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Effects of dietary protease on immune responses of weaned pigs

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Abstract

This experiment was conducted to investigate effects of dietary protease on immune responses of weaned pigs. Weaned pigs (n = 75; 7.06 ± 0.18 kg BW; 28 d old) were randomly assigned to 3 treatments (5 pigs/pen; 5 pens/treatment). Dietary treatments were positive control, a diet with required protein level (PC), negative control, a diet with lower protein level than PC (NC), and NC + 0.02% dietary protease (PRO). The dietary protease used in this experiment was a commercial product containing 75,000 protease units/g derived from *Nocardiopsis prasina* produced in *Bacillus licheniformis*. The dietary treatments did not contain any ingredients or additives that may provide antibacterial or physiological effects. Pigs were fed respective dietary treatments for 6 weeks. Blood was collected from randomly selected 2 pigs in each pen on d 1, 3, 7, and 14 after weaning. Measurements were number of white blood cells (WBC), tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1), and C-reactive protein (CRP). Pigs fed PRO had lower WBC on d 7 (14.84 vs 20.42 × 10^3/μL; $p < 0.05$) and TNF-α on d 7 (618 vs 889 pg/mL; $p = 0.085$) and 14 (437 vs 576 pg/mL; $p = 0.069$) than those fed NC, but there were no differences on WBC and TNF-α between PC and PRO. Pigs fed PRO had lower TGF-β1 on d 3 (630 vs. 1,588 and 1,396 pg/mL; $p < 0.05$) than those fed PC and NC. However, no differences were found on CRP among dietary treatments. In conclusion, addition of dietary protease reduced inflammatory immune responses of weaned pigs.

Keywords: Dietary protease, Immune response, Weaned pigs

INTRODUCTION

Weaner pig diets mainly consist of corn and soybean meal (SBM) and contain approximately 20% of crude protein (CP) [1], but the CP digestibility or availability in weaned pigs ranges is from 60% to 80% due to their immature digestive system [2]. Undigested protein can be a source for pathogenic bacteria in the gut to ferment consequently and can contribute to causing post-weaning diarrhea and

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Competing interests
No potential conflict of interest relevant to this article was reported.

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

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Conceptualization: Song M.
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Ethics approval and consent to participate
The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Korea (approval code: CNU-00611).

death of pigs [3–6]. Furthermore, undigested protein not used by pigs can be excreted to cause environmental pollution, such as nitrogen emission [7,8]. Due to these issues, the swine industry has been looking for the solutions to improve the availability of protein in pig diets.

Addition of dietary protease to weaner pig diets may be a solution to improve the protein availability of pigs after weaning [9–11]. Dietary protease is an enzyme that hydrolyzes high molecular weight polypeptides into lower molecular weight oligopeptides for further digestion by endogenous proteases [12]. Dietary protease properties include to break down protein-bound complexes with anti-nutritional factors and are typically used in swine and poultry diets as a mono-component or multi-enzyme products [13–15]. Several studies showed addition of dietary multi-enzymes with the protease in pig diets improved digestibility of protein and other nutrients as well as growth performance of pigs with different stages of age [15–17]. In addition, several recent studies reported dietary protease application as a mono-component in pig diets had positive effects on growth rate, protein digestibility, and gut health of pigs [15,18]. However, there was no information about immune responses of weaned pigs fed diets with dietary mono-component protease. Therefore, the purpose of this study was to verify effects of dietary protease in a diet based on corn and SBM on immune responses of weaned pigs.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Korea. This experiment was conducted at the Animal Research Center of Chungnam National University.

Experimental design, animals, and diets

Weaned pigs [Duroc × (Landrace × Yorkshire); n = 75; 7.06 kg of average body weight (BW)] were randomly allotted to 3 diets (3 barrows and 2 gilts per pen; 5 replicates per diet) in a randomized complete block design (block = BW and sex). The diets were a basal diet with corn and SBM basis to meet or exceed the requirement of crude protein [positive control diet (PC)], 2) a lower protein diet than PC [negative control diet (NC)], and 3) NC + 0.02% dietary protease (PRO). The dietary protease was a commercial product (Ronozyme® ProAct, DSM nutrition products, Kaiseraugst, Switzerland) containing 75,000 protease units/g derived from *Nocardiopsis prasina* produced in *Bacillus licheniformis*. The basal diet did not include any feed ingredients that may have any antibacterial or physiological effects, such as animal plasma, antibiotics, or zinc oxide (Table 1). All pigs were housed in an environmentally controlled room with a slatted plastic floor and allowed *ad libitum* access to diets and water for 6 weeks.

