Evaluation of Spray-Dried Porcine Plasma for Growth Performance, Production Yield, Immune Responses, and Total *Vibrio* Counts in Pond-Reared Giant Tiger Prawn (*Penaeus monodon*)

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ABSTRACT

Spray-dried porcine plasma (SDP) is a protein-rich feed ingredient derived from animal blood with potential health benefits. This study evaluated the effects of dietary SDP on growth, survival, and immune responses in pond-reared giant tiger prawn (*Penaeus monodon*). Six earthen ponds in a commercial farming system in Prachuap Khiri Khan Province, Thailand, were stocked with postlarvae at a density of 750,000 shrimp·ha^{-1} (75 shrimp·m^{-2}). Three ponds received a standard commercial diet (Control group), while the other three ponds were fed a pelleted diet containing 4.5% SDP (SDP group) for 30 days (days 30–60 post-stocking), after which all ponds were fed a standard diet until harvest. The inclusion of SDP improved feed conversion ratio (FCR) compared with the control group. Shrimp fed the SDP diet showed significant ($p<0.05$) increases in immune parameters and reduced total *Vibrio* counts in both the hepatopancreas and intestine. Unexpectedly, an outbreak of acute hepatopancreatic necrosis disease (AHPND) occurred on day 80, affecting all control ponds and one SDP pond, while the other two SDP ponds remained AHPND-negative. This suggested a potential protective effect of SDP. Although the AHPND outbreak limited conclusive assessment of growth and production, the observed improvement in FCR, immunity, and in *Vibrio* reduction indicates that dietary SDP may enhance shrimp health under farm conditions.

Keywords: Acute hepatopancreatic necrosis disease, Functional feed additive, *Penaeus monodon*, Shrimp health management, Spray-dried porcine plasma

INTRODUCTION

The giant tiger prawn (*Penaeus monodon*) is a commercially important crustacean that plays a significant role in aquaculture across Asian countries. However, the intensification of shrimp farming practices has increased the susceptibility of farmed populations to infectious diseases, particularly *Vibrio* infections, which represent a major obstacle to the sustainability and profitability of the sector (Chanratchakool *et al*., 1998; De Souza Valente and Wan, 2021; Haifa-Haryani *et al*., 2023).

Given the impact of bacterial diseases such as vibriosis on shrimp farming, the development of effective and sustainable disease management strategies is crucial. While improving biosecurity protocols and pond management practices is essential, nutritional approaches that enhance the innate immunity and host resilience provide a promising alternative. In this context, functional feed components that influence immune function and gut health are increasingly regarded as an alternative to conventional methods, such as antibiotic use, which raised concerns about bacterial resistance

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and potential environmental impact (Dawood *et al*., 2018; Rairat *et al*., 2022; Qiao *et al*., 2023).

Spray-dried porcine plasma (SDP) is a protein-rich by-product derived from the meat industry. It consists of functional proteins such as immunoglobulins, albumin, transferrin, bioactive peptides, and growth factors (Pérez-Bosque *et al*., 2016). In terrestrial animals, particularly weaned piglets, dietary SDP has been consistently linked to health benefits. These include improved growth performance, higher feed intake, enhanced nutrient absorption, better gut health, and reduced inflammation and disease susceptibility (Moretó and Pérez-Bosque, 2009; Peace *et al*., 2011; Pérez-Bosque *et al*., 2016; Kazimierska and Biel, 2023). Such benefits are largely attributed to its immunomodulatory components, especially immunoglobulins, which support gut barrier integrity and modulate mucosal immune responses (Moretó and Pérez-Bosque, 2009; Pérez-Bosque *et al*., 2016).

Beyond terrestrial animals, research on certain fish species, such as Nile tilapia (*Oreochromis niloticus*) (De Araújo *et al*., 2017) and gilthead sea bream (*Sparus aurata*) (Gisbert *et al*., 2015; Tapia-Paniagua *et al*., 2020; Fernández-Alacid *et al*., 2022), has also reported positive effects of dietary SDP on growth, feed efficiency, and immune function.

Although the advantages of SDP in animal production are well established (Moretó and Pérez-Bosque, 2009; Torrallardona, 2010; Pérez-Bosque *et al*., 2016; Campbell *et al*., 2019; Balan *et al*., 2021; Kazimierska and Biel, 2023), studies on shrimp aquaculture remain scarce. A preliminary trial suggested potential benefits for growth and survival in giant tiger prawn (Russell, 2000). More recently, laboratory studies with Pacific white shrimp (*Litopenaeus vannamei*) demonstrated that dietary inclusion of 4.5–6% porcine SDP enhanced growth, survival, feed efficiency, immune parameters, and resistance to *Vibrio parahaemolyticus* (Chuchird *et al*., 2021). Similarly, Cano *et al*. (2023) reported that laboratory-reared Pacific white shrimp fed pelleted feed containing 3% bovine SDP exhibited significantly improved body weight, survival rates, and total hemocyte counts compared to those on a control diet. Nevertheless, information on shrimp responses to SDP under pond-based commercial conditions remains limited.

