Effects of dietary probiotic, liquid feed and nutritional concentration on the growth performance, nutrient digestibility and fecal score of weaning piglets

Song Zhang¹², Dong Huy Yoo¹³, Xiang Ao¹, and In Ho Kim¹,*

Objective: This study was conducted to investigate the effects of dietary probiotic blend and liquid feed program at different nutritional densities on growth performance, nutrient digestibility, fecal score of weaning piglets.

Methods: A total of 120 weaning pigs with an initial body weight of 7.05±0.93 kg per pig (21 days of age) were randomly allocated into 1 of the following 8 dietary treatments (3 replicates per treatment with 5 pigs per replicate) in a 2×2×2 factorial arrangement (nutrition levels: apparent metabolic energy [AME] = 3,500 kcal/kg, crude protein [CP] = 20% vs AME = 3,400 kcal/kg, CP = 19.42%; feed types:dry vs wet; probiotics levels: 0 mg/kg vs 300 mg/kg).

Results: During d 5 to d 15, greater average daily gain (ADG) and average daily feed intake (ADFI) (p<0.05) were observed in probiotics treatments. During d 15 to d 25, gain:feed (G:F) ratio (p<0.05) were significantly improved in probiotics, wet feed and high nutrition diet. Moreover, two interactions i) between nutrition levels and feed types, and ii) between nutrition levels and probiotics were found in G:F ratio. Furthermore, there was a significant positive interaction on G:F among those 3 factors (p<0.05). Overall, increasing ADG, ADFI, and G:F ratio were detected in probiotics treatment significantly (p<0.05). Besides, an obvious reduction on fecal score was observed in probiotics treatment from d 0 to d 5 (p<0.05). There was an interactive effect on fecal score between feed types and nutrition concentrations from d 5 to d 25 (p<0.05).

Conclusion: These results indicated that probiotics supplementation could benefit growth performance and reduce the frequency of watery feces. Besides, wet feed program (feed:water = 1:1.25) could improve the G:F. The effect of liquid feed or probiotic could be influenced by dietary nutrition density in weaned piglets. An increased value of G:F was obtained when wet feeding a high nutrition diet (100 kcal higher than NRC 2012 recommendations) was supplemented with probiotics for 15 to 25 days.

Keywords: Dietary Probiotic Blend; Liquid Feeding Program; Nutrition Levels; Growth Performance; Fecal Score; Nutrient Digestibility; Weaning Pigs

INTRODUCTION

Gastrointestinal disturbances immediately post weaning can cause heavy economic loss in the pig industry. The weaning transition is a complex period during which the piglets have to cope with abrupt separation from their mothers and adapt to new environment where they are mixed with other litters. In addition, their diet will be switched from highly-digestible (liquid) milk to a less digestible and more-complex solid feed during this transition. Weaned at an early age (21 to 35 d) in intensive production systems has probably exacerbated the level of general stress in these immature animals [1].

In the past decade, various nutritional methods or solutions to minimize the weaning
losses have been tested in practice, some of which have been widely implemented in practice. Probiotics, which is a modulator to increase many active behaviors, plays an important role in gut-brain axis regulation [2]. Many references demonstrate different probiotics capacities can enhance productivity in weaning piglets and increase gain:feed (G:F) ratio [3], average daily gain (ADG) [4] and nutrient digestibility [5]. Direct action of the probiotics can achieve a higher bioavailability of feed nutrients, indirect gut health modulation (relieving weaning stress, preventing diarrhea, improving the intestinal microbiota profile, etc.) or perhaps a combination of both may be involved [6]. Liquid feed diets have been widely used in western and southern Europe for 20 years, especially in France and Italy [7]. There has been an increase in the use of fermented liquid and liquid feed in the European Union (EU) since the ban on the use of antibiotics as growth promoters in pig feed [8]. Piglets grew faster on the liquid diet due to a higher feed intake (FI), a better transition from milk feeding and lower risk of dehydration [9]. With the advancement of additives and feeding technology, probiotics and liquid feeding would be used in most swine farms. However, what nutritionists always focus on is the formulation designs. Various nutrition levels and the liquid feed program might influence the effects of additives in pigs. Therefore, we hypothesized there might be an interaction among nutrition levels, the liquid feeding program and probiotics. However, no research has been carried out to determine the interaction among probiotics, the liquid feeding program and nutrition designs. Consequently, the objective of the study is to determine effects of dietary probiotic blend and liquid feed program at different nutritional densities on growth performance, nutrient digestibility, fecal score in weaning piglets.