Sample collection and immune measurements

Blood samples from 2 weaned pigs (1 barrow and 1 gilt) in each pen were collected from the jugular vein of each pig with and without ethylenediaminetetraacetic acid (EDTA) tubes to yield whole blood and serum, respectively, on d 1, 3, 7, and 14 after weaning. The blood for serum was held to clot at room temperature for 2 h, kept at 4℃ overnight, and then centrifuged for 15 min at 3,000 × g at room temperature. The number of white blood cells (WBC) were analyzed using a multi-parameter, automated hematology analyzer calibrated for porcine blood (scil Vet abc hematology analyzer, scil animal care company, F-67120 Altorf, France). Inflammatory cytokines and C-reactive protein (CRP) were measured using porcine ELISA kits following the manufacturer's procedures [tumor necrosis factor-α (TNF-α; Genorise Scientific, Berwyn, PA, USA); transforming growth factor-β 1 (TGF-β 1; Genorise Scientific, Berwyn, PA, USA); CRP (Genorise Scientific, Berwyn,

Table 1. Composition of basal diet for weaned pigs (as-fed basis)

Items	PC	NC
Ingredients (%)		
Corn	56.09	58.09
Soybean meal (44%)	26.00	24.00
Soy protein concentrate	12.00	12.00
Soybean oil	3.00	3.00
Limestone	1.30	1.30
Monocalcium phosphate	1.20	1.20
Vitamin-mineral premix[1)]	0.04	0.04
L-Lysine-HCl	0.24	0.24
DL-Methionine	0.09	0.09
L-Threonine	0.04	0.04
Total	100.00	100.00
Calculated energy and nutrient contents		
Metabolizable energy (Mcal/kg)	3.53	3.42
Crude protein (%)	24.49	22.51
Calcium (%)	0.81	0.73
Phosphorus (%)	0.69	0.63
Lysine (%)	1.54	1.41

[1)]The vitamin-mineral premix provided the following quantities of per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 2,500 IU; vitamin E, 30 IU; vitamin K_3, 3 mg; D-pantothenic acid, 15 mg; nicotinic acid, 40 mg; choline, 400 mg; and vitamin B_{12}, 12 μg; Fe, 90 mg from iron sulfate; Cu, 8.8 mg from copper sulfate; Zn, 100 mg from zinc oxide; Mn, 54 mg from manganese oxide; I, 0.35 mg from potassium iodide; Se, 0.30 mg from sodium selenite.

PC, positive control; NC, negative control.

PA, USA)]. All the cytokine measurements of serum samples were based on the report of Song et al. [19]. The intra-assay coefficients of variation for TNF-α, TGF-β1, and CRP were 6%, 6%, and 6%, respectively. The inter-assay coefficients of variation for TNF-α, TGF-β1, and CRP were 8%, 11%, and 9%, respectively.

Statistical analysis

All data were analyzed using the PROC GLM procedure of SAS (SAS Inst., Cary, NC, USA) in a randomized complete block design. The experimental unit was the pen and blocks were BW and sex. The statistical model for the number of WBC, inflammatory cytokines (TNF-α and TGF-β1), and CRP included effects of diets as a fixed effect and BW and sex as covariates. In addition, pair-wise comparisons were performed among diets when a main effect of diet was found. Results are given as means ± SEM. Statistical significance and tendency were considered at $p < 0.05$ and $0.05 \leq p < 0.10$, respectively.

RESULTS AND DISCUSSION

Post-weaning period in pigs is the most stressful period due to nutritional, immunological, physiological, and environmental changes, and/or disease challenges [20]. Previous studies showed the stress factors can give a serious damage in gut health and impair immune functions of weaned pigs [21–24]. Further, the dysfunctional nutrient metabolism and impaired immune system caused by the post-weaning stressors can contribute to causing serious diarrhea and sudden mortality of weaned pigs [8,25]. As the present study showed, the concentrations of all inflammatory immune

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responses of weaned pigs were the highest maybe because of the post-weaning stress and decreased those gradually as pigs grew older. Under the conditions in present study, PRO decreased ($p < 0.05$) the number of WBC on d 7 after weaning compared with NC (Fig. 1). The number of WBC can be used as an indicator for systemic inflammation and thus the higher number of WBC than normal condition indicates an ongoing systemic inflammation [26]. The PRO tended ($p < 0.10$) to decrease serum TNF-α on d 7 and 14 compared with NC (Fig. 2). The TNF-α is one of the most important pro-inflammatory cytokines that stimulates systemic inflammation and acute phase reaction [21]. Interestingly, PRO decreased serum TGF-β1 on d 3 ($p < 0.05$) and tended to decrease it on d 7 and 14 ($p < 0.10$) compared with PC and/or NC (Fig. 3). The TGF-β1 has functions both immune-suppressive and immune-enhancing activities [27]. As an anti-inflammatory cytokine, TGF-β1 stimulates the proliferation and differentiation of T and B cells and deactivate monocyte/macrophage [28]. However, as a pro-inflammatory cytokine, TGF-β1 in the presence

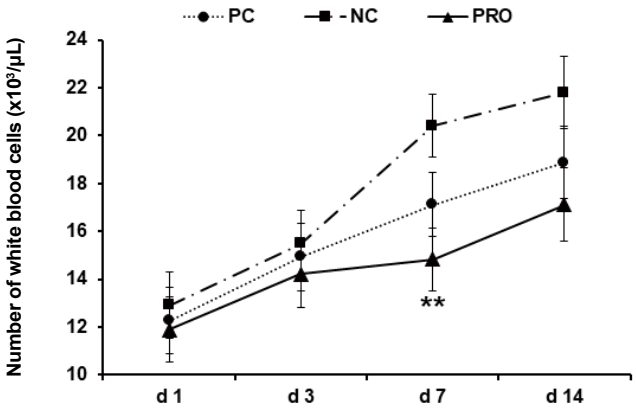

Fig. 1. Effect of dietary protease on number of white blood cells of weaned pigs. Each value is the mean of 5 replicates. **Difference between PRO and NC ($p < 0.05$). PC, positive control; NC, negative control; PRO, negative control + 0.02% dietary protease.

