Effect of enzymatic hydrolysate of cottonseed protein supplementation on growth performance and intestinal health of nursery pigs in Thailand

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Abstract
This study investigated the effects of enzymatic hydrolysate of cottonseed protein (EHCP) supplementation on the growth performance and intestinal health of nursery pigs in Thailand. A total of 180 newly weaned piglets were randomly allocated to 3 groups with 6 replicates in each group and 10 piglets per replicate. Nursery pigs were fed three diets containing 0, 1%, and 1.5% EHCP for 28–63 days of age. The results indicated that 1% EHCP supplementation increased average daily feed intake (ADFI) and average daily gain (ADG) and decreased feed conversion rate (FCR) in the numerical, suggesting that appropriate EHCP supplementation could numerically improve growth performance of nursery pigs in Thailand. Moreover, 1% EHCP supplementation significantly decreased intestinal crypt depth and diarrhea incidence and increased intestinal villus height to crypt depth ratio and fecal consistency, suggesting that optimum EHCP supplementation could improve intestinal morphology and decreased diarrhea incidence of nursery pigs in Thailand. Furthermore, 1% EHCP supplementation significantly improved intestinal glutathione (GSH) level and superoxide dismutase (SOD) activity and indicated that optimal EHCP supplementation could improve intestinal antioxidant capacity of nursery pigs in Thailand. Optimum EHCP supplementation numerically increased growth, significantly decreased diarrhea incidence, significantly improved intestinal morphology and antioxidant capacity of nursery pig in Thailand.

Keywords Enzymatic hydrolysate of cottonseed protein · Nursery pigs · Growth performance · Intestinal health

Introduction
The growth rate of piglets is a vital factor affecting the growth of finishing pig. (Ramakrishnan 2001). However, piglets are highly susceptible to weaning stress at 3 to 4 weeks of age, especially in tropical regions such as Thailand, because they are more likely subjected to stressors such as environmental, nutritional, and microbial unbalances (Nabuurs 1998). It has been reported that weanling stress induced decreased feed intake and growth, caused diarrhea, and impaired intestinal functions and morphology of early weaned pigs (Pluske et al. 1995; Pluske et al. 1997a, b). To solve these problems, immunoprotective—like probiotics or acidifiers (Kalbande et al. 1992)—and immunostimulatory—like yeast and β-glucans (Maneewan et al. 2012)—supplements have been used as feed supplements to improve growth performance and to control disease. The reason of using probiotics or acidifiers is that they could improve the intestinal microflora environment (Hashemi et al. 2012). It was reported that dietary acidifiers improved the growth performance of weaned piglets (Partanen and Mroz 1999). The reason of using yeast and β-glucans is that they could cause immunological stimulation in animals resulting in higher production because of greater stability of homeostasis (Maneewan et al. 2012). As another growth promotant,
peptides might solve postweaning problems, because peptides have a growth promotion effect. A previous study found that exogenous peptides could preserve immune system to improve growth of human (Genton and Kudsk 2003) and mice (Selsted and Ouellette 2005). Enzymatic hydrolysate of cottonseed protein (EHCP) contains numerous peptides. To now, no study has paid attention to the effect of EHCP on growth and intestinal health of piglets. It was reported that enzymatic hydrolysis soybean meal supplementation improved intestinal health of weaned pigs (Zhou et al. 2011). We inferred that EHCP might affect growth and intestinal health of piglets, which is worthy of investigation.

