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# Thermophile-fermented compost extract as a possible feed additive to enhance fecundity in the laying hen and pig: Modulation of gut metabolism

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**Recently, we reported that the oral administration of an extract of compost fermented with marine animal resources and thermophilic *Bacillus* species should confer health benefits in fish, pigs and rodents. Herein, the relations between fecundity and gut metabolites in laying hens and pigs on farms after oral exposure to compost were investigated. On the hen farms, the egg production of hens continuously administered the extract was maintained at significantly higher levels compared with the hens not administered the extract. On the swine farms, after the compost treatment, the shipping dates of fattening pigs were shortened, with an improvement in the death rate of the pigs. When the levels of fecal organic acids, such as short-chain fatty acids, lactate, and ammonium, as indicators of gut metabolism and energy sources for peripheral tissues, were examined, the levels of the acetate, propionate, and butyrate in the feces of the hens and pigs in the compost-treated group were not always different from those in the untreated control group. However, the levels of lactate were consistently low in the feces of both animals after the compost treatment. The fecal ammonium concentrations in old hens (age 597–672 days) and 2-month-old piglets from the compost-fed mother sows were low when compared with the untreated groups. The concentrations of free organic acids and their related compounds in the animal products (eggs and pig loins) were nearly equal to those in the untreated control products. Thus, the oral administration of the thermophile-fermented compost should improve the fecundity of hens and pigs by modifying their gut metabolism.**

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[**Key words:** Thermophiles; Compost; Feces; Lactate; Ammonium; Pig; Hen]

Probiotics are defined as microbial food ingredients and/or cultures that include potentially beneficial microbes for gut microflora. Probiotic bacteria, such as *Lactobacillus* sp. and *Bifidobacterium* sp., inhabit the animal gut and have been used to control the health conditions in animals. The physiological effects of these mesophilic microbes on the health of animals have been investigated (1–5). Members of the genus *Bacillus*, such as *Bacillus subtilis* and *Bacillus licheniformis*, are also considered inhabitants in the animal gut and members of probiotic bacteria (6,7). Interestingly, thermophilic *Bacillus* strains that reside in porcine feces have been shown to enhance composting (8,9). However, the physiological and environmental roles of thermophilic *Bacillus* species in the animal gut have been poorly understood.

Recently, we reported that the oral administration of compost or its extract, which including thermophile bacteria, increases the levels of free amino acids in the muscle of flatfishes and their survival in the fisheries (10), indicating that thermophiles can potentially act as probiotic bacteria. Because the compost used here is a fermented product of marine animal resources such as small fish, crabs, and shrimps and lacks value as a commercial food, it is called marine animal resources (MAR) compost (11). An analysis of its microbial structure has revealed that bacteria similar to *Bacillus thermoamylovorans* and *Bacillus thermocloacae* are the predominant inhabitants, and a bacterium related to *B. subtilis*, which produces a cyclic lipopeptide with antifungal activity, also exists in this compost. In stockbreeders, the oral administration of a water extract of MAR compost to animals (including chickens and pigs) decreases the volume of fecal matter (12). The feces of chickens and pigs are frequently composted, but unlike most other cases, the temperature during the fecal composting process was maintained at 70 °C or more by fermentation-associated self-heating on the swine farms where the MAR compost extract was administered, suggesting that the intake of thermophilic bacteria by pigs should contribute to the rapid and active composting of their feces, as previously reported by other researchers (8,9). In this study, when the long-term administration with MAR compost caused efficient fecundity in hen and swine farms, the effects on the levels of fecal organic acids were investigated. Short-chain fatty acids (SCFAs), e.g., acetate, butyrate, and propionate, were produced by the metabolism of gut microbiota and serve as energy sources for

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peripheral tissues in the gut (13,14). When the compost extract was administered, the levels of fecal lactate, a health index of gut metabolites (15–17), were particularly reduced in hens, and a similar effect was observed in pigs. These observations suggest that the oral administration of a MAR compost extract with thermophilic *Bacillus* species should influence the gut metabolism in livestock animals, thereby improving their fecundities.

