Effects of *Bacillus licheniformis* derived-protease supplementation, alone or in combination, with valine to low protein diet on growth performance and carcass quality grade in growing-finishing pigs

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**ABSTRACT**

This study was conducted to evaluate the effects of dietary supplementation of *Bacillus licheniformis* derived-protease, alone or in combination with valine to low protein diet on growth performance, nutrient digestibility and carcass quality grade in growing-finishing pigs. A total of 180 crossbred ([Landrace × Yorkshire] × Duroc) growing pigs with an initial body weight of 22.56 ± 1.59 kg were used in a 17-week feeding trial. Pigs were randomly allotted into three treatments: CON, basal diet; PROT, low protein diet with 0.05% protease; PROTV, PROT diet with 0.08% valine. During the finishing phase (wk 7–17) and overall period (wk 0–17), gain: feed (G:F) ratio was increased (P < 0.05) in pigs fed the PROTV diet compared with those fed the CON diet. Although the supplementation of *B. licheniformis* derived-protease in combination with valine to low protein diet increased the G:F ratio of finishing phase and overall, compared with CON, other indexes of growth performance, nutrient digestibility and carcass quality grade were not impacted. Dietary supplementation with a combination of *B. licheniformis* derived-protease and valine in a low crude protein diet had no deleterious effects on growth performance, nutrient digestibility and carcass quality grade in growing-finishing pigs.

**Introduction**

The prices of main feed grains (especially corn and soybean meal) are dramatically increasing due to the numerous demands by its inclusion in many dominant feed ingredients and human consumption industries, which reduced the profit margins of pork production (Niemi et al. 2010). Higher inclusion levels of soybean meal in the diet resulted in high crude protein (CP) levels which led to excesses of other essential amino acids and excesses nitrogen in excreta (Jacela et al. 2009; Remus et al. 2020). Moreover, the requirements of protein-rich feed ingredients in the swine industry are essentially a need for the availability of amino acids (AA; Gloaguen et al. 2014).

Recent studies have demonstrated that reducing dietary protein supplementation and simultaneously supplementing crystalline amino acids (CAA) could improve nitrogen utilization and feed efficiency (Zhou et al. 2016; Spring et al. 2020). A low protein diet supplemented with the first four limiting amino acids (LAA, lysine, threonine, methionine and tryptophan) can be fed to pigs without impairing the growth performance and carcass characteristics of pigs while reducing the cost of feed (Roux et al. 2011; Tous et al. 2014; Monteiro et al. 2017; Li et al. 2018). In recent studies, a further reduction of dietary CP necessitates the requirement for valine, as the fifth LAA, which can also contribute to the improvement of pig growth performance (Liu et al. 2015; Soumeh et al. 2015; Ma et al. 2019). Protease, as a stand-alone enzyme or as a part of enzyme cocktails, has shown positive effects on growth performance and nutrient digestibility to pigs fed low protein diets (Zuo et al. 2015; Tactacan et al. 2016; Lei et al. 2017; Nguyen et al. 2018). It is also one of the effective ways to increase nutrient digestibility, especially CP, by hydrolysing protein to peptides and amino (Guggenbuhl et al. 2012). To our knowledge, no data are available about the *Bacillus licheniformis* derived-protease in combination with valine in low protein diets of growing-finishing pigs. Therefore, we hypothesized that the inclusion of *B. licheniformis* derived-protease, alone or in combination with valine may have no negative effects on growth performance, nutrient digestibility and carcass quality grade in pigs fed on low protein diets.

**Material and methods**

The experimental protocol (DK-4-1715) describing the management and care of animals was subjected to approval by the Animal Care and Use Committee of Dankook University, Cheonan, South Korea. The protease used in the current experiment is manufactured by a commercial company (Ronozyme ProAct; DSM Nutritional Products, Ltd., Heerlen, The
Netherlands). This product is produced by submerged fermentation of *B. licheniformis* containing transcribed genes from *Nocardiopsis prasina*. Protease activity is 75,000 protease units (PRU) g⁻¹. One PRU is defined as the amount of enzyme that releases 1 μmol of p-nitroanilide from 1 μmol/L of substrate/min at pH 9.0 and 37°C.

