NOTE *Immunology*

Decreasing Traits of Fecal Immunoglobulin A in Neonatal and Weaning Piglets

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ABSTRACT. The concentration of fecal secretory immunoglobulin A (sIgA) in neonate and weaning piglets was measured daily from 1 day after birth to 50 days of age. The concentration of fecal sIgA started from the level of 10^4 μg/g wet feces 1 day after birth and then increased to a maximal value of up to 10^5 μg/g within a few days of birth. The values constantly declined to between 10^1 and 10^2 μg/g for the next 10 days and were relatively constant until weaning. The level of sIgA in the feces remained very low until at least 50 days of age. The vulnerability of pre- or post-weaning piglets can be explained, at least in part, by this low level of sIgA in the intestine.
KEY WORDS: feces, immunoglobulin A, piglet.

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Weaning piglets are often vulnerable to enteric pathogens, such as enterotoxigenic *Escherichia coli, Clostridium perfringens*, and rotavirus [8–10]. This vulnerability can be explained by factors such as an immature immune system.

Secretory immunoglobulin A (sIgA) is a protective molecule of the mucosal immune system [13]. It mediates the primary immunological defense line in the mucosal immune system. In the case of pigs, maternal sIgA is supplied to piglets by the maternal colostrum and milk. The luminal level of sIgA in newborn piglets may decrease constantly as the suckling period proceeds because the supply of sIgA from milk naturally decreases due to the reduction of milk consumption. In a previous study, we demonstrated that there is a period around weaning that is supposed to be critical for maturation of the host immune system in terms of immune deviation; in this period, the level of luminal sIgA decreases drastically and remains low for several days in experimental rodents [4]. In the case of rodents, which is different from that of pigs, a placental transfer of immunoglobulin G occurs. However, sIgA is supplied by the colostrum and maternal milk in rodents. Therefore, the evolution of luminal sIgA may be the same for both animals. However, research into the day-to-day evolution of the intestinal sIgA level of piglets after birth has been limited. In the previous study reported by Scharek *et al.* [12], the changes in fecal IgA in piglets was determined weekly from 7 days after birth until 70 days of age. Distinctive changes in fecal IgA of piglets were shown including a drop at day 14 after birth and a constant decrease from day 28 after birth. The reasons for such decline in the fecal, hence intestinal luminal, sIgA level seem to be related to dietary management, i.e., dry feed was offered to the piglets at 14 days of age and weaning at 28 days of age, although it was not conclusive due to the weekly sampling. Therefore, we will show in this report the daily changes in fecal sIgA during suckling and weaning piglets from birth to 50 days of age. An important finding was that the luminal sIgA level rapidly declined from 2 or 3 days after birth until 10 to 14 days of age, the level further declined to a very low level from 25 days of age to 30 or 32 days of age and remained low until at least 50 days of age at which time the piglet body weight was about 20 kg.

Four pregnant sows were used as donors of piglets. They were crossbred (Landrace x Large white) and inseminated with a Duroc boar. Three sows (#1, #2 and #3) were kept at an experimental farm of the KYODOKEN Institute (Fukuchiyama, Kyoto, Japan), and one sow (#4) was kept at the technical center of Toyohashi Feed Mills (Shinshiro, Aichi, Japan). They were kept under standard care conditions during the experiment. Three (Litter 4) or four (Litters 1, 2 and 3) newborn piglets were randomly selected and subjected to sampling of rectal stool. Litters 1 and 3 were composed of female piglets. Litters 2 and 4 were composed of male piglets. Daily sampling started at 1 day of age and continued up to 50 days of age. Gentle stimulation of the anus with a sterile cotton swab was performed when necessary. All piglets were weaned at 25 days of age by segregation from the sows. Litters were separated in pens. The sows were fed a commercially available formula feed (#1–3, BreeMeal Maxim, Nippon Formula Feed, Yokohama, Japan; #4, Super Livro Master, Toyohashi Feed Mills) throughout the experiment. Piglets at the KYODOKEN Institute (Litters 1, 2 and 3) were fed SDS No. 1 until 35 days of age and then SDS No. 2 (Nippon Formula Feed) until the end of experiment. The piglets at Toyohashi Feed Mills (Litter 4) were fed Pearl Milk™ (PM) 1 from 0 to 17 days of age, PM 2 from 14 to 32 days of age, PM 3 from 30 to 44 days of age and then PM 4 (Toyohashi Feed Mills) from 40 to 50 days of age. SDS Nos. 1 and 2 are both characterized by a high

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concentration of animal protein such as defatted milk, whey, protein and fish meal, mixed with other ingredients, such as grain powder and sucrose. These types of feed do not contain any antimicrobials and probiotics. PM1, 2, 3 and 4 are similar, but they contain avilamycin (40, 40, 30 or 20 g/t, respectively), colistin sulfate (40, 40, 30 or 20 g/t, respectively) and morantel citrate (30 g/t) as antimicrobials.

