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### NUTRITION

# Evaluation of a novel plant protein source on growth performance of weaning pigs

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# Abstract

A total of 100 weaning pigs [Duroc × (Yorkshire × Landrace)] with an average body weight of 8.28 ± 0.97 kg were used in a 5 wk trial to determine the effect of fermented soybean meal by *Bacillus spp.* on growth performance and gut health in weaning pigs. Pigs were randomly allowed to one of two dietary diets including 1) CON, a control group; 2) TRT, which replaced 5% of fermented soybean meal in the CON diet with 5% of a combination of high digestible Bacillus-fermented soybean meal with nucleotide, and functional amino acids blend. During 5 wk, pigs fed the TRT diet increased final average body weight, average, daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) by 13.80%, 24.433%, 17.65%, respectively. In conclusion, weaning pigs fed a combination of highly digestible Bacillus-fermented soybean meal with nucleotides, and functional amino acids increased final body weight, ADG, ADFI by 13.8%, 24.4%, 17.7%, respectively, and decreased FCR by 5.5%. The combination could be a potential-plant derived protein source to further enhance piglets performance.

Key words: Nucleotide; Inosine monophosphate, Fermented soybean meal, functional amino acids

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### Introduction

Protein sources derived from animal tissues or products, despite high costs, have been used extensively in feeding nursery pigs mainly due to relatively higher digestibility (Kim & Easter, 2001; Gottlob et al., 2006), palatability (Ermer et al., 1994), and negligible concentrations of anti-nutritional factors (Anderson & Wolf, 1995) compared with protein sources derived from plants. However, increasing concern about animal and meat safety remains as potential risk regarding feeding animal tissue-derived ingredients to animals, along with its future availability.

Plant proteins, such as soybean meal (SBM), are the major source of protein in swine diets and are relatively cheaper than animal protein sources. However, the use of plant proteins in piglet diets is often limited due to the presence of various anti-nutritional factors, which interfere with digestion, absorption and utilization of nutrients (Hong et al., 2004).

To overcome the issues, soy protein concentrate, extruded soybean meal, and enzyme pre-treated SBM (Kim et al., 2003, 2006) has been used in nursery pig diets replacing the conventional SBM. Among them, Bacillus-fermented SBM provides multiple benefits such as (1) partially digested soy proteins (high proportion of low-molecular-weight peptide) that are utilized better, (2) higher protein content than SBM due to fermentation of carbohydrate, (3) low anti-nutritional factors (removed or minimized trypsin inhibitors, glycinin,  $\beta$ -conglycinin, raffinose and stachyose), and (4) probiotic effect that is beneficial to nursery pigs by inhibiting intestinal colonization of pathogens (Kiers et al., 2003; Feng et al., 2007).

At weaning piglets face with abrupt separation from their dam, mixing with other litters, moving to

new environment and switch from highly digestible milk with high levels of nucleotides (Mateo et al., 2004) to a less digestible commercial feed (Lalles, 2008). It is highlighted that insufficient nucleotide concentrations (Martinez-Puig et al., 2007) reduces feed intake and growth performance (Salobir et al., 2005; Kim et al., 2010). Nucleotides are also known to prevent oxidative stress (Salobir et al., 2005), diarrhea (Martinez-Puig et al., 2007), and increased requirement during times of rapid growth or stress (Carver & Walker, 1995; Frankič et al., 2006).

More recently, the roles of functional amino acids (FAA) in supporting the immune system, maintaining the intestinal mucosal barrier, regulating the antioxidant defence, and synthesizing immune molecules have received greater attention (Le Floc'h et al., 2018). It has been suggested, therefore, that supplementation with key FAA may mitigate the negative effects of stressors. Supplementing Met, Thr, and Trp at a higher ratio than requirement has been suggested to modulate the immune status of grower pigs under poor sanitary conditions (van der Meer et al., 2016) or in weaned pigs challenged with enteric diseases such as Salmonella (Rodrigues et al., 2021).

Considering all potential benefits, we hypothesized that the combination of highly digestible Bacillus-FSBM, nucleotide, and functional amino acids blend could be used in young pigs' diets to replace conventional plant protein sources without adversely affecting pigs' growth. This commercial field test was conducted to evaluate the effect of this combination product on growth performance in weaning piglets.

