# Effects of Dietary *Lactobacillus brevis* Supplementation on Growth Performance, Dry Matter and Nitrogen Digestibilities, Blood Cell Counts and Fecal Odor Emission Compounds in Growing Pigs

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# 육성돈사료에 *Lactobacillus brevis*의 첨가가 성산성, 건물과 질소 소화율, 혈구수 및 분 내 악취 발생 물질에 미치는 영향

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## 요 약

본 시험은 육성돈 사료내 생균제 (*Lactobacillus brevis*, $3.4 \times 10^8$ CFU/g)의 첨가, 급여가 생산성, 건물과 질소 소화율, 혈구수 및 분 내 악취 발생 물질에 미치는 영향을 조사하기 위하여 실시하였다. 개시시 체중 24.60 ± 1.28 kg의 3원교잡종 [(Landrace × Yorkshire) × Duroc] 육성돈 96두를 공시하여 42일간 사양시험을 실시하였다. 시험설계는 옥수수–대두박 위주의 사료내 생균제를 첨가하지 않은 CON (basal diet), 생균제를 0.2% 첨가한 LB1 과 생균제를 0.4% 첨가한 LB2의 3개 처리구로 하여 처리당 8반복, 반복당 4두씩 완전임의 배치하였다. 전체 시험기간동안의 일당증체량, 일당사료섭취량 및 사료효율에서 있어서는 처리구간 유의한 차이를 나타내지 않았다(P<0.05). 질소 소화율에서 LB1 과 LB2 처리구가 대조구와 비교하여 유의적으로 증가하였다(linear effect, P<0.05). 그러나 건물 소화율에 있어서는 처리구간에 유의적인 차이를 보이지 않았다(P>0.05). 혈액내 WBC, RBC 및 lymphocyte 함량에 있어서는 처리구간에 유의적인 차이를 보이지 않았다(P>0.05). 분내 암모니아태 질소 및 황화수소의 함량은 LB2 처리구가 대조구와 비교하여 유의적으로 감소하였다(linear effect, P<0.05). 분내 acetic acid 와 propionic acid 함량에서는 BMS2 처리구가 대조구와 비교하여 유의적으로 감소하였다(linear effect, P<0.05), butyric acid 에서는 각 처리구간 유의적인 차이는 없었다(P>0.05). 결론적으로, 육성돈 사료내 0.4%의 *Lactobacillus brevis* ($3.4 \times 10^8$ CFU/g) 첨가는 질소 소화율 향상 및 분내 악취 발생 물질 함량을 감소 시키는 것으로 사료된다.

(**Key words** : *Lactobacillus brevis*, 소화율, 혈구수, 악취 발생 물질, 육성돈)

## Ⅰ. INTRODUCTION

It is commonly accepted that an optimum microbial balance in animal gastrointestine associated with good health and nutrition. Probiotics have been demonstrated to be useful in manipulating gut microbial balance (Fuller, 1989; Collins and Gibson, 1999). Due to this reason, the probiotics, which is also be defined as direct-fed microbials (DFM), has been received much consideration in

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recent years. The most widely used probiotics are lactic acid bacteria (LAB). Increasing evidences indicated that the presence of LAB in diet can maintain a favorable microbial ecosystem for livestock (Sandine, 1979). Data obtained from previous studies have shown that some of the LAB used as probiotics are capable of improving growth performance (Baird, 1977; Jasek et al., 1992), stimulating the immune system (Tortuero et al., 1995; Aattaouri et al., 2002) and affect the population of microflora in digestive tract (Jonsson and Conway, 1992).

Ammonia nitrogen ($NH_3$–N), hydrogen sulfide ($H_2S$) and volatile fatty acids (VFA) are the main components of pig manure contributing to environmental pollution (Zahn et al., 1997). With the increasingly restriction of environmental regulations, it is critical that more strategies should be provided on decreasing environmental pollution. Some recent focus about probiotics has been shifted from health promoting effects to decrease fecal emission of noxious gas content by manipulate intestinal microbial populations. Dietary addition of complex probiotics suggested decreasing fecal noxious gas emission (Hong et al., 2002; Chen et al., 2005; Chen et al., 2006). However, adverse results were also obtained by some other researchers (Spriet et al., 1987). As the LAB include various bacterial species, it is necessary to evaluate different probiotic preparations used in different conditions.