This study was conducted in a commercial farming environment, thereby incorporating environmental variability and the disease pressures typical to shrimp aquaculture. The objectives were to evaluate the effect of dietary porcine SDP on growth performance, production, immune responses, and *Vibrio* counts in the hepatopancreas and intestine of pond-raised giant tiger prawn.

MATERIALS AND METHODS

Experimental conditions

The on-farm trial was conducted at a commercial giant tiger prawn farm in Prachuap Khiri Khan Province, Thailand. Six earthen ponds, each ranging in size from 0.4 to 0.64 ha, were used for the experiment. Postlarvae (PL 10–12 stage, approximately 5 mg per shrimp), obtained from a single hatchery, were stocked into the grow-out ponds at a density of 75 shrimp·m^{-2} (750,000 shrimp·ha^{-1}). The animal study protocol was approved by the Kasetsart University Institutional Animal Care and Use Committee (IACUC approval number: ACKU66-FIS-016).

Experimental diets

The six ponds were randomly assigned to two dietary treatments, with three ponds per treatment. In the control group, shrimp were fed a standard commercial pelleted feed throughout the culture period. In the SDP group, shrimp were fed a pelleted feed supplemented with 4.5% porcine SDP (AP 820P, APC Europe, S.L., Granollers, Spain), partially replacing poultry and soybean meal. This inclusion level was selected based on previous findings (Chuchird *et al*., 2021).

Both control and SDP diets were formulated and manufactured by TRF Feedmill Co., Ltd. (Samut

Sakhon, Thailand). For the SDP diet, the plasma powder was mixed with other feed ingredients prior to pelleting. The pellets were produced by steaming at 100 °C for 30 min, followed by air-drying. The ingredient composition and proximate analysis of the two diets are presented in Table 1.

Shrimp in the SDP group received the supplemented diet for 30 days, from day 30 post-stocking. Before and after this period, they were fed the standard commercial feed. Feeding rates were adjusted according to estimated shrimp biomass in each pond, following the farm's standard practice.

Table 1. Ingredients and proximate chemical composition (%) of the experimental diets.

	Control diet	SDP diet
Ingredients (%)		
Spray-dried porcine plasma (SDP)	-	4.50
Shrimp shell meal 45%	4.17	4.17
Fish meal 65% crude protein	3.75	3.75
Fish meal 60% crude protein	7.50	7.50
Fermented soybean meal	2.08	2.08
Squid meat meal	1.67	1.67
Squid viscera	1.46	1.46
Squid liver meal	1.67	1.67
Poultry meal 64%	20.00	17.75
Chicken protein powder	1.25	1.25
Soybean meal high protein	17.03	14.78
Full fat soybean meal	4.38	4.38
Wheat gluten	1.04	1.04
Corn protein concentrate	1.67	1.67
Wheat flour	16.67	16.67
Wheat grain	4.17	4.17
Premix vitamin and mineral	3.89	3.89
L-lysine	0.10	0.10
DL-methionine	0.10	0.10
Additive and preservative	0.54	0.54
Liquid attractant	6.88	6.88
Chemical composition (%)		
Protein (%N ×6.25)	43.94	44.26
Fat (%)	8.30	7.74
Fiber (%)	2.92	2.67
Moisture (%)	9.38	9.85
NaCl (%)	1.39	1.70
Ash (%)	11.07	11.72
Digestibility (%)	80.49	83.49

Note: The feed raw materials were obtained from TRF Feedmill Co., Ltd. (Samut Sakhon, Thailand). Both diets were pelleted and produced at TRF Feedmill Co., Ltd.

Experimental design

After stocking the postlarvae into earthen ponds, all shrimp were initially fed the control diet for 30 days (day 1 to day 30). From day 31 to day 60, shrimp in the SDP group were fed the SDP diet, after which (day 61 onwards) they were switched back to the control diet until the end of the feeding trial. This feeding strategy was intended to minimize production costs while maximizing the benefits of SDP by limiting its use to the period when the risk of disease outbreaks is typically highest. Shrimp in the control group were fed the control diet throughout the entire experimental period.

Shrimp were randomly sampled at three time points: day 30 (pre-SDP feeding), day 60 (post-SDP feeding), and day 75 post-stocking (15 days after cessation of SDP feeding). At each sampling, 20 shrimp were randomly collected from each pond and transported live to the Aquaculture Business Research Center, Faculty of Fisheries, Kasetsart University (Bangkok, Thailand) for immunological assays and *Vibrio* counts. Additionally, on days 30, 60, and 90, another 10 shrimp per pond were sampled for histological examination. Notably, an outbreak of acute hepatopancreatic necrosis disease (AHPND) occurred on day 80 post-stocking (see Results). The day 90 sampling was therefore used to assess histopathological changes in the hepatopancreas and lymphoid organs of the infected shrimp.