Fecal samples were immediately frozen and transported to Kyoto Prefectural University. After they were thawed, an appropriate amount of cold sterile phosphate-buffered saline was added and mixed well. The supernatant was recovered from the mixture after centrifugation at 15,000 rpm for 15 min at 4°C. A pig IgA ELISA Quantitation Kit (Bethyl, Montgomery, TX, U.S.A.) was used to determine the total amount of sIgA in the fecal extract. The above-mentioned supernatants were further diluted to obtain appropriate dilutions (200 to 400 fold), 100 μl of which was assayed. The procedures were carried out according to the manufacturer's instructions. *O*-Phenylenediamine (0.4 mg/m*l*) in a 0.24 mM citrate buffer (pH 5.0) with 0.2% H_2O_2 was used as the substrate for the IgA-horseradish peroxidase-conjugated antibodies. The absorbance was measured at 490 nm with the Model 550 microplate reader (Bio-Rad, Tokyo, Japan). The fecal sIgA levels were statistically analyzed by one-way ANOVA with post hoc comparison by Scheffe's F test using the Statcel software [17].

The fecal sIgA levels of the piglets are shown in Fig. 1. The values in this figure are expressed in a logarithmic form because of the remarkable decline in the values from the beginning of the experiment. The values were ca. 10^4 μg/g wet feces 1 day after birth, and they then increased to maximal values of up to 10^5 μg/g wet feces within a few days after birth. They steadily declined to between 10^1 and 10^2 μg/g wet feces for the next 10 days and were relatively constant until weaning. However, there was another decline a few days after weaning. The level of sIgA in the feces remained very low until the end of the experiment. The first and second declines in the fecal IgA level appeared to be much larger than those previously reported [12]. There seemed to be a difference in the rate of the first decline in the fecal sIgA level between Litter 4 and other three litters. The values of Litter 4 were significantly lower ($p<0.05$) than those of Litters 1 and 3 on days 3 and 4 after birth. The value of Litter 4 was significantly lower ($p<0.05$) than that of Litter 1 on day 5 after birth. The dietary composition did not seem to significantly affect development of mucosal immunity in the piglets because the piglets from Litter 4 showed similar traits, except for the 3 day period just after birth, as those observed in the other 3 litters that were fed diets free from antimicrobials. However, the limited number of animals might have interfered with detection of a statistical significance. Further elucidation of the effect of diet and sex of piglets should be considered.