*Lactobacillus brevis* is a heterofermentative gram-positive organism which suggested to have generally regarded as safe (GRAS) status and to be able to survive through the gastrointestinal tract (Elina et al., 2003). Therefore, the present study was conducted to investigate whether the probiotic of *Lactobacillus brevis* supplementation at different levels (0.2% and 0.4%) would affect growth performance, DM and N digestibilities, blood cell counts and fecal odor emission compounds in growing pigs.

## Ⅱ. MATERIALS & METHODS

### 1. Experimental design, animals and diets

Ninety six [(Landrace × Yorkshire) × Duroc] pigs with an initial BW of 24.60 ± 1.28 kg were used during a six weeks feeding trial to evaluate the effects of dietary *Lactobacillus brevis* ($3.4 \times 10^8$ CFU/g) supplementation on growth performance, DM and N digestibilities, blood cell counts and fecal odor emission compounds in growing pigs. At the beginning of the experiment, pigs were allotted on the basis of initial BW to three dietary treatments in a completely randomized design. There were eight replicate pens per treatment with four pigs per pen. Dietary treatments included: 1) CON (basal diet); 2) LB1 (basal diet + *Lactobacillus brevis* 0.2%) and 3) LB2 (basal diet + *Lactobacillus brevis* 0.4%). Diets were provided in mash form and formulated to meet or exceed NRC (1998) recommendations for all the nutrients regardless of treatment. Pigs were housed in an environmentally controlled facility and room temperature was maintained approximately at 24°C. Each pen was equipped with a self-feeder and nipple waterer to allow for *ad libitum* access to feed and water throughout all the experimental period.

### 2. Sampling and measurements

Pigs were weighted at the last day of experiment and pen feed disappearance was also recorded at the completion of the 42-d growing period. Those data were utilized in the determination of ADG, ADFI, and gain/feed using initial BW as a covariate.

On d 35 of the experiment, pigs were fed diets containing 0.20% chromic oxide ($Cr_2O_3$). At the end of experiment (d 42), fecal grab samples were taken randomly from at least two pigs in each pen to determine the digestibilities of DM

Table 1. Formula and chemical compositions of diets (as-fed basis)

| Ingredients (%) | CON | LB1 | LB2 |
|---|---|---|---|
| Ground corn | 59.93 | 59.73 | 59.53 |
| Soybean meal | 23.75 | 23.75 | 23.75 |
| Rice bran | 5.00 | 5.00 | 5.00 |
| Molasses | 4.00 | 4.00 | 4.00 |
| Animal fat | 2.61 | 2.61 | 2.61 |
| Rapeseed meal | 2.00 | 2.00 | 2.00 |
| Defl. phosphate | 1.16 | 1.16 | 1.16 |
| Calcium carbonate | 0.44 | 0.44 | 0.44 |
| L-Lysine (78%) | 0.34 | 0.34 | 0.34 |
| Probiotics (*Lactobacillus brevis*) | – | 0.20 | 0.40 |
| Salt | 0.15 | 0.15 | 0.15 |
| Vitamin premix[1)] | 0.10 | 0.10 | 0.10 |
| Mineral premix[2)] | 0.25 | 0.25 | 0.25 |
| DL-methionine (98%) | 0.10 | 0.10 | 0.10 |
| Choline chloride (60%) | 0.08 | 0.08 | 0.08 |
| L-Threonine (98%) | 0.09 | 0.09 | 0.09 |
| Chemical composition[3)] | | | |
| Digestible energy (kcal/kg) | 3,447 | 3,447 | 3,447 |
| Crude protein (%) | 17.72 | 17.72 | 17.72 |
| Lysine (%) | 1.02 | 1.02 | 1.02 |
| Calcium (%) | 0.70 | 0.70 | 0.70 |
| Phosphorus (%) | 0.59 | 0.59 | 0.59 |

[1)] Provided per kg of complete diet: 4,000 IU of vitamin A; 800 IU of vitamin $D_3$; 17 IU of vitamin E; 2 mg of vitamin K; 4 mg of vitamin $B_2$; 1 mg of vitamin $B_6$; 16 μg of vitamin $B_{12}$; 11 mg of pantothenic acid; 20 mg of niacin and 0.02 mg of biotin.