The intestine is regarded as the most important antistress and immune organ (Shen et al. 2009); particularly, anti-weanling stress was closely associated with intestinal health (Stier et al. 2014). To improve the intestinal health, the host has developed intestinal mucosal immune system (Eckmann et al. 1995) that is basically consisted of immune barrier and physical barrier (Moretó and Pérez-Bosque 2009). The intestinal maturity was directly apparent to determine intestinal physical barrier health of pigs (Shirkey et al. 2006). It was reported that the peptide could affect intestinal maturity of human (Jeppesen et al. 2009). However, no study has paid attention to the effect of EHCP on intestinal maturity of piglets. Moreover, it was reported that the intestinal physical barrier is particularly vulnerable to oxidative damage of pigs (Pearce et al. 2013). To combat the oxidative damage, pigs have developed antioxidant system, which includes non-enzymatic compounds (glutathione) and enzymatic antioxidant compounds (like superoxide dismutase (SOD), catalase (CAT), and glutathione-dependent enzymes) (Zhu et al. 2012). In vitro, a previous study found that antioxidative peptides play important roles in antioxidative activity (Chen et al. 1996). Bendich (1990) revealed that the regeneration of an antioxidant peptide is closely related to the glutathione in vivo. We hypothesized that EHCP supplementation might improve intestinal physical barrier through increasing antioxidant peptide levels of piglets. This possibility is worthy of investigation.

Except for the intestinal physical barrier, pigs also developed immune barrier in the intestinal mucosal immune system (Oswald 2006). The intestinal immune barrier of pigs primarily relies on local immunity, which is closely related to intestinal immunoglobulins, such as intestinal immunoglobulin G (IgG) and immunoglobulin A (IgA) (Le Dividich et al. 2005). It is reported that tetrameric tripeptide, as a member of the peptide’s family, could be bound to the constant portion of IgG to affect immune response in a mouse (Marino et al. 2000). Hence, we speculated that EHCP supplementation might affect the intestinal immune barrier through taking part in the immunoglobulin composition in the gut of animals. However, to date, studies to investigate the effects of EHCP supplementation on intestinal immune barrier function in piglet have not been carried out, which needs investigation.

The aim of the study was to assess the production results and health status of weaners modulated by supplementation of EHCP.

Materials and methods

Feed additive

Enzymatic hydrolysate of cottonseed protein (EHCP) was obtained from Chengdu Mytech Biotech Co., Ltd. (Chengdu, China). The brand name was “Fortide-C.” EHCP consisted of nutrient peptides and was a high-quality raw material. It was prepared from carefully selected soybean meal and was processed by enzymatic hydrolysis into small peptide. For the nutritional value of EHCP (Table 1), the total antioxidant capacity and peptides were determined. The peptides were determined using the trichloroacetic acid precipitation method by Yvon et al. (1989). Moreover, the total antioxidant capacity of EHCP using ABTS assay according to Re et al. (1999).

Feed mixture

Experimental animals were fed two diets prepared for the first period after weaning (about 3 weeks)—mixture 1—and for the second one (successive 2 weeks)—mixture 2. The composition and nutritive value of feed mixtures is given in Table 2. The basal diets were based on corn-soybean meal. EHCP was added to the basal mixtures in three doses: 0, 1%, and 1.5%. The chemical composition of experimental mixtures is presented in Table 3.

Experimental design or animals

The study was conducted using completely randomized experimental design (CRD). All newly weaned piglets were divided into 3 dietary treatments. A total of 180 nursery pigs (Duroc × Landrace × Yorkshire, 28 days of age) with similar body weight (7.74 ± 1.08 kg initial weight) were randomly allocated to three treatments with 6 replications per treatment and 10 piglets per replication and per replication is one pen (10 piglets in per pen).

Table 1 The nutritional value of EHCP

| Item                  | EHCP |
|-----------------------|------|
| Moisture (%)          | ≤ 8.00 |
| Crude protein (%)     | ≥ 45.00 |
| Crude fat (%)         | 1.50 |
| Crude ash (%)         | ≤ 15.00 |
| Peptide (%)           | ≥ 28.00 |
| Total antioxidant capacity | 0.84 |
The housing was an evaporative cooling system house. Feed and water were provided ad libitum throughout the experimental period.

The following production data were collected during the experiment: body weight (BW) at 28 (weaning or begin of the experiment), 49 (feed change), and 63 (the end of the experiment) days of age and feed intake (FI). To estimate health status and immunity, fecal ammonia content, diarrhea incidents, and fecal consistency were estimated and some tissue sample were taken.