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### **Material and Methods**

The experimental protocols describing the management were prepared and reviewed by following the guidelines of the ethics committee

#### Animal, experimental design and housing

In the present experiment a total of 100 weaning piglets [Duroc × (Yorkshire × Landrace)] with an average body weight of  $8.28 \pm 0.97$  kg were selected for a 5-wk feeding trial. Pigs were randomly allocated into 2 dietary treatments with 50 pigs per treatment. Dietary treatments included:

Items	Diets
CON	Basal diet
TRT	Replace 5% of fermented soybean meal in the CON diet with 5% of a combination of high digestible Bacillus-fermented soybean meal with nucleotide, and functional amino acids blend



# Results

#### Growth performance

The performance response of pigs is presented in Table 1. The final average body weight was increased by 13.80%, which equals 2.54kg higher in weaning pigs fed TRT diet compared to CON diet after 35 day-feeding trial. Feeding weanling pigs with TRT diet had a greater ADG and ADFI by 24.433% and

17.65%, respectively, compared to those fed a CON diet after 35 day-feeding trial. The trial results also show that the FCR was decreased by 5.5% in weanling pigs fed the TRT diet compared to those fed the CON diet after 35 day-feeding trial.





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The economic benefit of feeding the treatment diet was calculated based on pigs' total feed cost and growth rate (Table 1). The feeding treatment diet provided \$4.04 additional profit over the control feed due to increased growth of pigs albeit the treatment diet has increased total feed cost per pig.

Table 1.	Effect of treatment diet on economic benefit calculated based on the
	total feed cost and growth of pigs

Expense	CON	TRT
Feed price (\$/kg)	0.696	0.716
Feed intake (kg, 35days)	17.01	19.985
Total feed cost (\$/pig)	11.839	14.309
Save cost (\$, A)		-2.47
Live weight (kg)	18.40	20.94
Live hog price (2.565 \$/kg)	47.196	53.7111
Additional profit (\$, B)		6.5151
Total additional profits (\$/pig, A+B)		4.045
1,000 pigs farm profit (\$, 35 days)		4,045
1,000 pigs farm profit (VND, 35 days)		93,030,400

### Discussion

Soybean meal (SBM) is the major source of protein in swine diets. However, its application in piglet diets is limited due to the presence of anti-nutritional factors and antigenic proteins, which interfere with digestion, absorption and utilization of nutrients (Holm et al., 1992; Hong et al., 2004). Plasma protein, fish meal or dried porcine intestinal cells are better protein resources, but they are expensive. Thus, this commercial field test was conducted to evaluate the combination of highly digestible fermented soybean meal by *B. subtilis*, nucleotide, and functional amino acids for weanling pigs to reduce the gap between plant-derived protein and animal-derived protein.

The fermentation of SBM by *Bacillus subtilis* in this study increased CP concentrations (48 to 55%) without affecting concentrations of the major limiting AA for pigs, increased proportion of the low-molecular-weight-peptide (<30 kDa) from 20% to 67.3%, which can be beneficial to newly weaned pigs with potentially limited gastric HCl secretion needed for protein digestion.

Fermentation of SBM by *Bacillus subtilis* also helps to reduce anti-nutritional factors, such as glycinin and  $\beta$ -conglycinin because most of the subunits composing glycinin and  $\beta$ -conglycinin are greater than 20 kDa (Rickert et al., 2004; Deak et al., 2006). Glycinin and  $\beta$ -conglycinin are potential antigenic and allergenic compounds (Holzhauser et al., 2009) for newly weaned pigs, causing villus atrophy and crypt hyperplasia in the small intestine (Li et al., 1990); thus, reduction of glycinin and  $\beta$ -conglycinin in SBM can be beneficial to nursery pigs.

In addition, soybean oligosaccharides including stachyose and raffinose are also named flatulence factors, because they cannot be digested by the animal in the intestine and are fermented by microbes in the gastro-intestine which can cause flatulence and diarrhea (Zhang et al., 2003). The presence of soybean oligosaccharides can reduce nutrient digestibility by increasing osmolality in the gut and the passage rate of nutrients through the intestine (Wiggins, 1984). Including stachyose and raffinose in the diets also can cause intestinal disor-



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ders in weaning piglets because pigs do not have endogenous enzymes capable of digesting specific oligosaccharides (Veldman et al., 1993). It was observed that supplementation of 1% pure stachyose stimulated lactobacillus spp., however, adding 2% pure stachyose significantly reduced intestinal lactobacteria and bifidobacteria (Zhang et al., 2003). The author also observed that supplementation of stachyose at an inclusion level of 1 and 2% reduced the growth rate of weanling piglets. Smiricky et al. (2002) observed that the addition of soy solubles containing a high amount of raffinose and stachyose to a soy protein concentrate-based diet reduced N and AA digestibility in growing pigs. The recommended level of soybean galactooligosaccharides (stachyose+raffinose) in weaning piglets diet is not to exceed 1% when SBM is used as the main protein source (Choct et al., 2010). Therefore, removing stachyose and raffinose by fermentation with Bacillus subtilis was one of the efficient ways to increase the nutritional value of soybean meal. Trypsin inhibitor concentration in SBM can also be reduced after fermentation (Hong et al., 2004), which can improve protein digestion in the small intestine. Albeit we did not measure nutrient digestibility or intestinal viscosity, reducing galacto-oligosaccharides partly contributed to the improved performance in the treatment group.