**Experimental design, diets and management**

A total of 180 crossbred ([Landrace × Yorkshire] × Duroc) growing pigs with an average body weight (BW) of 22.56 ± 1.59 kg were used in a 17-week growth trial. Pigs were allotted to 1 of 3 treatments in a randomized complete block design. Each dietary treatment consisted of 12 replications with 5 pigs per pen. A 2-phase (wk 0–6, and wk 7–17) feeding programme was employed in the current study, which consisted of growing (wk 0–6) and finishing (wk 7–17) periods. The experimental dietary treatments: CON, basal diet (growing, and finishing diets containing 17.56%, and 15.89% CP, respectively); PROT, low protein diet (growing, and finishing containing diets containing 16.83%, and 15.15% CP, respectively) with 0.05% protease; PROTV, PROT diet with 0.08% valine. The CON diet contained adequate nutrient concentrations for growing-finishing pigs which were formulated according to NRC (2012) recommendations, and the other two groups contained equivalent nutrient concentrations with the exception of low protein. Diet PROT and PROTV were similar to diet CON in terms of all nutrients except for lower CP contents with similar concentrations of indispensable amino acids. The composition of the experimental diets is presented in Table 1. All pigs were housed in an environmentally controlled room with a slatted plastic floor. Temperature and humidity were 25°C and 60%, respectively. Each pen was equipped with a one-sided self-feeder and one nipple waterer that allowed for ad libitum access to the feed and water throughout the experiment.

**Sampling and measurements**

Individual BW was measured at the beginning and the end of each dietary phase and feed consumption was recorded on a pen basis during the experiment to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain/feed ratio (G:F). Chromium oxide (Cr₂O₃) was added to the diet as an indigestible marker at 0.20% of the diet, for 7 days prior to faecal collection at wk 6 and wk 17 for apparent total tract digestibility (ATTD) calculation of dry matter (DM), CP and energy (Kong and Adeola 2014). The faecal grab samples were collected randomly from at least two pigs in each pen (1 barrow and 1 gilt) at the end of each phase. Faecal samples were dried at 70°C for 72 h in the oven, after which they were pulverized to pass through a 1-mm screen. Dietary DM and CP were analysed according to the procedures described by AOAC International (AOAC 2007). The CP was determined by Kjeltec 2300 Nitrogen Analyser (Foss Tecator AB, Hoeganaes, Sweden). And the chromium was determined by UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). The energy was determined by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL).

At the end of the experiments, all pigs were transferred to a local commercial slaughterhouse and slaughtered by exsanguination after being electrically stunned (feed was withheld 24 h before slaughtering). Backfat thickness was measured from each pen (2 pigs per pen, 1 gilt and 1 barrow; 24 pigs per treatment) using a real-time ultrasound instrument (Piglot 105, SFK Technology, Herlev, Denmark) and carcass weight was measured at tenth ribs after slaughter. The carcass quality grade was determined according to the Korea Institute for Animal Products Quality Evaluation (KAPE 2010). The quality of pork carcasses was graded as grade 1* = 3, grade 1 = 2 and grade 2 = 1 based on the marbling, lean colour and conditions of belly streaks.

**Statistical analysis**

Experiment data on growth performance and nutrient digestibility were based on the pen, whereas data on carcass grade were based on the individual animal. The data were subjected to one-way ANOVA followed by the Bonferroni test for multiple comparisons using the statistical software package SPSS (version 20; IBM, New York, NY). The significance level was set at a probability of 0.05.