At the end of 49 and 63 days of age, 200 g fresh fecal of each replication was collected for fecal ammonia content analysis by stream distillation (Apha 1985) and fecal consistency evaluation. Fecal consistency scoring was based on the following index used by Cho et al. (2007): 0, normal (feces firm and well formed); 1, soft consistency (feces soft and formed);
2, mid-diarrhea (fluid feces, usually yellowish); and 3, severe diarrhea (feces watery and projectile). The incidence of diarrhea was recorded throughout the experiment and was calculated according to Xiong et al. (2014).

All experimental procedures were approved by the Animal Science Department of Kasetsart University.

### Sampling and chemical analyses

#### Feed

Representative samples of experimental diets were collected from each batch, pooled together, and kept in the freezer for nutrient composition analysis (Helrick 1990).

#### Animals

All pigs were weighed at the 28, 49, and 63 days of age of the experiment. The feed offered and the feed refused were recorded periodically for each replicate. All data of average daily feed intake (ADFI), average daily gain (ADG), and feed conversion efficiency (FCR) were calculated. Two pigs from each replication (a total of 12 pigs) were humanely sacrificed at the end of experiment, one to take sample for histology and the second one to prepare scrapings.

At the end of the study, one pig (the body weight close to the average weight of each treatment group) from each replication was humanely sacrificed for gut histology study (middle of the duodenum, jejunum, and ileum) including villi height and width, crypt depth, villi height to crypt depth ratio, and villi surface area. Gut morphology was measured according to the methods of Brown et al. (2006).

At the end of the study, one pig from each replication was humanely sacrificed for intestinal tissue collection. Duodenal scrapings were all collected from the pig pylorus on a 10- to 30-cm distance, and snap frozen in liquid nitrogen for immune and antioxidant capability analysis: the secretory IgA assay using ELISA Kit (Cusabio), thiobarbituric acid reactive species (TBARs) (Biovision), total antioxidant capacity (T-AOC) (Abcam), activity of superoxide dismutase (SOD) (Abcam), catalase (CAT) (Abcam), and GSH (by Kit) (Biovision).

### Statistical analysis

Statistical analyses were done using the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Duncan’s new multiple range test was used to assess differences between means and values and $P < 0.05$ were considered statistically significant. Count data groups were analyzed using chi-square test. Results were expressed as the mean ± standard deviation (SD).

### Results

#### Production results

The influence of EHCP on growth performance of nursery pigs is shown in Table 4. There were no significant differences in final body weight, ADFI, ADG, and FCR among dietary treatments during experimental time. However, ADFI and ADG were higher numerically in piglet fed 1% EHCP than the control group by 7.0% and 11.9% at 28–49 days of age, and fed 1.5% EHCP than the control group by 6.4% and 11.6% at 50–63 days of age. FCR numerically decreased fed 1% EHCP by 3.6% at 28–49 days of age, and fed 1.5% EHCP by 4.9% at 47–63 days of age. At 28–63 days of age, ADFI and ADG were higher numerically in piglet fed 1.5% and 1% EHCP than the control group by 7.0% and 10.2%, respectively, as well as FCR was lower in fed 1% EHCP by 3.3% of nursery pigs.

| Item          | EHCP (%) (28–49 days of age) | EHCP (%) (50–63 days of age) |
|---------------|------------------------------|-----------------------------|
|               | 0   | 1   | 1.5 | 0   | 1   | 1.5 |
| Moisture (%)  | 9.44| 9.24| 9.35| 9.89| 9.82| 9.70|
| Protein (%)   | 18.95| 18.83| 18.86| 18.32| 18.73| 18.45|
| Fiber (%)     | 2.18| 2.15| 2.08| 2.16| 2.19| 2.22|
| Fat (%)       | 4.96| 4.78| 4.65| 4.31| 4.71| 3.98|
| Ash (%)       | 6.81| 7.08| 6.97| 6.72| 6.42| 6.35|
| Calcium (%)   | 0.19| 0.24| 0.20| 0.19| 0.19| 0.20|
| Phosphorus (%)| 0.78| 0.80| 0.78| 0.85| 0.77| 0.84|
| Gross energy (kcal/kg) | 4522| 4492| 4454| 4457| 4401| 4468|
The effects of EHCP supplementation on fecal ammonia and diarrhea incidence of nursery pigs are shown in Table 5. The diarrhea incidence was significantly decreased with increasing EHCP supplementation levels up to 1% diet \( (P < 0.05) \), and plateaued thereafter \( (P > 0.05) \) at 28–49 days of age. However, EHCP supplementation had no effects on ammonia at 28–63 days of age and diarrhea incidence at 50–63 days of age \( (P > 0.05) \).