At harvest, shrimp body weight, average daily gain (ADG), feed conversion ratio (FCR), survival rate, and production yield per unit area were determined. The indices were calculated as follows:

$$\text{ADG } (g \cdot day^{-1}) = (\text{Final body weight} - \text{Initial body weight}) / \text{Culture period (day)};$$

$$\text{FCR} = \text{Total feed consumed} / \text{Weight gain};$$

$$\text{Survival rate } (\%) = (\text{Number of shrimp at harvest} / \text{Initial number of shrimp}) \times 100$$

Immune parameters

The immune parameters studied consisted of total hemocyte count (THC), phagocytic activity, phenoloxidase (PO) activity, and superoxide dismutase (SOD) activity. Each assay was conducted in two replicates per pond, resulting in six replicates per treatment group.

For THC and phagocytic activity assays, 0.5 mL of hemolymph was withdrawn using a syringe preloaded with 1 mL of anticoagulant (K-199+5% L-cysteine). The K-199 solution contained 1% M-199, 1.88 M NaCl, 0.06 M $CaCl_2 \cdot 2H_2O$, 0.1 M L-glutamine, 9.14 mM HEPES, and a 10% salt mixture (0.05 M KCl, 0.16 M $MgCl_2 \cdot 6H_2O$, 0.12 M $MgSO_4 \cdot 7H_2O$, and 3.2 mM $NaH_4PO_4 \cdot 2H_2O$). Hemolymph from four shrimp was pooled to form one replicate. A 100-µL aliquot was used for THC determination with a hemocytometer under a light microscope. Another 200-µL aliquot was used to assess phagocytic activity following the method of Itami *et al*. (1994). Briefly, hemocytes were rinsed with shrimp saline (28.4 $g \cdot L^{-1}$ NaCl, 1 $g \cdot L^{-1}$ $MgCl_2 \cdot 6H_2O$, 2 $g \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$, 2.25 $g \cdot L^{-1}$ $CaCl_2 \cdot 2H_2O$, 0.7 $g \cdot L^{-1}$ KCl, 2.38 $g \cdot L^{-1}$ HEPES, and 1 $g \cdot L^{-1}$ glucose) and allowed to adhere to cover slips for 20 min. After three rinses with shrimp saline, 2 mL of heat-killed yeast (5×10^8 cells$\cdot mL^{-1}$) was added, and samples were incubated for 2 h. The cover slips were then rinsed five times, fixed with methanol, stained with eosin and methylene blue, and mounted using Permount Mounting Medium. A total of 200 hemocytes per sample were counted, and phagocytic activity was expressed as the percentage of phagocytosing hemocytes relative to the total hemocytes.

For PO activity, 0.5 mL of hemolymph was collected using a syringe preloaded with 1.5 mL of anticoagulant (K-199 + 5% L-cysteine). Hemolymph from three shrimp was pooled to form one replicate. Following Söderhäll and Hall (1984), 200 µL of the hemolymph-anticoagulant supernatant (centrifuged at 10,000 rpm, 4 °C, 10 min) was mixed with 200 µL of 0.1% trypsin in cacodylate buffer. After incubating for 2–3 min at

room temperature, 200 µL of L-DOPA (4 mg·mL^{-1}) was added, and absorbance was measured at 490 nm using a spectrophotometer. Protein concentration was determined following Lowry *et al*. (1951), and PO activity was expressed as ΔA_{490}·min^{-1}·mg protein^{-1}.

For SOD activity, 1 mL of hemolymph was collected using a syringe preloaded with 1 mL of 10% sodium citrate. Hemolymph from three shrimp was pooled to form one replicate. SOD activity was measured using a SOD Assay Kit (SIGMA19160-1KT-F, Sigma-Aldrich, USA), following the manufacturer's protocol.

Total Vibrio counts

The hepatopancreas and intestine were aseptically dissected from sampled shrimp. For each tissue, samples from 10 shrimp were pooled to form one replicate, with six pooled replicates per group (two replicates per pond). Tissues were weighed, homogenized in sterile saline, and serial ten-fold dilutions were prepared. An aliquot (0.1 mL) of each dilution was spread on Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar and incubated at 37 °C for 24 h. Colonies of *Vibrio* spp. were enumerated, and results were expressed as colony-forming units per gram of tissue (CFU·g^{-1}).

Histological analysis

Shrimp were fixed in Davidson's fixative for 24–48 h and processed following standard histological procedures (Bell and Lightner, 1988). Tissues were dehydrated through a graded ethanol series, cleared in xylene, infiltrated and embedded in paraffin wax. Sections (5 µm) were cut using a microtome, mounted on glass slides, stained with hematoxylin and eosin (H&E), and examined under a light microscope.