[2)] Provided per kg of complete diet: 220 mg of Cu; 175 mg of Fe; 191 mg of Zn; 89 mg of Mn; 0.3 mg of I; 0.5 mg of Co and 0.4 mg of Se.

[3)] Calculated values.

and N. Chromic oxide was used as an indigestible marker in diets to calculate digestibility coefficients. After collection, fresh samples were frozen in refrigerator at −20°C until they were analyzed. Before chemical analysis, fecal samples were dried at 70°C for 72 hours and subsequently ground to pass through a 1-mm screen. All the fecal samples, along with feed samples, were analyzed for DM and N according to the AOAC procedures (AOAC, 1995). Chromium was analyzed by UV absorption spectrophotometry (Shimadzu, UV-1201, Japan). Nitrogen was determined by a Leco NS 2000 Nitrogen Analyzer (LECO Corporation, St. Joseph, MI, USA).

At the beginning of experiment, one pig was randomly chosen from each pen (n = 24) and bled via jugular venipuncture to obtain whole blood samples for determining WBC, RBC and

lymphocyte. Same pigs were bled again at the ending of experiment. Blood samples were collected into 5-ml $K_3$EDTA vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and stored in refrigerator (4°C) until further analysis. When the measurements were performed, RBC, WBC and lymphocyte were all analyzed by the automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA).

One day before the end of experiment (day 41), fecal grab samples were also collected and frozen for analyzing $NH_3$–N, and VFA concentrations. The $NH_3$–N concentration was determined according to the method of Chaney and Marbach (1962). The VFA measured in this experiment included acetic acid, propionic acid and butyric acid. Analysis method was as follow: previously frozen fecal samples were thawed and 2 g samples were taken. Each sample was diluted with 8 mL of distilled water and added two drops of concentrated HCl. Then samples were mixed and centrifuged at 17,400 × g for 10 min at 4°C. The supernatant was filtered using a 0.22-μm filter (Millipore Co., Bedford, MA, USA) and pipetted in to 2-mL gas chromatography vials (Supelco, Inc. No.27265, Bellefonte, PA, USA). The VFA concentrations were analyzed by gas chromatography (Hewlett Packard 6890 Plus, USA) according to the method of Otto et al. (2003). For analysis fecal $H_2S$ concentration, fresh fecal samples were also collected from at least two pigs in each pen at the day 41. When the analysis was performed, 300 g fresh fecal samples were transfer in to a sealed box and fermented for 30h in an incubator (35°C). Fermented samples were analyzed by gas search probe (Gastec Corp., Kanagawa, Japan).

### 3. Statistical analyses

In this experiment, all statistical analyses were performed as a completely randomized design using GLM procedures of SAS (1996). Pen was considered as the experimental unit for the data of growth performance and fecal analysis, whereas individual pig data were used as the experimental unit in the blood analysis. In addition, CON treatment was compared to LB treatments by the polynomial regression (Peterson, 1985) method to determine linear and quadratic effects. Variability in the data is expressed as standard error (SE) of the mean and a probability level of $P<0.05$ was considered statistically significant.

## Ⅲ. RESULTS & DISCUSSION

### 1. Growth performance

Table 2 shows the effects of dietary *Lactobacillus brevis* on growth performance in growing pigs. Inclusion of *Lactobacillus brevis* ($3.4 \times 10^8$ CFU/g)

Table 2. Effects of *Lactobacillus brevis* on growth performance in growing pigs[1)]

| Items | CON[2)] | LB1[2)] | LB2[2)] | SE[3)] | P values | |
|---|---|---|---|---|---|---|
| | | | | | Linear | Quadratic |
| ADG (g) | 728 | 754 | 759 | 24 | 0.33 | 0.68 |
| ADFI (g) | 1,658 | 1,720 | 1,625 | 71 | 0.65 | 0.34 |
| Gain/feed | 0.439 | 0.438 | 0.467 | 0.026 | 0.36 | 0.51 |