The effect of EHCP supplementation on fecal consistency of nursery pigs is shown in Table 6. There were significant differences on the number of nursery pigs scored 0, 1, 2, and 3 points at 28–49 and 50–63 days of age among dietary treatments \( (P < 0.05) \). The number of nursery pigs scored 0 points (normal) fed EHCP supplementation was higher than the control group, but the other scoring results were contrary.

### Intestinal maturity status

The effect of EHCP supplementation on intestinal morphology of nursery pigs is shown in Fig. 1a–e. The crypt depth in the duodenum was tending to decline fed 1% EHCP supplementation when compared with the control group \( (P = 0.093) \). Moreover, crypt depth in the jejunum and ileum were significantly decreased with EHCP supplementation levels up to 1% diet \( (P < 0.05) \) and gradually increased. The villi height/crypt depth in the jejunum and ileum were significantly increased with increasing EHCP supplementation levels.

### Table 4 Production results of weaners

| Item                  | EHCP (%) | SEM  |
|-----------------------|----------|------|
|                       | 0        | 1    | 1.5 |
| Initial weight (kg)   | 7.57 ± 0.79 | 7.73 ± 1.26 | 7.93 ± 1.19 | 0.25 |
| Final weight (kg)     | 21.10 ± 1.93 | 22.63 ± 3.89 | 22.56 ± 3.70 | 0.75 |
| Weight gain (kg)      | 13.53 ± 1.31 | 14.91 ± 2.68 | 14.63 ± 2.59 | 0.53 |
| 28–49 days of age     |           |      |     |
| FI (kg)               | 8.60 ± 1.28 | 9.21 ± 1.21 | 9.09 ± 1.23 | 0.28 |
| ADFI (g/d)            | 409.6 ± 60.99 | 438.4 ± 58.17 | 432.8 ± 59.01 | 13.50 |
| ADG (g/d)             | 298.5 ± 47.07 | 333.9 ± 59.89 | 311.2 ± 66.03 | 13.37 |
| FCR                   | 1.38 ± 0.14 | 1.33 ± 0.09 | 1.42 ± 0.16 | 0.03 |
| 50–63 days of age     |           |      |     |
| FI (kg)               | 11.80 ± 1.28 | 12.52 ± 2.45 | 12.56 ± 2.32 | 0.47 |
| ADFI (g/d)            | 842.8 ± 91.42 | 894.1 ± 175.2 | 896.9 ± 165.9 | 33.54 |
| ADG (g/d)             | 518.3 ± 52.20 | 564.0 ± 106.9 | 578.2 ± 97.50 | 20.62 |
| FCR                   | 1.63 ± 0.12 | 1.59 ± 0.10 | 1.55 ± 0.10 | 0.03 |
| 28–63 days of age     |           |      |     |
| FI (kg)               | 20.40 ± 2.51 | 21.73 ± 3.67 | 21.83 ± 3.37 | 0.73 |
| ADFI (g/d)            | 582.9 ± 71.81 | 620.7 ± 104.8 | 623.5 ± 96.28 | 20.87 |
| ADG (g/d)             | 386.4 ± 37.56 | 425.9 ± 76.82 | 418.0 ± 74.05 | 15.04 |
| FCR                   | 1.51 ± 0.06 | 1.46 ± 0.07 | 1.50 ± 0.05 | 0.01 |