The period characterized by a low intestinal sIgA level is

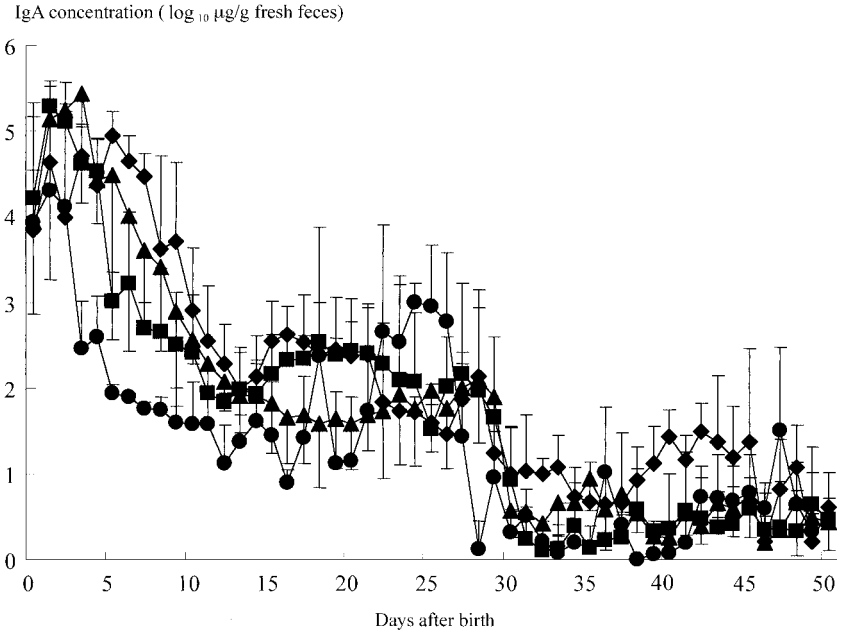

Fig. 1. Changes in of the fecal IgA concentration (μg/g) of piglets weaned at 25 days after birth. Values are expressed in logarithmic form and means with standard deviations. ◆, Litters (n=4) from sow #1; ■, Litters (n=4) from sow #2; ▲, Litters (n=4) from sow #3; ●, Litters (n=3) from sow #4. Litters 1 and 3 were composed of female piglets. Litters 2 and 4 were composed of male piglets. For details, see text. The values for Litter 4 were significantly lower ($p<0.05$) than those for Litters 1 and 3 on days 3 and 4. The value for Litter 4 was significantly lower ($p<0.05$) than that for Litter 1 on day 5.

as short as a few days in the case of the rodent model [4, 5]. Bacteria colonize intensively in the intestine during this period [4, 5]. This colonization may help development of the mucosal immune system in weaning pups. Due to development of the piglets' own mucosal immunity, the increase in bacterial colonization seems to cease at 30 days of age. In the case of piglets, we also observed intensive colonization by bacteria, which is similar to the findings in rodents. However, the bacterial diversity still increased even at 50 days of age [6]. It has, therefore, been suggested that the mucosal immune system of weaning piglets does not develop well even at 50 days of age when piglets weigh in excess of 20 kg. This point was confirmed in this experiment, in which the mucosal immunity of weaned piglets at 50 days of age had not yet developed. In the case of the rodent model, pups start to eat fiber-rich solid food from their dams before weaning. Indeed, they consumed highly digestible ingredient diets (SDS No. 1 or PM1 or 2). Early development of mucosal immunity in rodents can obviously be explained by rapid maturation; however, it must also be stimulated by consumption of a fiber-rich, less digestible diet.

Irrespective of the presence of antimicrobials, the luminal sIgA level remained very low 3 weeks after weaning. The prevalence of some of the enteropathogens, such as enterotoxigenic *E. coli* and enterotoxemic *E. coli*, in post-weaning piglets is well known [2, 15]. The high prevalence of these enteropathogens can be explained by the lower sIgA concentration during this period. If the nasal sIgA level showed similar traits to those observed in fecal sIgA, this may explain the prevalence of several respiratory diseases in post-weaning piglets, such as atrophic rhinitis [1], Glasser's disease [11] and Mycoplasmal disease [14]. This point needs further elucidation, but the low sIgA level on the nasal and bronchial mucosa likely occurs. Nasal and bronchial sIgA is also supplied from the maternal colostrum in newborn animals [3], and serum IgA in piglets would be exhausted after gut closure due to constant secretion to the surface of the mucosa. Diet is one of the factors affecting development of mucosal immunity of weaning animals, as evidenced in an experiment using a rodent model. Obviously, the composition of the diet for weaning piglets is determined by nutritional requirements to realize maximal performance in piglets. Therefore, it is not reasonable to modify the dietary components to reduce the energy content. However, if routinely used diets, particularly post-weaning diets that are used for animals up to 20–30 kg of body weight, delay development of mucosal immunity in piglets, nutritional requirements and development of mucosal immunity must somehow be balanced. This viewpoint is likely important because routine use of dietary antimicrobials will not be acceptable to consumers.

In our recent experiment using a rodent model, a decline in the luminal sIgA level at around weaning was significantly alleviated by an oral dose of *Lactobacillus johnsonii* NCC533 [7]. This observation was confirmed in post-weaning piglets by oral administration of *L. plantarum* Lq80 [16], although one report has indicated the ineffectiveness of probiotic *Enterococcus faecium* in relation to development of the immune system of piglets [12]. In our previous experiments using rodents or piglets, we orally administered live bacteria with a syringe at a dose of 10^{10} cells/shot. This dosage was much larger than that used in the experiment with *E. faecium*, which was included in the diet at a level of 10^9 cells/kg feed. Use of a probiotic is a potential solution to stimulate development of mucosal immunity; however, the appropriate dosage and choice of species remain to be studied.

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