Water quality parameters

Water quality parameters, including pH, alkalinity, total ammonia nitrogen, nitrite, calcium, and magnesium, were measured biweekly on-farm using commercial test kits. Salinity was determined using a salinometer.

Statistical analysis

Data on immune parameters (THC, phagocytosis, PO, and SOD activities) and total *Vibrio* counts were analyzed using independent-samples t-tests in SPSS version 27 (IBM Corporation, Armonk, NY, USA). Results are presented as mean±standard deviation.

RESULTS

Shrimp production data from each pond are presented in Table 2. Shrimp in all ponds exhibited normal growth until day 80 post-stocking, when significant mortality occurred. Subsequent PCR testing confirmed acute hepatopancreatic necrosis disease (AHPND) in all three control ponds and in pond SDP 3 (data not shown). In contrast, pond SDP 1 and SDP 2 remained unaffected. This disease outbreak inevitably confounds the experimental outcomes, making direct comparisons between dietary treatments difficult.

Regardless of diet, ponds affected by AHPND produced substantially lower yields (2,219–4,419 kg·ha^{-1} or 0.22–0.44 kg·m^{-2}) compared to the healthy ponds (7,563–12,919 kg·ha^{-1} or 0.76–1.29 kg·m^{-2}). Shrimp from the three AHPND-affected control ponds were harvested between days 102–105 post-stocking. Although pond SDP 2 remained free of AHPND, it was located adjacent to pond SDP 3, which was harvested early at day 90 due to disease. To prevent potential economic loss, the farmer decided to harvest SDP 2 at day 100. In contrast, pond SDP 1, which was separated from infected ponds and confirmed AHPND-negative by PCR testing, was maintained until day 150, allowing shrimp to reach marketable size.

The average final body weight varied among ponds, with pond SDP 1 reaching 20.00 g after the longest culture duration. Shrimp in pond SDP 2 averaged 12.50 g, while those in the control ponds ranged between 11.76 and 12.82 g. The lowest average final body weight (10.00 g) was recorded in pond SDP 3, although these differences were not statistically analyzed due to the occurrence of AHPND disease in some ponds. ADG was within

a narrow range of 0.11 to 0.13 g·day^{-1} across all ponds. These observations suggest that differences in production outcomes were more closely related in growth rate.

Although interpretation is complicated by the AHPND outbreak, the FCR values observed in the SDP-fed ponds (1.11–1.67) tended to be lower than those in the control ponds (1.76–2.18). This may indicate a possible improvement in feed utilization associated with the inclusion of SDP in the diet. Nevertheless, given that the real difference of FCR between the control and SDP groups could not be statistically evaluated, the apparent improvement in the SDP group should be treated with caution.

Shrimp immunity was assessed as summarized in Table 3. Prior to SDP feeding, all immune parameters were comparable between the two groups. After 30 days of SDP supplementation, THC (8.37±2.73×10^7 cells·mL^{-1}), phagocytosis (71.67±1.25%), PO activity (77.00±21.36 ΔA_{490}

Table 2. Growth performance, feed efficiency, survival, production yield, and harvest day of giant tiger prawn (*Penaeus monodon*) reared in ponds under control and spray-dried porcine plasma (SDP) dietary treatments. Ponds marked with an asterisk (*) were affected by acute hepatopancreatic necrosis disease (AHPND) on day 80 post-stocking.

Pond	Body weight	Average daily gain (ADG)	Feed conversion ratio (FCR)	Survival rate	Production yield	Harvest day
Control 1*	11.90 g	0.12 g·day^{-1}	1.76	41.26 %	3,681 kg·ha^{-1} or 0.37 kg·m^{-2}	102
Control 2*	12.82 g	0.12 g·day^{-1}	2.18	45.96 %	4,419 kg·ha^{-1} or 0.44 kg·m^{-2}	105
Control 3*	11.76 g	0.11 g·day^{-1}	1.80	33.45 %	2,950 kg·ha^{-1} or 0.30 kg·m^{-2}	105
SDP 1	20.00 g	0.13 g·day^{-1}	1.67	86.14 %	12,919 kg·ha^{-1} or 1.29 kg·m^{-2}	150
SDP 2	12.50 g	0.13 g·day^{-1}	1.38	80.64 %	7,563 kg·ha^{-1} or 0.76 kg·m^{-2}	100
SDP 3*	10.00 g	0.11 g·day^{-1}	1.11	29.58 %	2,219 kg·ha^{-1} or 0.22 kg·m^{-2}	90

Table 3. Immune responses of giant tiger prawn (*Penaeus monodon*) fed control and spray-dried porcine plasma (SDP) diets at different sampling times.