| Item | Growing (0–6 weeks) | Finishing (6–17 weeks) |
|------|---------------------|------------------------|
| **Ingredients %** | **CON** | **PROT** | **CON** | **PROT** |
| Corn | 34.73 | 36.64 | 38.31 | 40.24 |
| Wheat | 15.00 | 15.00 | 15.00 | 15.00 |
| Rice | 10.00 | 10.00 | 10.00 | 10.00 |
| Soybean meal | 21.19 | 19.31 | 15.94 | 14.06 |
| Canola meal | 4.00 | 4.00 | 5.00 | 5.00 |
| Sesameseed meal | 2.00 | 2.00 | 2.00 | 2.00 |
| Palm kernel meal | 2.00 | 2.00 | 3.00 | 3.00 |
| Yellow grease | 4.60 | 4.50 | 4.40 | 4.30 |
| Molasses | 3.00 | 3.00 | 3.00 | 3.00 |
| Limestone | 1.21 | 1.21 | 1.15 | 1.15 |
| MDCP | 0.60 | 0.63 | 0.54 | 0.56 |
| Salt | 0.32 | 0.32 | 0.30 | 0.30 |
| DL-methionine | 0.09 | 0.08 | 0.07 | 0.06 |
| L-lysine HCL | 0.50 | 0.50 | 0.51 | 0.51 |
| L-threonine | 0.10 | 0.10 | 0.11 | 0.10 |
| L-tryptophan | 0.01 | 0.01 | 0.02 | 0.02 |
| L-valine | 0.20 | 0.18 | 0.20 | 0.18 |
| Vit/Mn premix | 0.20 | 0.20 | 0.50 | 0.50 |
| Phytase | 0.05 | 0.05 | 0.05 | 0.05 |
| Protease | 0.05 | 0.05 | 0.05 | 0.05 |
| Others | 0.40 | 0.40 | 0.40 | 0.40 |

**Calculated composition**

| Item | Growing (0–6 weeks) | Finishing (6–17 weeks) |
|------|---------------------|------------------------|
| Moisture | 12.19 | 12.22 | 12.19 | 12.23 |
| CP | 17.56 | 16.83 | 15.89 | 15.15 |
| Ether extract | 7.29 | 7.23 | 7.34 | 7.27 |
| Crude fibre | 2.75 | 2.72 | 2.81 | 2.77 |
| Crude ash | 5.37 | 5.30 | 5.03 | 4.95 |
| Starch and sugar | 43.27 | 44.15 | 44.89 | 45.80 |
| NDF | 10.23 | 10.21 | 10.83 | 10.81 |
| Calcium | 0.74 | 0.74 | 0.70 | 0.70 |
| Dig. phosphorus | 0.28 | 0.28 | 0.26 | 0.26 |
| NE swine | 10.35 | 10.35 | 10.35 | 10.35 |
| SID lysine | 0.99 | 0.95 | 0.89 | 0.84 |
| SID Met + Cys | 0.58 | 0.56 | 0.53 | 0.50 |
| SID threonine | 0.63 | 0.60 | 0.58 | 0.54 |
| SID tryptophan | 0.18 | 0.17 | 0.17 | 0.16 |
| SID valine | 0.69 | 0.66 | 0.62 | 0.59 |

Notes: CON, Basal diet; PROT, low protein diet with 0.05% protease; PROTV, low protein diet with 0.05% protease and 0.08% valine. 
*Provided per kg of complete diet: 6500 IU vitamin A, 950 IU vitamin D₃, 27 IU vitamin E, 2.0 mg vitamin K₃, 3.6 mg vitamin B₁₂, 1.3 mg vitamin B₆, 15 mg pantothenic acid, 26.0 mg niacin and 0.03 mg biotin; 50 mg Mn (as MnO₂), 70 mg Zn (as ZnO), 54 mg Cu (as CuSO₄·5H₂O), 0.5 mg I (as Ca(IO₃)₂), 0.5 mg Co (as Co₂O₃·7H₂O), and 0.25 mg Se (as Na₂SeO₃·5H₂O).
quality grade was based on individual pigs. All data were analysed using the mixed procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). Differences among treatment means were determined using Duncan’s multiple range tests with a \(P < 0.05\) indicating significance. Variability in the data was expressed as the pooled SEM.

**Results**

**Growth performance**

Throughout the experiment, there was no difference in BW among dietary treatments (Table 2). Also, no differences were detected among dietary treatments in ADG, ADFI and G:F during the growing phase (wk 0–6). During the finishing phase (wk 7–17) and overall period, ADG and ADFI were not affected by dietary treatments; however, increased \(P < 0.05\) G:F was obtained in pigs fed PROTV diet when compared with pigs fed CON and PROT diets.