MATERIALS AND METHODS

The protocols used for the current experiment were approved by the Animal Care and Use Committee of Dankook University, Korea.

Animal, diet, experimental design

A total of 120 weaning pigs (21 days of age) with an initial body weight (BW) of 7.05±0.93 kg per pig were randomly allocated into 1 of 8 dietary treatments (3 replicates per treatment with 5 pigs per replicate) in a 2×2×2 factorial arrangement with 2 levels of nutrition density apparent metabolic energy (AME) = 14.63 kJ/kg or 3,500 kcal/kg, CP = 20% vs AME = 14.23 kJ/kg or 3,400 kcal/kg, CP = 19.42%), 2 types of feed (dry vs wet), the liquid feed was prepared by mixing meal and

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Statistical analyses
Data were analyzed as a completely randomized block design, with a 2×2×2 factorial arrangement, using general linear model procedure [14]. Variability in the data was expressed as the pooled standard error of mean, and p<0.05 was considered statistically significant, whereas p<0.10 was considered a tendency.

RESULTS

Growth performance and nutrient digestibility of dry matter, energy and nitrogen
The results of growth performance and nutrient digestibility of DM, energy and nitrogen are presented in Table 2, 3, respectively. In the phase 2, ADG and ADFI were greater (p<0.05) in probiotics treatments comparing to the non-probiotics treatments. In the phase 3, probiotics, wet feed and high nutrition diet significantly improved G:F (p<0.05). Besides, those pigs fed high nutrition diet appeared to have greater ADG and G:F than that fed low nutrition diet (p<0.05). Moreover, the interactive influence on G:F appeared not only between nutrition levels and feed types but also between nutrition levels and probiotics (p<0.05). Interestingly, there was a significant probiotics × feed type × nutrition density interaction on G:F (p<0.05). Piglets fed the diet containing probiotics had increased ADG, ADFI, and G:F comparing to that receiving the diet without probiotics (p<0.05). Besides, high nutrition diet significantly raised ADG and ADFI (p<0.05). No interactive response was found through the entire experiments (p>0.05). There was no difference in nutrient digestibility of DM, energy and nitrogen as well (p>0.05).

Fecal score
The results of fecal score are appeared in Table 4. An obvious reduction on fecal score was observed in probiotics treatment from d 0 to d 5 (p<0.05). There was an interactive effect on fecal score between feed types and nutrition concentrations from d 5 to d 25 (p<0.05).

DISCUSSION
The results of this study showed that dietary probiotics blend can increase the ADG, ADFI, and G:F ratio throughout the trial. And the results are accordance with the previous paper of our laboratory [15], which evidenced positive effects on growth performance in the overall period associated with the inclusion of multi-strain probiotics (B. licheniformis and B. subtilis) in the diets. Generally, probiotics or probiotics mixtures could improve ADG [16-18] and increase G:F ratio [19, 20] in post-weaned piglets. However, the influence of probiotics on ADFI is inconsistent. In agreement with our results, Nguyen et al [21] documented that increasing the inclusion of the probiotics mixture (Bacillus coagulans, B. licheniformis, B. subtilis, and Clostridium butyricum) levels in the diets linearly increased the ADG and ADFI for day 0 to 7 as well as

Table 1. Diet compositions (as fed basis)

| Item                     | High nutrition diet | Low nutrition diet |
|--------------------------|---------------------|--------------------|
| Yellow corn              | 33.32               | 36.95              |
| Extruded corn            | 20.00               | 20.00              |
| Whey powder (78%)        | 7.00                | 7.00               |
| Soybean meal (46%)       | 8.28                | 5.98               |
| Fermented soybean meal   | 5.00                | 5.00               |
| Extruded soybean meal    | 5.00                | 5.00               |
| Skimmed milk powder      | 7.00                | 7.00               |
| Fish meal                | 3.00                | 3.00               |
| Sugar                    | 2.00                | 2.00               |
| Glucose                  | 2.00                | 2.00               |
| SDPP                     | 3.00                | 3.00               |
| Soy oil                  | 1.33                | 0.00               |
| Limestone                | 0.55                | 0.55               |
| MCP                      | 0.68                | 0.72               |
| Salt                     | 0.10                | 0.10               |
| Lysine-HCL (98.5%)       | 0.44                | 0.42               |
| DL-methionine (99%)      | 0.30                | 0.29               |
| L-threonine (98.5%)      | 0.20                | 0.19               |
| L-tryptophan (10%)       | 0.30                | 0.30               |
| Choline (50%)            | 0.10                | 0.10               |
| Vitamin premix           | 0.20                | 0.20               |
| Mineral premix           | 0.20                | 0.20               |
| Total                    | 100.00              | 100.00             |