### Table 5 Fecal ammonia and diarrhea incidence during the experimental time

| Item                    | EHCP (%) | SEM  |
|-------------------------|----------|------|
|                        | 0        | 1    | 1.5 |
| 28–49 days of age       |          |      |     |
| Ammonia (ppm)           | 851.0 ± 177.4 | 805.0 ± 241.1 | 809.2 ± 285.1 | 56.23 |
| Diarrhea incidence (%)  | 4.84 ± 1.57<sup>a</sup> | 2.86 ± 1.50<sup>b</sup> | 1.51 ± 0.69<sup>b</sup> | 0.44 |
| 50–63 days of age       |          |      |     |
| Ammonia (ppm)           | 834.9 ± 228.68 | 741.9 ± 124.4 | 715.3 ± 182.9 | 42.69 |
| Diarrhea incidence (%)  | 3.04 ± 1.36 | 2.51 ± 1.76 | 1.19 ± 1.20 | 0.38 |

<sup>a, b</sup> Means in the same rows with different superscripts are significantly different \( (P ≤ 0.05) \)

### Table 6 Results of fecal estimation during the experimental period

| Item                    | EHCP (%) | SEM  |
|-------------------------|----------|------|
|                        | 0        | 1    | 1.5 |
| 28–49 days of age<sup>1</sup> |      |      |    |
| Score (%)               | 0        | 72.71 ± 7.81 | 70.28 ± 6.29 | 78.18 ± 5.20 | 1.65 |
|                        | 1        | 19.07 ± 7.95 | 24.94 ± 5.79 | 19.50 ± 5.96 | 1.61 |
|                        | 2        | 7.34 ± 2.11  | 4.54 ± 1.43  | 2.31 ± 1.19  | 0.62 |
|                        | 3        | 0.88 ± 1.36  | 0.25 ± 0.60  | 0.00 ± 0.00  | 0.21 |
| 50–63 days of age<sup>1</sup> |      |      |    |
| Score (%)               | 0        | 37.04 ± 7.54 | 43.73 ± 5.17 | 46.57 ± 5.33 | 1.66 |
|                        | 1        | 59.18 ± 8.46 | 53.52 ± 6.75 | 52.14 ± 4.56 | 1.67 |
|                        | 2        | 3.64 ± 1.54  | 2.44 ± 3.35  | 1.29 ± 1.29  | 0.55 |
|                        | 3        | 0.14 ± 0.34  | 0.31 ± 0.47  | 0.00 ± 0.00  | 0.08 |

<sup>1</sup> Means were significant differences in fecal consistency scores among different treatment groups \( (P ≤ 0.05) \)
up to 1% diet ($P < 0.05$) and plateaued thereafter ($P > 0.05$). However, there were no significant differences on villus height, villus width, and villus surface area in the duodenum, jejunum, and ileum, crypt depth in the duodenum, villi height/crypt depth in the duodenum among dietary treatments at 63 days of age of nursery pigs ($P > 0.05$).

**Intestinal immunity status**

The effects of EHCP on immune and antioxidant status of nursery pigs are shown in Table 7. The glutathione (GSH) levels and superoxide dismutase (SOD) activity in the intestine were significantly enhanced with EHCP supplementation levels up to 1.5% diet ($P < 0.05$). However, EHCP
supplementation had no significant effect on levels of sIgA and TBARs, T-AOC, and activity of catalase (CAT) at 63 days of age of nursery pigs (P > 0.05).

**Discussion**

**EHCP supplementation numerically improved growth performance of nursery pigs**

No differences in average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) of nursery pigs were observed among dietary treatment groups. Although there is no significant difference in biostatistics, in the production practice, farmers seem to be more concerned about the numerical increase. In the present study, the ADFI and ADG for 1.5% EHCP supplementation group were much higher by 7.0% and 8.2%, but the FCR was lower by 0.7% than those of the 0.0% EHCP supplementation group. Similar observations in ADG with fermented soybean meal, containing peptides, to weaned pig diets were made by Zhou et al. (2011) and Szuba-Trznadel et al. (2014). The slightly improving growth performance was probably related to the digestive and absorptive capacity of intestine in animal. It was reported that peptides could be directly absorbed and elevate protein synthesis in the intestine (Stevenson 2000), which were beneficial to the growth of piglets (Wang et al. 2007). Moreover, piglet growth was largely dependent on its health status, which was reflected by fecal parameters (Nyachoti et al. 2006).

