acids are seldom detected in normal fermentation in the large intestine because they are typically intermediate metabolites and are quickly converted to propionate or acetate by acid-utilizing bacteria [17]. There were less than 2 to 4 mmol/kg of lactic and succinic acids in the piglet feces of those grown normally (Fig. 1-A, B). Abnormal fermentation in the large intestine often leads to lactic or succinic acid accumulation, such as in cases of short-bowel syndrome among human subjects [2], and when an acute change occurs in the diets of weaning piglets [5], or when rats ingest indigestible oligosaccharides [9]. Therefore, a rapid and large influx of a readily fermented substrate, such as starch, can induce lactic acid accumulation in the large intestine of piglets. In this study, lactic acid accumulation in diarrheal feces as a result of dyspepsia might have been caused by a rapid growth of amylolytic lactic acid producers such as *Streptococci* and the disappearance of lactic acid-utilizing bacteria, which are caused by a lowered pH as occurs in the rumen [4]. The same phenomenon was observed in *in vitro* models of pig cecal fermentation [21]. On the other hand, succinic and lactic acid accumulations in AAD can be observed when the intestinal microflora was predominated by Enterobacteriaceae and Lactobacillli due to the elimination of other bacteria such as acid-utilizing bacteria by the presence of antibiotics [18]. The accumulation of these acids in diarrheal feces of AAD suggests that AAD may be categorized as a fermentative diarrhea. Lactic and succinic acids are very slowly absorbed by epithelial cells; therefore, these acids accumulate easily in the large intestine when there are few lactic and succinic acid-utilizing bacteria [20]. A high concentration of lactic and succinic acids in the large intestine leads to diarrhea [15, 16] because luminal accumulation of lactic and succinic acids leads to an osmotic load for water secretion from the mucosa in the large intestine [9]. Lactic and succinic acids are strong organic acids that are believed to lower luminal pH [6]. In addition, lactic and succinic acids do not lead to bicarbonate...
secretion from the mucosa. This may cause further acidification of the lumen [20]. It has been postulated that low luminal pH, i.e., pH 5.0, causes such serious damage to the large intestine in rats and pigs that normal functions such as water absorption cannot be maintained [1, 15]. However, diarrheal feces do not necessarily have low pH. In a preliminary study, there was no difference in pH between the lactic acid-accumulated diarrheal feces in dyspepsia and normal solid feces free from lactic acid (data not shown). Moreover, in this study, fecal pH in AAD piglets did not correlate with lactic and succinic acid concentration (data not shown). Feces contain many buffering substances in different amounts that may affect fecal pH. It seems difficult therefore to establish absolute criteria for fecal pH that allow for distinctions in the nature of diarrhea.

In contrast to dyspepsia and AAD, colibacillosis diarrhea is caused by virulence of enterobacteria. Excessive water secretion from the small intestine due to an enterobacterial toxin is the primary cause of diarrhea [11].

It is noteworthy that there was little difference in the total concentration (mmol/kg feces) of organic acids among the three diarrheal illnesses (Fig. 1-C), although there was a significant difference in lactic or succinic acid concentration in feces. Organic acids, especially short-chain fatty acids, are major bacterial fermentation products in the large intestine [3]. Therefore, fermentation in the large intestine still occurs under all conditions except when lactic or succinic acid accumulates.

Lactic or succinic acid accumulation in diarrheic feces is therefore a sign of fermentative diarrhea in dyspepsia or AAD and does not correlate with colibacillosis, although the animals excreted loose or diarrheic feces that were similar in both conditions. During the initial stage of diarrheal illness in which pigs maintain normal activity, it is hard to distinguish colibacillosis from non-pathogenic diarrhea without bacteriological determinations. This is a time-consuming task, however the results of the present study suggest that the analysis of lactic and succinic acids in feces would provide a possible method for rapidly diagnosing diarrhea caused by the pathogenic *E. coli*.

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