[1)] Ninety six pigs with an average initial and final BW of 24.60 ± 1.28 and 55.95 ± 2.44 kg, respectively.
[2)] Abbreviations: CON, control diet; LB1, control diet + 0.2% *Lactobacillus brevis*; LB2, control diet + 0.4% *Lactobacillus brevis*.
[3)] Pooled standard error.

in growing diets at either 0.2 or 0.4% had no significant effect (P>0.05) on ADG, ADFI and gain/feed during the entire experimental period. This result is consistent with the published research of Kornegay et al. (1990) who reported that addition of *Lactobacillus acidophilus* had no effects on growth rate of growing pigs. Similarly, Apgar et al. (1993) reported that no effects of lactic acid-producing microbe (*Bifidobacterium globosum* A) on ADG, ADFI and gain/feed in growing-finishing pigs. In contrast, our early study found an improvement of ADG when diet supplemented with 0.2% complex probiotics (*Lactobacillus acidophilus, Saccharomyces cerevisiae and Bacillus subtilis*) in growing pigs (Chen et al., 2005). Baird (1977) suggested that supplementation of *Lactobacillus* increased ADG and feed efficiency. Different results may attribute to several aspects. Firstly, the age of animal should be considered. Population of gastrointestinal bacteria altered during the first few months of an infant's life, while the composition of bacteria becomes more stable on adults (Heilig et al., 2002). Therefore, it is reasonable that studies conducted for probiotics found beneficial effects in nursery pigs more frequently (Bomba et al., 2002). Second, the property and validity of probiotic preparations are various. Third, the environment situation and animal healthy status may also affect the results (Hays, 1969).

## 2. Dry matter and nitrogen digestibilities

Effects of dietary *Lactobacillus brevis* on DM and N digestibilities are reported in Table 3. The DM digestibility was not affected by the addition of *Lactobacillus brevis* (P>0.05). Inclusion of either 0.2 or 0.4% *Lactobacillus brevis* improved N digestibility significantly (linear effect, P<0.05).

In the review reported by Wenk (2000), he suggested that *lactobacilli* can stimulate and stabilize the digestion processes. Burgestaller et al. (1984) also reported that probiotics can influence digestive processes by enhancing the population of beneficial micro-organisms and by improving microbial enzyme activity. Current results are consistent with Maxwell et al. (1983) who reported improved DM and N digestibilities by addition complex probiotic preparation (Feed-Mate 68: *Lactobacillus acidophilus, Streptococcus faecium* and *Lactobacillus planatarium* or Primalac: *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, Torulopsis* and *Aspergillus oryzae*). On the contrary, Shon et al. (2005) reported supplementation of *Lactobacillus reuteri*-based probiotics had not effects on DM and N digestibilities in growing pigs. Hale and Newton (1979) also suggested DM and N digestibilities were not affected by diet included a nonviable *Lactobacillus* fermentation product in growing pigs. According to Jonsson and Conway (1992)

Table 3. Effects of *Lactobacillus brevis* on nutrients digestibility in growing pigs[1)]

| Items (%) | CON[2)] | LB1[2)] | LB2[2)] | SE[3)] | P values | |
|---|---|---|---|---|---|---|
| | | | | | Linear | Quadratic |
| DM | 76.37 | 76.37 | 77.59 | 0.60 | 0.17 | 0.41 |
| N | 75.67[b] | 78.10[a] | 79.92[a] | 0.80 | 0.002 | 0.76 |

[1)] Ninety six pigs with an average initial BW of 24.60 ± 1.28 kg.
[2)] Abbreviations: CON, control diet; LB1, control diet + 0.2% *Lactobacillus brevis*; LB2, control diet + 0.4% *Lactobacillus brevis*.
[3)] Pooled standard error.
[a, b] means in the same row with different superscripts differ (P<0.05).

who suggested that the feeding probiotics may probable influence microflora in the digestive tract. However, they also reported that the so-called balancing of the flora is difficult for analyzing and may not always be clearly connected with those proposed beneficial effects.