**EHCP supplementation decreased diarrhea incidence and improved fecal consistency of nursery pigs**

In animals, diarrhea incidence and fecal consistency were used as important fecal parameters of reflection of intestinal health (Nyachoti et al. 2006). Current study showed that optimal EHCP supplementation significantly decreased diarrhea incidence during 28–49 days of age and improved fecal consistency during 28–63 days of age of nursery pigs. Similarly, it was reported that enzymatic hydrolysate of wheat gluten and glutathione reduced diarrhea incidence of weaned pigs (Wang et al. 2011; Kandil et al. 1995; Reeds and Burrin 2001). In the current study, EHCP supplementation increased glutathione levels in the intestine of nursery pigs. Above data suggested that EHCP supplementation decreasing diarrhea incidence and improving fecal consistency may be attributed to increase glutathione levels of nursery pigs. Further findings were that diarrhea incidence of piglet was largely associated with integrity of intestinal morphology (Pluske et al. 1997a, b). Therefore, we next investigated the effect of EHCP supplementation on intestinal morphology of nursery pigs.

**EHCP supplementation improved intestinal morphology of nursery pigs**

The present study showed that 1% EHCP supplementation significantly decreased crypt depth and increased villus height to crypt depth ratio in the jejunum and ileum of nursery pigs, suggesting that EHCP supplementation could significantly improve intestinal morphology of nursery pigs. Previous studies have shown that glutathione could repair intestinal morphology and increase number of enterocytes (Kandil et al. 1995; Reeds and Burrin 2001). This study found that EHCP supplementation increased intestinal glutathione levels of nursery pigs. Hence, this study suggested that EHCP supplementation improving intestinal morphology may be partly related to increase glutathione level. The integrity of intestinal morphology largely depended on the intestinal immune system of piglets (McLamb et al. 2013). Therefore, we next investigated the effect of EHCP supplementation on the intestinal immune system of nursery pigs.
EHCP supplementation significantly improved intestinal immune system of nursery pigs

The intestinal mucosal immune system is basically consisted of immune barrier and physical barrier in animals (Moretò and Pérez-Bosque 2009), which were closely related to gut immunologic substance like gut secretory immunoglobulin A (sIgA) and intestinal antioxidant system included non-enzymatic compounds glutathione (GSH) and enzymatic antioxidants compounds (like SOD, CAT, and glutathione-dependent enzymes) (Zhu et al. 2012). The present study showed that EHCP supplementation improved intestinal SOD activity and glutathione (GSH) level nursery pigs. Zhang et al. (2012) found that peptide could elevate utilization of mineral substance like copper and zinc in animals. It was reported that copper and zinc are parts of the synthesis of superoxide dismutase (SOD) in animals (Zelko et al. 2002). Hence, EHCP supplementation could improve intestinal antioxidant system like improving SOD activity via elevating utilization of mineral substance like copper and zinc, and then improved intestinal immune system of nursery pigs. However, the underlying mechanisms require further investigation.

Conclusion

In summary, this study indicated that 1% EHCP supplementation numerically improved growth performance of nursery pigs in Thailand. Furthermore, this study demonstrated for the firstly time that (1) the optimal EHCP supplementation decreased intestinal crypt depth and diarrhea incidence and improved intestinal villus height to crypt depth ratio and fecal consistency, suggesting that optimal EHCP supplementation improved intestinal morphology and decreased diarrhea of nursery pigs in Thailand. (2) The optimal EHCP supplementation improved intestinal antioxidant capacity, thereby elevating intestinal immune system, and finally improved intestinal health of nursery pigs in Thailand.

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Compliance with ethical standards All experimental procedures were approved by the Animal Science Department of Kasetsart University.

Conflict of interest The authors declare that they have no conflict of interest.

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