## MATERIALS AND METHODS

**Treatment of MAR compost** MAR compost and its extract, which were used in this study, were made as previously described (10–12). In brief, the compost was made from marine animals and coffee residues by a repeated fed-batch fermentation system with three bioreactors (11). Bacteria populations in this compost were composed mainly of *Bacillaceae*. The compost was diluted 1/100 with potable water (as v/v), and the resulting suspension was incubated aerobically at 60 °C for 10–24 h (10,12). The compost suspension was used as the compost extract. The compost extract contained <99.9% $H_2O$, <0.1% protein, <0.1% lipid, <0.1% fiber, <0.1% ash, <50 ppm lactate, and <50 ppm butyrate, indicating that the compost extract itself included negligible nutrient as previously described (10,12). The number of thermophilic bacteria in the compost extract was assessed as $<5.0 \times 10^6$ CFU/ml (10,12).

**Hens, their breeding conditions, and sampling** A flock of hens comprising approximately 10,000–30,000 birds was reared in an environmentally controlled windowless house. All treatments of hens were conducted in accordance with the institutional animal care guidelines for the poultry farms Motoki Poultry Farm (Chiba, Japan) (farm A) and Miyakoji Farm (Ibaraki, Japan) (farm B). A flock of the *Julia Lite* strain of hens was reared on farm A, and a flock of the *Isa White* strain of hens was reared on farm B. The hens of the MAR compost group on farm A were continuously given approximately 0.5% (v/v) compost extract in potable water *ad libitum*. The thermophile-fermented MAR compost used in this study was made from marine animal resources by a fed-batch fermentation system with three bioreactors (Miroku Co., Ltd. and Keiyo Plant Engineering Co., Ltd., Japan). The MAR compost grain was diluted 1/100 with potable water (as v/v), and the resulting suspension was incubated aerobically at 60 °C for 10–24 h as previously described (10,12). The diluted extract was used as a compost extract. In contrast, the hens of the compost group in farm B were continuously fed approximately 0.1% (v/v) MAR compost grain with the commercial feeds. Fresh fecal matter from the hens was collected and stored at −80 °C. The samples of eggs were directly supplied from the farm and stored at −80 °C after homogenization. The rate of egg production was the proportion of hens that were laying among the total reared hens, and this proportion was estimated daily.

**Pigs, their breeding conditions, and sampling** Sows (crossbred Landrace × Large White) and their piglets (crossbred sows × Duroc) were conventionally maintained at two Japanese farms: Oosaki Swine Business, Ltd., Japan (Gunma, Japan) (farm C) and Yabe Farm (Chiba, Japan) (farm D). All pigs were orally administered commercial feeds and potable water *ad libitum*. All treatments of the pigs were conducted in accordance with the institutional animal care guidelines for the farms. The management was according to individual farm-specific guidelines for the most efficient production of piglets (12). The pigs were given 0.4% (v/v) MAR compost extract, which was supplied by a water pipe at the swine farm, as previously described (12). Fresh fecal matter from the sows and piglets was collected and stored at −80 °C. The loin samples were supplied from the meat processing plant and stored at −80 °C after homogenization.

**Examination of feces, eggs, and loins** The stored samples were subjected to a capillary electrophoresis analysis for the determination of SCFAs, lactate, and ammonium, as previously described (18). Three grams of fecal matter or egg were suspended in 10 ml of 99.5% EtOH; then, 20 ml $H_2O$ was added. This extract was stirred for 10 min and centrifuged at 3000 ×*g* for 10 min. The supernatant (1.5 ml) was poured into a Microcon 3000 column (Millipore Co., Ltd., USA) and was centrifuged at 14,000 ×*g* for 10 min. The filtrates, including molecules of less than 3000 Da, were analyzed with a capillary electrophoresis system (CE system G1600A) equipped with a fused silica capillary (l = 104 cm, L = 112 cm, and id = 50 μm) with a basic anion buffer (Agilent Co., Ltd., USA). The samples were electrophoresed at −30 kV (reversed polarity) under 300 mbar × s (50 mbar for 6.0 s) of pressure, and the temperature of the capillary was controlled at 20 °C (19). The peak area in the resulting chromatograms was calculated with ChemiStation software, and the SCFAs, lactate, and ammonium contents were determined. The levels of $H_2O$ in the fecal matter were estimated by the subtraction of the dry weight from the fresh weight of the fecal matter. The free amino acids and related compound contents of eggs from farm A and loins (pork shoulder muscles) from farm C were measured by an amino acid analyzer (JLC 500/v) (JEOL Ltd., Japan). In brief, the egg samples and loin samples, which were homogenized, deproteinized and filtered according to the manufacturer's protocol (20), were analyzed.