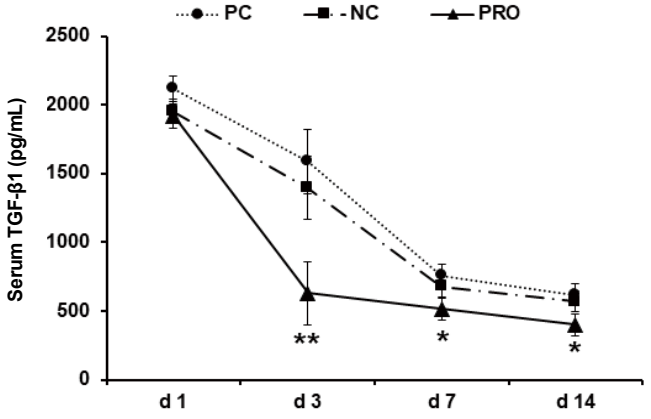

Fig. 3. Effect of dietary protease on serum TGF-β1 of weaned pigs. Each value is the mean of 5 replicates. *Compared with PC and/or NC ($p < 0.10$). **Difference between PRO and PC and/or NC ($p < 0.05$). PC, positive control; NC, negative control; PRO, negative control + 0.02% dietary protease.

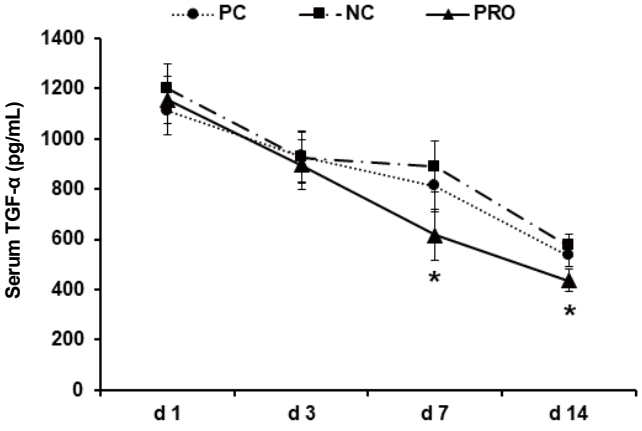

Fig. 2. Effect of dietary protease on serum TNF-α of weaned pigs. Each value is the mean of 5 replicates. *Difference between PRO and NC ($p < 0.10$). PC, positive control; NC, negative control; PRO, negative control + 0.02% dietary protease.

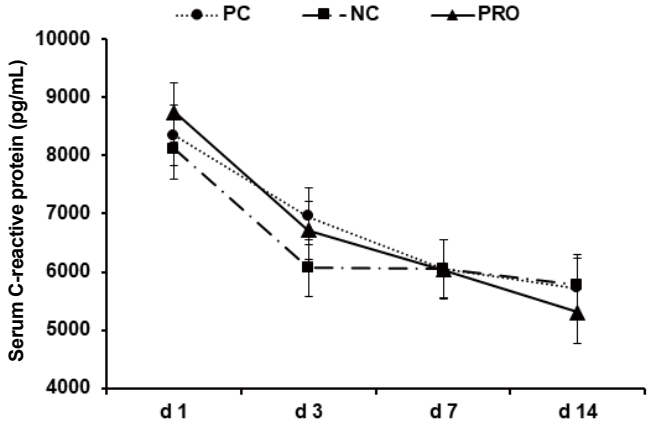

Fig. 4. Effect of dietary protease on serum C-reactive protein of weaned pigs. Each value is the mean of 5 replicates. PC, positive control; NC, negative control; PRO, negative control + 0.02% dietary protease.

of different cytokines can induce the differentiation of diverse T helper cells, which promote further tissue inflammation [29]. The concentration of serum CRP of all dietary treatments was getting decreased from d 1 to 14 after weaning (Fig. 4), but no protease effects were found. The CRP can be used as an inflammatory response indicator and pro-inflammatory cytokine such as TNF-α are critical inducers of the synthesis of CRP [24,28,29]. Based on the results, present study observed dietary protease provided anti-inflammatory effects for weaned pigs by attenuating inflammatory immune responses. Further, dietary protease may contribute to reduction of post-weaning stress and mortality by alleviating inflammation of weaned pigs.

CONCLUSION

The present study showed addition of dietary protease reduced inflammatory immune responses, such as number of WBC and serum TNF-α and TGF-β1, of weaned pigs.

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