Calculated composition (%)

| Item | High nutrition diet | Low nutrition diet |
|------|---------------------|--------------------|
| CP   | 20.00               | 19.42              |
| Crude fat | 4.70               | 3.42               |
| Ash  | 5.49                | 6.05               |
| AME (kcal/kg) | 3500               | 3400               |
| Ca   | 0.7                 | 0.7                |
| AP   | 0.5                 | 0.5                |
| Lys  | 1.58                | 1.55               |
| Apparent ileal digestible amino acid | |
| SID-Lys | 1.48               | 1.45               |
| ME/CP | 175                | 175                |
| CP/SID-Lys | 13.79              | 13.79              |
| SID-TSAA/SID-Lys | 0.60           | 0.60               |
| SID-Thr/SID-Lys | 0.62              | 0.62               |
| SID-Trp/SID-Lys | 0.17              | 0.17               |

5DPP, spray-dried porcine plasma; MCP, monocalcium phosphate; AME, apparent metabolic energy; SID, standard ileal digestibility; CP, crude protein; TSAA, total sulfur amino acid.

1) Supplied per kg diet: 4,000 IU vitamin A, 800 IU vitamin D3, 171 IU vitamin E, 2 mg vitamin K, 4 mg vitamin B12, 1 mg vitamin B6, 16 μg vitamin B12, 11 mg pantothenic acid, 20 mg niacin and 0.08 mg biotin.

3) Supplied per kg diet: 220 mg Cu, 175 mg Fe, 191 mg Zn, 89 mg Mn, 0.3 mg I, 0.5 mg Co and 0.4 mg Se.

3) Calculated values.

of individual pigs and signs of stool consistency in the pen.
Table 2. Effects of feeding program on growth performance in weaning pigs

| Items                      | TRT1  | TRT2  | TRT3  | TRT4  | TRT5  | TRT6  | TRT7  | TRT8  | SEM          | p-value  |
|----------------------------|-------|-------|-------|-------|-------|-------|-------|-------|--------------|----------|
|                            | High nutrition density |        |       |        | Low nutrition density |        |       |       |              |          |
|                            | Dry type | Wet type |     |       | Dry type | Wet type |     |       |              |          |
| Body weight (kg)           | NC     | Probiotics | NC | Probiotics | NC     | Probiotics | NC | Probiotics | NC     | Probiotics |
| Initial                   | 7.08   | 7.08   | 7.07 | 7.05 | 7.04   | 7.04   | 7.02 | 7.02 | 0.61         | 0.930    | 0.963    |
| Phase1                    | 8.09   | 8.16   | 8.13 | 8.16 | 8.16   | 8.09   | 8.03 | 8.11 | 0.63         | 0.940    | 0.966    |
| Phase2                    | 10.75  | 10.95  | 10.82 | 11.22 | 10.12  | 10.35  | 10.20 | 10.54 | 0.69         | 0.216    | 0.755    |
| Phase3                    | 13.56  | 15.15  | 14.38 | 15.84 | 12.79  | 14.11  | 12.93 | 14.31 | 1.02         | 0.118    | 0.535    |
| Phase1                    | ADG (g) | 201    | 216   | 213   | 221   | 224    | 211   | 201   | 218          | 0.970    | 0.980    |
| ADH (g)                   | 212    | 217    | 213   | 237   | 234    | 233    | 219   | 239   | 20           | 0.739    | 0.515    |
| G:F                      | 0.968  | 0.998  | 0.889 | 0.930 | 0.960  | 0.907  | 0.917 | 0.916 | 0.032        | 0.366    | 0.663    |
| Phase2                    | ADG (g) | 266    | 279   | 269   | 307   | 196    | 225   | 217   | 243          | <0.001   | 0.248    |
| ADH (g)                   | 332    | 349    | 314   | 424   | 266    | 315    | 290   | 329   | 24           | 0.005    | 0.183    |
| G:F                      | 0.800  | 0.802  | 0.859 | 0.723 | 0.738  | 0.727  | 0.751 | 0.738 | 0.046        | 0.099    | 0.986    |
| Phase3                    | ADG (g) | 281    | 419   | 356   | 461   | 267    | 376   | 273   | 376          | 0.078    | 0.232    |
| ADH (g)                   | 418    | 623    | 529   | 669   | 452    | 551    | 403   | 565   | 61           | 0.141    | 0.486    |
| G:F                      | 0.668  | 0.674  | 0.672 | 0.690 | 0.587  | 0.684  | 0.676 | 0.666 | 0.010        | 0.005    | 0.006    |
| Overall                   | ADG (g) | 259    | 323   | 292   | 352   | 230    | 283   | 269   | 291          | 0.017    | 0.220    |
| ADH (g)                   | 342    | 432    | 385   | 484   | 334    | 393    | 321   | 405   | 28           | 0.031    | 0.256    |
| G:F                      | 0.756  | 0.747  | 0.763 | 0.726 | 0.687  | 0.720  | 0.736 | 0.719 | 0.016        | 0.011    | 0.482    |