**Carcass quality grade**

The effect of dietary supplementation with protease alone or in combination with valine on carcass quality grade is shown in Table 4. Pigs fed PROT diet and PROTV diet had no effects \((P > 0.05)\) on carcass weight, backfat thickness and carcass grade.

**Discussion**

A considerable amount of research has been conducted in recent years on the effects of low protein diets supplemented with CAA in pigs (Cline et al. 2016; Apple et al. 2017). It has also been suggested that valine may limit growth performance when dietary protein is reduced by more than 4% in growing pigs (Figuerola et al. 2002). The *B. licheniformis* derived-protease has the ability to increase protein utilization, which could exert beneficial effects on growth performance and nutrient digestibility in finishing pigs fed low protein diets (Upadhaya et al. 2016; Lei et al. 2017; Nguyen et al. 2018). As mentioned above, the combination of *B. licheniformis* derived-protease and valine may counteract the negative effects on growing-finishers fed low protein diets. Therefore, it is worth investigating to evaluate the effect of dietary supplementation of *B. licheniformis* derived-protease, alone or in combination with valine to low protein diet on growth performance, nutrient digestibility and carcass quality grade in growing-finishers. In order to avoid the negative effects of low protein diets in pigs, one effective approach to decrease the content of dietary CP is supplementing with CAA to balance for an ideal protein pattern (Tous et al. 2014; Monteiro et al. 2017). In the current study, except for lysine:valine ratios were lower in the PROTV group than in the CON group, the ratios of lysine to other LAAs (threonine, methionine and tryptophan) used in PROT group and PROTV group were similar with CON group.

In the current study, the addition of *B. licheniformis* derived-protease alone or in combination with valine to a low protein diet did not result in significant differences in BW, ADG, ADFI and nutrient digestibility of growing or finishing. However, the inclusion of *B. licheniformis* derived-protease in combination with valine in the current experiment increased the G:F ratio of the growing-finishers. In the present study, pigs fed PROTV diet showed a greater G:F ratio when compared with pigs fed CON diet during the finishing period. Our results were in accordance with CAA in pigs (Cline et al. 2016; Apple et al. 2017). With the supplementation of protease and valine in low protein diet, the carcass quality grade was not significantly different. In addition, the combination of protease and valine in low protein diet on growth performance, nutrient digestibility and carcass quality grade in growing-finishers was not significantly different.

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**Table 2.** Effect of protease alone, and in combination with valine supplementation in low protein diet on growth performance in growing-finishers pigs.

| Items            | CON   | PROT  | PROTV | SEM*  | p-value |
|------------------|-------|-------|-------|-------|---------|
| BW, kg Initial   | 22.56 | 22.55 | 22.56 | 0.002 | 0.763   |
| Week 6           | 52.34 | 52.91 | 53.18 | 0.39  | 0.314   |
| Week 7           | 113.49| 114.75| 116.49| 1.33  | 0.295   |
| ADG, g           | 709   | 723   | 729   | 9.27  | 0.312   |
| ADFI, g          | 1600  | 1632  | 1625  | 20.00 | 0.500   |
| G:F              | 0.443 | 0.443 | 0.449 | 0.003 | 0.377   |

**Table 3.** Effect of protease and valine supplementation in low protein diet on nutrient digestibility in growing-finishers pigs.

| Items              | CON   | PROT  | PROTV | SEM*  | p-value |
|--------------------|-------|-------|-------|-------|---------|
| Week 6             |       |       |       |       |         |
| DM                 | 78.85 | 79.27 | 79.83 | 0.71  | 0.621   |
| Crude protein      | 78.29 | 78.98 | 78.85 | 0.88  | 0.840   |
| Energy             | 78.46 | 79.43 | 79.55 | 0.78  | 0.570   |
| Week 17            |       |       |       |       |         |
| DM                 | 71.17 | 71.99 | 72.46 | 0.62  | 0.356   |
| Crude protein      | 70.88 | 71.86 | 72.16 | 0.81  | 0.519   |
| Energy             | 71.07 | 71.76 | 72.77 | 0.67  | 0.235   |