Pond	Day 30 (pre-SDP feeding)	Day 60 (during SDP feeding)	Day 75 (post-SDP feeding)
	Total hemocyte count (10^7 cell·mL^{-1})		
Control group	5.18±0.30^a	5.89±1.95^b	5.43±0.19^a
SDP group	5.55±0.24^a	8.37±2.73^a	5.89±0.07^a
	Phagocytosis (%)		
Control group	59.56±5.57^a	61.78±3.62^b	58.00±2.23^a
SDP group	58.00±2.23^a	71.67±1.25^a	58.00±1.40^a
	Phenoloxidase activity (ΔA_{490}·min^{-1}·mg protein^{-1})		
Control group	59.81±11.98^a	51.25±4.97^b	52.10±5.30^a
SDP group	54.92±9.52^a	77.00±21.36^a	62.60±12.42^a
	Superoxide dismutase (% inhibition)		
Control group	45.57±5.25^a	47.52±5.25^b	47.78±4.88^a
SDP group	46.56±4.66^a	55.03±4.82^a	47.66±5.10^a

Note: The data are presented as mean±SD; Different superscripts within a column indicate statistically significant differences ($p<0.05$).

·min^{-1}·mg protein^{-1}), and SOD activity (55.03±4.82% inhibition) in the SDP-fed shrimp were significantly higher than those in the control group (5.89±1.95×10^{7} cells·mL^{-1}, 61.78±3.62%, 51.25±4.97 ΔA_{490}·min^{-1}·mg protein^{-1}, and 47.52±5.25% inhibition, respectively). By day 75 (15 days after cessation of SDP feeding), however, the immune responses of the SDP group had declined to baseline levels and no longer differed significantly from those of the control group.

Total *Vibrio* counts were assessed on days 30, 60, and 75 (Table 4). On day 30 (before SDP feeding), there were no significant differences ($p>0.05$) in *Vibrio* counts between the control and designated SDP groups in either the hepatopancreas or the intestine. On day 60 (after 30 days of SDP feeding), *Vibrio* counts increased substantially compared to the pre- feeding period in both hepatopancreas (31.75±10.76×10^{4} CFU·g^{-1}) and intestine (25.72±9.95×10^{2} CFU·g^{-1}) of the control group. In contrast, the SDP-fed group showed only a slight increase, but values were significantly lower ($p<0.05$) than those of the control shrimp in both hepatopancreas (7.84±3.84×10^{4} CFU·g^{-1}) and intestine (5.13±1.34×10^{2} CFU·g^{-1}). This trend persisted for 15 days after cessation of SDP feeding (day 75). At that time, total *Vibrio* counts in the hepatopancreas of the control and SDP group were 45.83±14.77×10^{4} CFU·g^{-1} and 5.70±2.92×10^{4} CFU·g^{-1}, respectively, and those in the intestine were 20.18±6.52×10^{2} CFU·g^{-1} and 5.88±1.80×10^{2} CFU·g^{-1}, respectively.

Histological examination of the hepatopancreas and lymphoid organ provided insights into shrimp health status. On day 30, the hepatopancreas structure appeared normal in shrimp from all groups (Figure 1). On day 60, the hepatopancreas of the control shrimp contained fewer lipid droplets, whereas those of the SDP group remained unchanged (Figure 1). By day 90 (10 days after the outbreak), the hepatopancreas of shrimp from all three control ponds and the AHPND-positive SDP pond (SDP 3) showed clear signs of atrophy, reduced lipid accumulation, and sloughing of tubule epithelial cells. In addition, prominent lymphoid organ spheroids were observed in all AHPND-affected shrimp but not in SDP 1 and the SDP 2 groups (Figure 2).

For reference, a healthy hepatopancreas displays well-defined tubules with intact epithelium, tightly packed structures, a clear lumen, and no evidence of necrosis, atrophy, or hemocyte infiltration. Similarly, a normal lymphoid organ consists of tubules with a central lumen, stromal matrix cells, and interstitial sinuses.

The ranges of water quality parameters in the six ponds are presented in Table 5. All values were similar between the two groups and generally within acceptable limits for marine shrimp culture (Jory, 2019; Songsangjinda, 2019). Although no statistical comparison was made between the groups, dietary SDP did not appear to influence water quality parameters.

Table 4. Total *Vibrio* counts (mean±standard deviation, n = 6) in the hepatopancreas and intestine of giant tiger prawn (*Penaeus monodon*) fed control and spray-dried porcine plasma (SDP) diets at different sampling times.

Pond	Day 30 (pre-SDP feeding)	Day 60 (during SDP feeding)	Day 75 (post-SDP feeding)
	Hepatopancreas (×10^{4} CFU·g^{-1})		
Control group	6.67±2.19^{a}	31.75±10.76^{b}	45.83±14.77^{b}
SDP group	6.24±2.55^{a}	7.84±3.84^{a}	5.70±2.92^{a}
	Intestine (×10^{2} CFU·g^{-1})		
Control group	4.99±0.56^{a}	25.72±9.95^{b}	20.18±6.52^{b}
SDP group	4.52±0.62^{a}	5.13±1.34^{a}	5.88±1.80^{a}

Note: The data are presented as mean±SD; Different superscripts within a column indicate statistically significant differences ($p<0.05$).