SEM, pooled standard error of means; NC, negative control; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain:feed.

ADG for day 8 to 21. However, Zhao et al. [22] believed probiotics (Lactobacillus reuteri and Lactobacillus plantarum) blend could not affect ADFI. Different probiotics strains and concentration of probiotics might be an important factor affecting the ADFI. Especially, since probiotics also live on nutrient, they might compete with host for nutrients in the diet or make hosts require more feed.

The key factor underlying the poor postweaning performance is the immediate reduction in FI due to the abrupt transition from liquid milk to less digestible feeds [23,24].

Table 3. Effects of feeding program on digestibility in weaning pigs

| Items (%)                     | TRT1  | TRT2  | TRT3  | TRT4  | TRT5  | TRT6  | TRT7  | TRT8  | SEM          | p-value  |
|-------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|--------------|----------|
|                               | High nutrition density |        |       |       | Low nutrition density |        |       |       |              |          |
|                               | Dry type | Wet type |     |       | Dry type | Wet type |     |       |              |          |
| Dry matter                   | NC     | Probiotics | NC | Probiotics | NC     | Probiotics | NC | Probiotics | NC     | Probiotics |
| 5 d                           | 83.31  | 84.21  | 84.38 | 84.77 | 84.21  | 84.25  | 83.50 | 83.97 | 0.53         | 0.245    | 0.315    |
| Nitrogen                      | 82.64  | 83.23  | 83.81 | 82.58 | 83.03  | 83.65  | 83.64 | 83.69 | 0.91         | 0.156    | 0.267    |
| Energy                        | 83.86  | 84.35  | 84.75 | 85.36 | 84.68  | 83.79  | 85.31 | 85.22 | 0.77         | 0.265    | 0.543    |
| 10 d                          | 84.50  | 84.93  | 83.51 | 85.06 | 84.44  | 83.79  | 85.04 | 83.89 | 0.95         | 0.854    | 0.453    |
| Nitrogen                      | 83.57  | 82.65  | 83.62 | 84.31 | 83.65  | 84.31  | 82.86 | 83.06 | 0.59         | 0.804    | 0.654    |
| Energy                        | 83.83  | 84.35  | 84.75 | 85.36 | 84.32  | 85.28  | 83.31 | 85.16 | 0.80         | 0.647    | 0.169    |
| 25 d                          | 84.34  | 85.94  | 85.08 | 86.89 | 84.32  | 84.78  | 85.43 | 84.66 | 0.69         | 0.122    | 0.174    |
| Nitrogen                      | 82.61  | 84.85  | 83.83 | 85.05 | 82.97  | 83.41  | 83.60 | 83.41 | 0.78         | 0.187    | 0.361    |
| Energy                        | 83.97  | 84.80  | 84.45 | 86.01 | 82.84  | 84.10  | 84.42 | 83.83 | 0.80         | 0.078    | 0.189    |

SEM, pooled Standard error of means; NC, negative control.