**Table 4.** Effect of protease and valine supplementation in low protein diet on carcass quality grade in growing-finishers pigs.

| Items              | CON   | PROT  | PROTV | SEM*  | p-value |
|--------------------|-------|-------|-------|-------|---------|
| Carcass weight, kg | 91.1  | 91.3  | 91.0  | 0.7   | 0.967   |
| Backfat thickness, mm | 18.7 | 18.9  | 19.1  | 0.4   | 0.787   |

**Notes:** CON, Basal diet; PROT, low protein diet with 0.05% protease; PROTV, low protein diet with 0.05% protease and 0.08% valine. Values represent the means of 12 pens \((n = 24)\) per treatment.

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\*Standard error of means.

\(a\)Means in the same row with different superscript differ significantly \((P < 0.05)\).
with Lei et al. (2017), who reported that the supplementation of *B. licheniformis* derived-protease alone in a low protein diet allowed pigs to obtain similar growth performance and nutrient digestibility to those fed CON diet. Previously, Choe et al. (2017) suggested that when supplemented alone, *B. licheniformis* derived-protease has been shown to increase growth performance in growing-finishing pigs. Moreover, Lei et al. (2017) reported that growth performance and nitrogen digestibility were negatively decreased by a low protein diet. Therefore, the possible reason for numerically increased nutrient digestibility in the PROTV group may be due to the combination of *B. licheniformis* derived-protease and valine could exhibit some compensation or synergism for the decreased dietary protein levels. However, Nguyen et al. (2018) reported that dietary *B. licheniformis* derived-protease supplementation improved growth performance and nutrient digestibility in growing-finishing pigs fed low protein diet. The reason for these differences could be attributed to the different dietary protein levels or different feed formulations.

As the ratios of lysine to other LAA (threonine, methionine and tryptophan) were similar with the CON group, the G:F ratio was statistically higher and the CP digestibility was numerically higher in the PROTV group than in both the CON group and PROT group, which may point at a synergism between *B. licheniformis* derived-protease and valine in pigs fed low protein diet. The present study showed no differences in growth performance and nutrient digestibility between CON and PROT dietary treatments. However, Lei et al. (2017) demonstrated that the inclusion of 0.05% *B. licheniformis* derived-protease in finishing pigs fed low protein (12.94%) diets led to a reduction in ADG. Moreover, previous studies found that the ADFI was reduced when pigs received a low protein diet deficient in valine and the ADG was increased by increasing the valinelysine ratio (Gloaguen et al. 2011; Liu et al. 2015). The possible reasons to explain the different results could be different extents of CP reduction in diet, different valine supplementation levels or the synergism between *B. licheniformis* derived-protease and valine. Taken together, these findings suggested that *B. licheniformis* derived-protease in combinations with valine may contribute to the growth performance of a low protein diet by reducing the use of protein feed and/or other synthetic AA. Further investigation is needed to clarify the mechanisms of this synergism.

Supplementation with protease alone or in combination with valine to low protein diet in this study did not show any significant adverse effects on carcass weight, backfat thickness and carcass grade in pigs. No comparisons could be made with other studies, because there was a scarcity of information on the effects of *B. licheniformis* derived-protease alone or in combination with valine supplementation on meat quality in growing-finishing pigs. Kerr et al. (2003) reported that dietary low protein had no significant effects on carcass characteristics. O’Shea et al. (2014) reported that the inclusion of protease in growing-finishing diets had no significant effect on carcass weight and backfat thickness. Analogous results were obtained when protease was added to the diet of growing-finishing pigs (Choe et al. 2017). Finite relative researches available on the effects of *B. licheniformis* derived-protease alone or in combination with valine on carcass characteristics of pigs fed low protein diet, further investigation is needed.

**Conclusion**

In conclusion, compared with supplementation of *B. licheniformis* derived-protease alone, the combination of *B. licheniformis* derived-protease and valine showed increased G:F ratio and did not show any adverse effects in growing-finishing pigs fed low protein diet. Therefore, *B. licheniformis* derived-protease in combination with valine may potentially reduce the side effects in pigs fed low protein diet.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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