## 3. Blood cell counts

Blood cell counts of RBC, WBC and lymphocytes were not affected (P>0.05) by the inclusion of dietary *Lactobacillus brevis* (Table 4). Present results are in agreement with Kil et al. (2004) who reported that no effect of complex probiotics (*Saccharomyces species*, *Enterococcus faecalis*, *Phaffia rhodozyma*, *Rodopseudomonas species* and *Bacillus species*) on WBC, IgG and IgA in pigs. Our early studies used complex probiotics and *Enterococcus faecium* also didn't find any influences on WBC, RBC and lymphocyte in growing and finishing pigs, respectively (Chen et al., 2005; Chen et al., 2006). However, large previous studies conducted in nursery pigs investigated influence of probiotics on blood profiles and immune system (Toruero et al., 1995). Therefore, we suggested that probiotics may affect some of blood characteristics in nursery pigs while as such effect was hardly performed in growing-finishing pigs.

## 4. Fecal odor emission compounds

Table 5 shows the effects of dietary *Lactobacillus brevis* on fecal odor emission compounds in growing pigs. Fecal $NH_3$–N and $H_2S$ concentrations of pigs were significant decreased (linear effect, P<0.05) when diets supplemented with *Lactobacillus brevis* ($3.4 \times 10^8$ CFU/g) at the level of 0.4%. Fecal VFA concentrations of acetic acid and propionic acid were also significant reduced with

Table 4. Effects of *Lactobacillus brevis* on blood cell counts in growing pigs[1)]

| Items | CON[2)] | LB1[2)] | LB2[2)] | SE[3)] | P values | |
|---|---|---|---|---|---|---|
| | | | | | Linear | Quadratic |
| RBC ($\times 10^6$/mm$^3$) | | | | | | |
| 0 day | 6.06 | 6.36 | 6.12 | 0.14 | 0.77 | 0.15 |
| 42 days | 6.36 | 6.60 | 6.25 | 0.18 | 0.67 | 0.20 |
| Difference | 0.30 | 0.24 | 0.13 | 0.23 | 0.60 | 0.91 |
| WBC ($\times 10^3$/mm$^3$) | | | | | | |
| 0 day | 20.72 | 20.98 | 19.52 | 1.99 | 0.68 | 0.73 |
| 42 days | 19.21 | 23.58 | 17.36 | 2.06 | 0.54 | 0.06 |
| Difference | −1.51 | 2.60 | −2.15 | 2.82 | 0.87 | 0.22 |
| Lymphocyte (%)[4)] | | | | | | |
| 0 day | 43.00 | 35.00 | 47.25 | 4.73 | 0.54 | 0.10 |
| 42 days | 51.50 | 52.25 | 59.75 | 3.55 | 0.12 | 0.45 |
| Difference | 8.50 | 17.25 | 12.50 | 6.09 | 0.65 | 0.38 |

1) Ninety six pigs with an average initial BW of 24.60 ± 1.28 kg.
2) Abbreviations: CON, control diet; LB1, control diet + 0.2% *Lactobacillus brevis*; LB2, control diet + 0.4% *Lactobacillus brevis*.
3) Pooled standard error.
4) Percentage of total white blood cell counts.

Table 5. Effects of *Lactobacillus brevis* on fecal odor emission compounds in growing pigs[1)]

| Items (ppm) | CON[2)] | LB1[2)] | LB2[2)] | SE[3)] | P values | |
|---|---|---|---|---|---|---|
| | | | | | Linear | Quadratic |
| $NH_3-N$ | 1,150[a] | 993[ab] | 873[b] | 65 | 0.01 | 0.82 |
| $H_2S$ | 204[a] | 160[ab] | 107[b] | 28 | 0.03 | 0.89 |
| Volatile fatty acids | | | | | | |
| Acetic acid | 3,513[a] | 3,160[ab] | 1,799[b] | 184 | 0.02 | 0.99 |
| Propionic acid | 2,383[a] | 2,230[ab] | 1,924[b] | 175 | 0.002 | 0.43 |
| Butyric acid | 1,582 | 1,509 | 1,252 | 139 | 0.12 | 0.60 |

[1)] Ninety six pigs with an average initial BW of 24.60 ± 1.28 kg.
[2)] Abbreviations: CON, control diet; LB1, control diet + 0.2% *Lactobacillus brevis*; LB2, control diet + 0.4% *Lactobacillus brevis*.
[3)] Pooled standard error.
[a, b] means in the same row with different superscripts differ (P<0.05).

the addition of 0.4% of *Lactobacillus brevis* (linear effect, P<0.05). Fecal butyric acid was not affected by the inclusion of either 0.2 or 0.4% *Lactobacillus brevis* (P>0.05).