In the postweaning period, high undigested protein can predispose pigs to postweaning diarrhea and proliferation of enteric pathogens (Rist et al., 2013). It is reported that 74% of the reduction in growth due to enteric pathogen challenge was due to feed efficiency (i.e., nutrient utilization) and not due to the decrease in feed intake. It has been shown that immune stimulation increases requirements for selective amino acids for growth, including methionine and cysteine (Litvak et al., 2013; Rakhshandeh et al., 2014), threonine (Jayaraman et al., 2015; Wellington et al., 2018), and tryptophan (Le Floc'h et al., 2009; de Ridder et al., 2012), suggesting that supplementation with these AAs may improve performance in disease-challenged pigs. Wellington et al. (2019) reported that supplementing dietary threonine at 20% above requirements for growth improved the growth performance of 22 kg pigs.

In addition, support for an altered AA profile during disease challenge is provided by the observation that the positive effects of dietary AA supplementation on ADG and feed efficiency of *Salmonella* Typhimurium-inoculated pigs were achieved without a concurrent increase in ADFI. This is in line with the results of a meta-analysis performed by Pastorelli et al. (2012) which showed the immune stimulation increases the requirements of threonine (Wellington et al., 2018), methionine (Litvak et al., 2013), and tryptophan (de Ridder et al., 2012) for growth.

Recently, both the dietary protein content and the supplementation of methionine, threonine, and tryptophan have been shown to modulate the immune status of grower pigs under poor sanitary conditions (van der Meer et al., 2016). It is also reported that diet supplementation with key FAA, specifically threonine, methionine, and tryptophan, above estimated requirements for growth improves growth performance and immune status of pigs under Salmonella Typhimurium challenge (Rodrigues et al., 2021). Collectively, the improved performance seen in the current study is explained, at least partly by the functional effects of additional amino acid blends in commercially hosed pigs where the presence of environmental and bacterial challenges is the norm.

Nucleotides are low-molecular-weight, intracellular compounds that are functional in numerous biochemical processes. They are essential for regenerating cells, particularly those in the immune system and gastrointestinal tract (Wang et al., 2008). In healthy conditions dietary nucleotides are probably not essential, however, during the period affected by stress, disease or limited nutrient intake, dietary nucleotides may become essential (Dancey et al., 2006). Typically, substantial amounts of nucleotides are obtained through de novo synthesis, but supplemental nucleotides may be beneficial during times of rapid growth or stress (Carver & Walker, 1995; Frankič et al., 2006). Before weaning, piglets consume high levels of nucleotides from sow's milk (Mateo et al., 2004). However, weaning diets may have insufficient nucleotide concentrations for growth and health. At this point, piglets no longer receive a nucleotide



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source from their mother's milk but demand remains high; Therefore, nucleotide demand increases during periods of stress and rapid growth, such as when piglets are weaned and need nucleotides for maintenance, growth, intestinal development, immune system support, and reducing oxidative stress (Mateo, 2005; Salobir et al., 2005; Sauer et al., 2012). Weaver and Kim, (2014) have shown that dietary supplementation of the nucleotide as a supplement improved the gain and feed intake of the newly weaned pigs. Consumption of the nucleotides also resulted in a tendency for decreased systemic inflammation and oxidative stress as measured by TNF $\alpha$  and 8-OHdG. It is speculated that nucleotides act on the immune system through their interaction with T cells and eventually increase feed intake and growth performance.

### Conclusions

Weaner pigs fed a combination of highly digestible Bacillus-fermented soybean meal with nucleotides, and functional amino acids increased final body weight, ADG, ADFI by 13.8%, 24.4%, 17.6%, respectively, and decreased FCR by 5.5%. The growth-promoting effects observed in this commercial farm study may be attributed to the synergistic effect of a combined supplementation of 1) Bacillus-fermented soybean with highly digestible low-molecular-weight peptide, the eliminated anti-nutritional factors during fermentation, and the beneficial microbes existing in FSBM to inhibit the pathogenic microbial proliferation; 2) Functional amino acids supplemented above the estimated requirements to enhance mucosal immunity, antioxidant capacity and block the inflammatory cell signaling; and 3) Nucleotide supplementation to support rapid epithelial cell regeneration. As demonstrated in this particular commercial farm study the combination of FSBM, functional AAs blend, and nucleotides could be a potential protein source that can replace other protein sources.

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