**Statistical analyses** All data were statistically analyzed using the Student *t* test, Mann–Whitney U test or Kruskal–Wallis test for comparisons between subjects. The statistical analyses were performed with StatView-J 4.02 software and Microsoft Excel 2011 for Macintosh.

## RESULTS

**Fecundity of hens and their feces** The rate of egg production and the egg weight of the hens with and without the MAR compost extract were measured in two hen houses on farm A. As seen in Table 1, the egg production rates were not different between the control group and the MAR compost group until the age of 200 days. However, the egg production in the MAR compost group significantly increased thereafter, but the egg weight decreased when compared with the control group before forced molting (before 424 days of age). Because forced molting was performed at 425–455 days, the egg production was largely decreased in both groups. However, the tendency of increased egg production and decreased egg weight in the MAR compost group was also observed after forced molting (after 480 days of age). Generally, egg production rather than egg weight is preferable in terms of efficiency; therefore, the observations in the compost group were better, from the viewpoint of fecundity, for the laying hen industry. In addition, old hens should produce large eggs and fewer eggs, as observed in the untreated control group. Because eggs of size 58 g–64 g (referred to as M size in the Japanese

**TABLE 1.** Egg production rates and egg weights in the hens on farm A, $n = 11{,}524$–$12{,}507$.

| Age in days (d) | Control group | | Compost extract group | |
|---|---|---|---|---|
| | Production (%) | Egg weight (g) | Production (%) | Egg weight (g) |
| 115 | 0 | 0 | 0 | 0 |
| 130 | 0.3 ± 0.1 | 33.1 ± 2.6 | 0.3 ± 0.1 | 33.1 ± 2.7 |
| 144 | 17.9 ± 3.4 | 41.6 ± 0.4 | 17.5 ± 3.3 | 41.7 ± 0.4 |
| 158 | 63.7 ± 2.9 | 46.1 ± 0.2 | 64.9 ± 3.2 | 45.8 ± 0.2 |
| 172 | 80.7 ± 0.5 | 50.8 ± 0.3 | 80.9 ± 0.3 | 50.3 ± 0.3 |
| 186 | 87.8 ± 0.7 | 52.9 ± 0.3 | 87.2 ± 0.8 | 52.7 ± 0.2 |
| 200 | 92.7 ± 0.2 | 56.6 ± 0.4 | 92.6 ± 0.1 | 55.3 ± 0.1 |
| 214 | 92.8 ± 0.1 | 59.0 ± 0.1 | 93.7 ± 0.1[a] | 57.5 ± 0.2[a] |
| 228 | 93.0 ± 0.1 | 61.1 ± 0.1 | 94.0 ± 0.1[a] | 60.2 ± 0.1[a] |