Many kinds of compounds have been identified in swine manure as being potential contributors to swine odor. Among those compounds, $NH_3$–N, $H_2S$, phenols, indoles and VFA considered to be main proportion of noxious gas emission from swine facility (Avery et al., 1975; Heber *et al*., 1997). Han et al. (2001) reviewed several studies using feed additives and suggested that probiotics can indirectly contribute to reduce environmental pollutants from animal manure by improving feed efficiency or nutrients retention. Decreased fecal $NH_3$–N in current study might be due to increased digestibility of nitrogen. Elsden et al. (1946) and Franklin et al. (2002) demonstrated that VFA production related with intestinal bacterial populations. Imoto and Namioka (1978) also showed the major site of VFA production in the pig to be the large intestine. Hydrogen sulfide was produced through both *in vivo* fermentation in the hindgut and *in vitro* anaerobic fermentation of manure slurry during storage (Kadota and Ishida, 1972; Banwart and Bremner, 1975). Therefore, decreased volatile compounds in our experiment are probable due to the improvement of hindgut microbial ecosystem balance by the supplementation of *Lactobacillus brevis*. Ji and Kim (2002) reported that addition of 0.2% probiotics complex (*Lactobacillus acidophilus, Bacillus species and Aspergillus oryzae*) significant decreased the ammonia production of pigs. Hong et al. (2002) also found increased DM and N digestibilities and reduced fecal $NH_3$–N and VFA concentrations by addition of probiotics (*Saccharomyces cerevisiae*) in finishing pigs. These previous results are in agreement with our present study.

## Ⅳ. IMPLICATIONS

This study demonstrated that dietary supplementation *Lactobacillus brevis* ($3.4 \times 10^8$ CFU/g) at the rate of at 0.4% (as-fed basis) to growing pigs diet improved nitrogen digestibility and decreased the concentrations of fecal odor emission compounds. Therefore, present investigations provide a practical strategy for decreasing swine odor which associated with the problem of environmental pollution.

## Ⅴ. ABSTRACT

This study was conducted to investigate the

effects of dietary *Lactobacillus brevis* ($3.4 \times 10^8$ CFU/g) supplementation on growth performance, DM and N digestibilities, blood cell counts and fecal odor emission compounds in growing pigs. Ninety six crossbred [(Landrace × Yorkshire) × Duroc] pigs with an initial BW of 24.60 ± 1.28 kg were used for 42-d feeding trial according to a completely randomized design. Three corn- soybean meal based dietary treatments included: 1) CON (basal diet); 2) LB1 (basal diet + *Lactobacillus brevis* 0.2%) and 3) LB2 (basal diet + *Lactobacillus brevis* 0.4%). There were three dietary treatments with eight replicate pens per treatment and four pigs per pen. Through the entire experimental period, ADG, ADFI and gain/feed had no significant differences among treatments (P>0.05). Nitrogen digestibility was increased in LB1 and LB2 treatments compared to CON treatment (linear effect, P<0.05), however, DM digestibility had no significant difference among all the treatments (P>0.05). The WBC, RBC and lymphocyte concentrations in whole blood were not affected by treatments (P>0.05). Fecal $NH_3$–N and $H_2S$ concentrations were significant decreased in LB2 treatment compared to CON treatment (linear effect, P<0.05). Fecal VFA (acetic acid and propionic acid) concentration was also reduced in LB2 treatment compared to CON treatment (linear effect, P<0.05). In conclusion, *Lactobacillus brevis* ($3.4 \times 10^8$ CFU/g) supplementation at the level of 0.4% can improve nitrogen digestibility and decrease the concentrations of fecal odor emission compounds in growing pigs.
(**Key Words** : *Lactobacillus brevis*, Digestibility, Blood Cell Counts, Odor Emission Compounds, Growing Pigs)

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