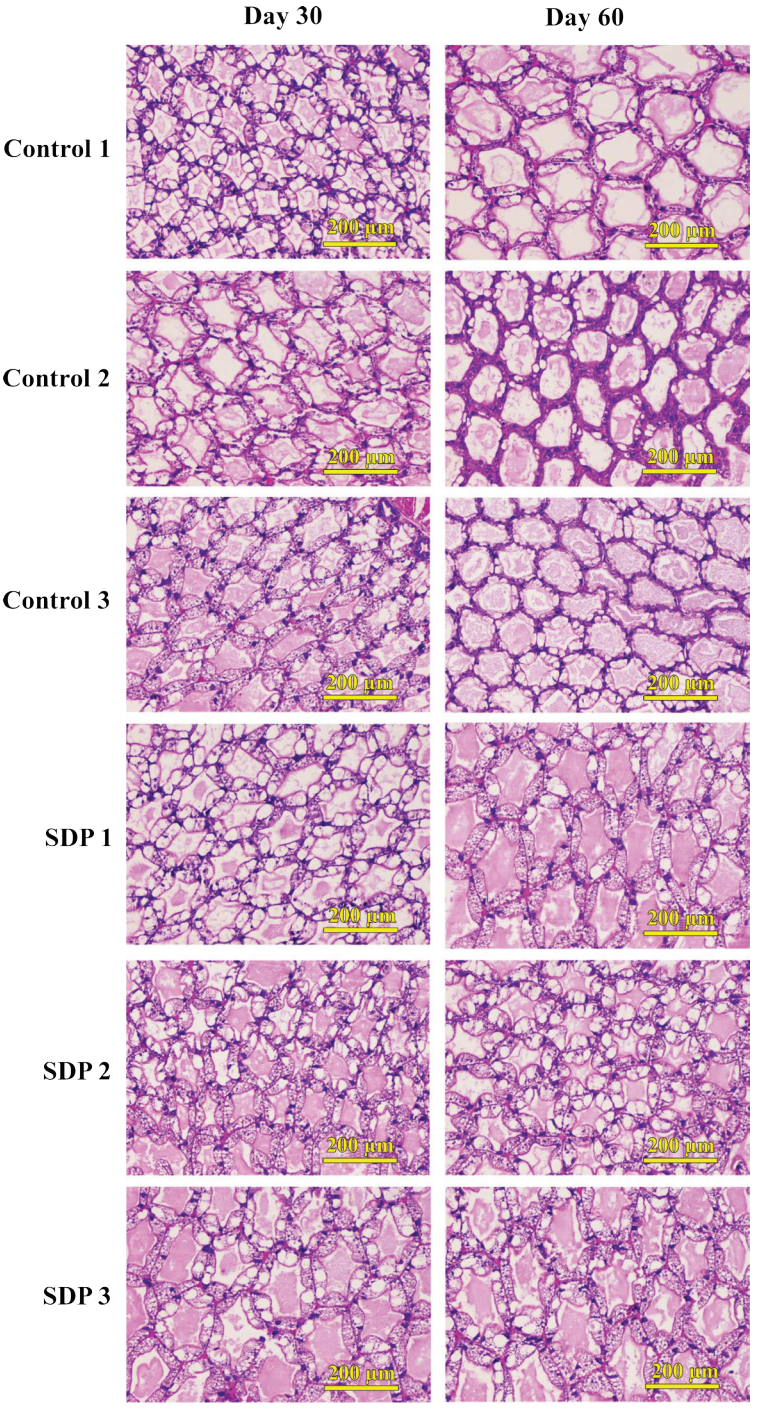

Figure 1. Histological sections of the hepatopancreas of giant tiger prawn (*Penaeus monodon*) from control and spray-dried porcine plasma (SDP) groups on day 30 (pre-SDP feeding) and day 60 (during SDP feeding). Control shrimp exhibited reduced lipid droplets at day 60, whereas the hepatopancreas of SDP-fed shrimp appeared unchanged. Sections were stained with hematoxylin and eosin (H&E). Scale bar = 200 µm.