| 242 | 92.7 ± 0.1 | 61.7 ± 0.1 | 93.5 ± 0.1[a] | 60.8 ± 0.1[a] |
| 256 | 92.5 ± 0.1 | 62.8 ± 0.1 | 93.4 ± 0.1[a] | 61.6 ± 0.1[a] |
| 270 | 92.7 ± 0.1 | 62.9 ± 0.1 | 93.7 ± 0.1[a] | 62.0 ± 0.1[a] |
| 284 | 92.9 ± 0.1 | 63.3 ± 0.0 | 93.9 ± 0.1[a] | 62.6 ± 0.1[a] |
| 298 | 92.6 ± 0.2 | 63.6 ± 0.1 | 93.8 ± 0.2[a] | 63.1 ± 0.1[a] |
| 312 | 92.3 ± 0.1 | 63.3 ± 0.0 | 93.4 ± 0.3[b] | 62.6 ± 0.1[c] |
| 326 | 91.6 ± 0.1 | 63.9 ± 0.1 | 93.1 ± 0.1[a] | 63.4 ± 0.1[a] |
| 340 | 90.7 ± 0.1 | 63.6 ± 0.1 | 91.4 ± 0.1 | 63.5 ± 0.0 |
| 354 | 91.0 ± 0.1 | 64.0 ± 0.1 | 92.1 ± 0.1[a] | 63.9 ± 0.0 |
| 368 | 90.4 ± 0.1 | 64.2 ± 0.1 | 91.3 ± 0.1[a] | 64.3 ± 0.1 |
| 382 | 90.1 ± 0.1 | 64.3 ± 0.0 | 91.1 ± 0.1[b] | 63.8 ± 0.1[a] |
| 396 | 89.1 ± 0.1 | 64.5 ± 0.0 | 90.1 ± 0.1[a] | 64.3 ± 0.0[a] |
| 410 | 87.6 ± 0.1 | 65.0 ± 0.0 | 89.0 ± 0.1[a] | 64.4 ± 0.1[a] |
| 424 | 87.0 ± 0.1 | 65.2 ± 0.1 | 88.8 ± 0.1[a] | 64.7 ± 0.1[a] |
| 438 | 28.6 ± 9.9 | 65.2 ± 0.0 | 28.8 ± 10.0 | 64.5 ± 0.0[a] |
| 452 | 0.2 ± 0.1 | 65.2 ± 0.0 | 0.1 ± 0.1 | 64.5 ± 0.0[a] |
| 466 | 16.4 ± 4.8 | 63.6 ± 0.5 | 14.5 ± 4.4 | 63.0 ± 0.4[a] |
| 480 | 75.7 ± 2.7 | 66.1 ± 0.5 | 75.6 ± 3.1 | 66.3 ± 0.4 |
| 494 | 88.3 ± 0.4 | 67.1 ± 0.3 | 89.8 ± 0.3[b] | 66.7 ± 0.3 |
| 508 | 88.3 ± 0.2 | 66.7 ± 0.0 | 89.2 ± 0.2[b] | 64.7 ± 0.1[a] |
| 522 | 87.2 ± 0.2 | 63.2 ± 0.0 | 87.8 ± 0.3 | 62.5 ± 0.0[a] |
| 536 | 87.2 ± 0.2 | 63.8 ± 0.2 | 88.1 ± 0.3[c] | 62.8 ± 0.2[b] |
| 550 | 87.1 ± 0.2 | 64.3 ± 0.1 | 88.0 ± 0.2[b] | 62.6 ± 0.0[a] |
| 564 | 86.4 ± 0.1 | 64.4 ± 0.1 | 88.3 ± 0.2[a] | 62.5 ± 0.2[a] |
| 578 | 85.9 ± 0.2 | 64.6 ± 0.1 | 87.7 ± 0.1[a] | 63.5 ± 0.1[a] |
| 592 | 85.5 ± 0.2 | 65.1 ± 0.1 | 87.5 ± 0.2[a] | 64.4 ± 0.0[a] |
| 606 | 83.7 ± 0.2 | 65.5 ± 0.1 | 86.3 ± 0.2[a] | 64.1 ± 0.2[a] |
| 620 | 82.1 ± 0.2 | 66.7 ± 0.0 | 85.2 ± 0.3[a] | 64.0 ± 0.0[a] |
| 634 | 81.0 ± 0.3 | 64.7 ± 0.1 | 84.2 ± 0.2[a] | 64.3 ± 0.1[a] |
| 648 | 79.9 ± 0.2 | 65.4 ± 0.0 | 82.6 ± 0.3[a] | 64.7 ± 0.1[a] |
| 662 | 78.4 ± 0.1 | 65.6 ± 0.0 | 81.3 ± 0.1[a] | 65.1 ± 0.0[a] |
| 676 | 76.8 ± 0.2 | 65.2 ± 0.0 | 80.3 ± 0.2[a] | 64.9 ± 0.0[a] |

a, $p < 0.001$; b, $p < 0.01$; c, $p < 0.05$; vs. control group at the same age (day), respectively. Forced molting was performed between 425 and 455 days of age.

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