New methods of feeding program: TRT1, high nutrition density feed × dry type × none; TRT2, high nutrition density feed × dry type × probiotics; TRT3, high nutrition density feed × wet type × none; TRT4, high nutrition density feed × wet type × probiotics; TRT5, low nutrition density feed × dry type × none; TRT6, low nutrition density feed × dry type × probiotics; TRT7, low nutrition density feed × wet type × none; TRT8, low nutrition density feed × wet type × probiotics.

* p < 0.05 was considered statistically significant, whereas p < 0.10 was considered a tendency.
* Phase 1, 0 to 5days; Phase 2, 5 to 15 days; Phase 3, 15 to 25 days.
therefore, sufficient FI is a big challenge for subsequent growth performance. Previous studies indicated liquid feeding re-constituted to 13% DM and fed via liquid feeders in the early weaning period improves FI which resulted in greater body weight gain [25,26]. Whereas, this study showed liquid feed which were re-constituted to 25% DM only enhanced G:F ratio. The difference might result from the different DM. Geary et al [27] reported DM content in the range of 255 to 149 g/kg had no significant effect on DM intake post-weaning 4 weeks. Similarly, when Yang et al [28] fed the piglet liquid feed in a ratio of 1:2 from d 0 to d 28, no difference was found in ADFI, but there was an enhanced G:F. There are three possible reasons for this result. Firstly, water plays a crucial role in the muscle growth, which is a major part of the com- position of organ and tissues, so enough water intake could be a reason for better G:F ratio. Secondly, a better transition from milk feeding can reduce the weaning stress and lead to a better G:F ratio. Thirdly, comparing with dry feed treatment, a lower fecal score and a tendency of better energy digestibility in wet feed group presented in this study also contributed to the improved G:F ratio.

Probiotics and nutrition density showed an interactive relation- ship in G:F ratio, which meant probiotics improved G:F ratio more dramatically in the high-nutrition diet. Similarly, our previous studies of Meng et al [29] and Yan et al [30] reported supplementation probiotics in high nutrition diets raised nutrient digestibility and reduced fecal gas emissions in growing pigs. And they believed that the interactive effect could be the increased microflora balance, which led to a better metabolism and transformation of feed into body mass. In our viewpoint, piglets fed relatively higher nutrition diet are more likely to suffer nutritional diarrhea, which results from that indigestible substrate inducing an explosive growth of bacteria and a disturbance of the colonization resistance [31]. Normally transient Escherichia coli strains in the gut [32] can multiply and attach. This study confirmed the reason for positive effect in relatively high nutrition diet might be that probiotics play a role in balancing gut microflora, benefitting intestinal integrity to relieve intestinal stress under high nutrition [33]. However, Lan et al [34] whose paper reported the beneficial effects of probiotics complex supplementation on ADFI is more dramatic with low nutrient density (3,850 kcal/kg vs 4,000 kcal/kg). Believed pigs were able to get same energy by increasing FI when low energy diets were provided. The difference interaction between nutrition level and probiotics might be caused by the actual energy in those trial diets and the different growth stages. There was a positive fecal score interaction between high nutrition diet and liquid feed. Relatively, the G:F ratio was improved more dramatically with liquid feed in the high-nutrition diet. Those two results might imply that comparing to NRC [10] nutrition recommendation, a higher nutrition formula should be considered when liquid feed is fed to piglets. Interestingly, there was an interaction on G:F among nutri- tion levels, probiotics and liquid feed in phase 3. Therefore, supplementation probiotics into liquid diet at high nutrition might be a whole solution to improve growth performance and health status in post-weaning pigs. When piglets are fed in different feeding programs or at various nutrition designs, additives applications should be considered specifically. How- ever, the interaction could not be found in overall growth performance.

**CONCLUSION**

These results indicated that probiotics in a supplementation diet could benefit growth performance (ADG, ADFI, and G:F) and reduce the frequency of wetty feces. Besides, a wet feed program (feed:water = 1:1.25) could improve the G:F. Because there were two positive interactions: one between liquid program and nutrition density, the other between sup- plementation probiotics and nutrition density, the effect of liquid feed or probiotic could be influenced by dietary nutrition density in weaned piglets. An increasing value of G:F was
obtained when wet feeding a high nutrition diet (100 kcal higher than NRC [10] recommendations) was supplemented with probiotics for 15 to 25 days.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript. Song Zhang is an employee of Kemin Industries (China) Co., Ltd. and Yoo DH is an employee of All The Best Co., Ltd.

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