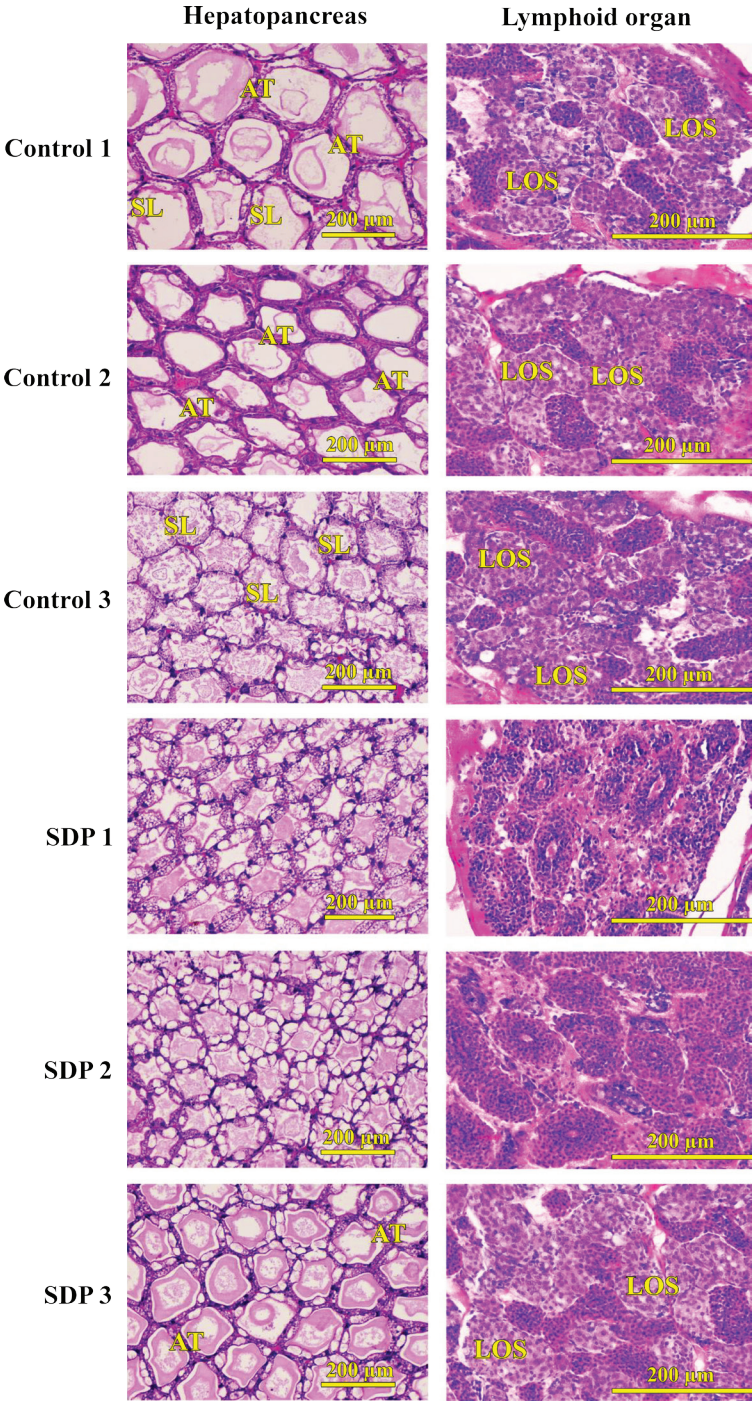

Figure 2. Histological sections of the hepatopancreas and lymphoid organ of giant tiger prawn (*Penaeus monodon*) at day 90 post-stocking. Control ponds and the AHPND-positive SDP pond (SDP 3) showed hepatopancreatic atrophy (AT), epithelial sloughing (SL), reduced lipid storage, and prominent lymphoid organ spheroids (LOS). In contrast, shrimp from SDP 1 and SDP 2 displayed normal hepatopancreatic tubules and lymphoid organs without spheroid formation. Hematoxylin and eosin (H&E) staining; scale bar = 200 µm.

Table 5. Ranges of water quality parameters in ponds during the culture period.

Pond	pH	Alkalinity ($mg \cdot L^{-1}$ $CaCO_3$)	Total ammonia nitrogen ($mg \cdot L^{-1}$)	Nitrite ($mg \cdot L^{-1}$)	Calcium ($mg \cdot L^{-1}$)	Magnesium ($mg \cdot L^{-1}$)	Salinity (ppt)
Control 1	7.6–8.0	85–119	0.50–3.00	0.0–0.01	280–440	825–1175	35–41
Control 2	7.7–8.0	119–153	0.00–3.00	0.0–0.50	280–440	850–1225	36–40
Control 3	7.6–8.0	85–153	0.00–3.00	0.0–0.03	240–480	775–1200	36–41
SDP 1	7.6–8.0	85–119	0.00–0.25	0.0–0.10	280–480	800–1150	33–38
SDP 2	7.5–8.2	51–153	0.00–3.00	0.0–1.0	320–560	775–1150	35–36
SDP 3	7.6–8.1	85–136	0.00–0.10	0.0–0.50	240–440	750–1050	35–39

DISCUSSION

The advantages of dietary SDP in aquafeeds for fish (Gisbert *et al*., 2015; De Araújo *et al*., 2017; Tapia-Paniagua *et al*., 2020; Fernández-Alacid *et al*., 2022) and shrimp (Chuchird *et al*., 2021; Cano *et al*., 2023) have been increasingly recognized. However, most of these studies were conducted under laboratory conditions. While such experiments provide compelling evidence of SDP's efficacy, practical validation under commercial farming environments remains necessary. Building on our previous laboratory study in shrimp (Chuchird *et al*., 2021), the present work was designed to evaluate whether similar benefits occur under farming conditions.

Our findings demonstrated positive effects of dietary SDP (4.5%) on feed efficiency and shrimp health, broadly consistent with earlier studies in Pacific white shrimp (Chuchird *et al*., 2021; Cano *et al*., 2023). In those studies, optimum SDP inclusion rates were reported as 4.5–6% (Chuchird *et al*., 2021) and 3% (Cano *et al*., 2023). Similarly, effective dietary concentrations of SDP in fish feed were generally in the range of 3–6% (Gisbert *et al*., 2015; De Araújo *et al*., 2017; Tapia-Paniagua *et al*., 2020; Fernández-Alacid *et al*., 2022). Higher inclusion levels may not be advantageous. For example, pacu (*Piaractus mesopotamicus*) fed 13% SDP showed no growth improvement (Bacchetta *et al*., 2020), while olive flounder (*Paralichthys olivaceus*) fed 19.7% SDP exhibited reduced body weight (Sim *et al*., 2023), likely due to excessive replacement of fishmeal.

The beneficial effects of SDP may be attributed to its high digestibility and nutritional value (Bureau *et al*., 1999; Cheng *et al*., 2004; Kazimierska and Biel, 2023), as well as improved gut health and reduced proinflammatory cytokine activity (Moretó and Pérez-Bosque, 2009; Torrallardona, 2010; Campbell *et al*., 2019; Balan *et al*., 2021). I mmunoglobulins, especially IgG, are considered key active components (Pierce *et al*., 2005; Pérez-Bosque *et al*., 2006; Pérez-Bosque *et al*., 2010). By binding to bacterial toxins or lipopolysaccharide (Han *et al*., 2009; Hedegaard *et al*., 2016; Jung *et al*., 2017), IgG reduces pathogen activity, thereby conserving host energy for growth and nutrient assimilation. Consistent with previous reports, the beneficial effects of SDP are often more pronounced in stressed or diseased animals than in healthy ones (Coffey and Cromwell, 1995; Pérez-Bosque *et al*., 2016; Chuchird *et al*., 2021).

Thermal processing during pelletization could potentially degrade IgG; however, the observed health benefits suggest that degradation was limited, or that other heat-stable compounds contributed to the effects. Some studies indicate that sugars and amino acids may help stabilize Ig during heating (Chen *et al*., 2000; Jaradat and Marquardt, 2000; Indyk *et al*., 2008). In addition, improved performance in trout fed diets containing SDP either before or after extrusion (Campbell *et al*., 2014) suggests that functionality can be retained. Nevertheless, further study is needed to determine how commercial feed processing affects IgG stability and whether other bioactive compounds play a central role.

The immunomodulatory effect of SDP has been reported in several studies (Gisbert *et al*., 2015; De Araújo *et al*., 2017; Chuchird *et al*., 2021; Fernández-Alacid *et al*., 2022; Cano *et al*., 2023), even though the exact mechanisms remain unclear. In the present study, shrimp fed a 4.5% SDP diet for 30 days showed significant enhancement of innate immune parameters. However, the effect was transient, with THC, phagocytosis, PO, and SOD activity returning to baseline within 15 days of feeding cessation. This suggests that continuous dietary inclusion may be required to maintain elevated immune function. Further studies should examine gene expression profiles of immune-related molecules (e.g., lectin, prophenoloxidase, lysozyme, catalase) to provide a clearer understanding of shrimp immune responses.

The reduction of *Vibrio* counts in the hepatopancreas and intestines of SDP-fed shrimp indicates a possible link between improved immunity and bacterial suppression. Direct antimicrobial effects of IgG in SDP (Han *et al*., 2009; Hedegaard *et al*., 2016; Jung *et al*., 2017) may also have contributed. Interestingly, the lower *Vibrio* counts persisted even at 15 days after SDP feeding ceased, which may reflect improved gut barrier function or shifts in the microbiota that competitively exclude *Vibrio*. This speculation, however, requires further study.

As in many field studies, experimental variability and unexpected disease outbreaks could not be controlled. An AHPND outbreak occurred on day 80, resulting in major losses in the affected ponds (all three control ponds and one SDP pond). This inevitably confounded growth and yield comparisons, but feed utilization benefits were still evident, as shown by lower FCR values and greater lipid storage in the hepatopancreas of SDP-fed shrimp. Notably, two SDP ponds were not affected by the outbreak, suggesting a possible protective effect under farm conditions. While this observation is consistent with laboratory challenge results in Pacific white shrimp (Chuchird *et al*., 2021), further controlled studies are required to confirm whether dietary SDP enhances resilience to AHPND in pond culture.

CONCLUSIONS

This study demonstrated that dietary inclusion of 4.5% SDP for 30 days, prior to pelleting, improved FCR, enhanced immune responses, and reduced *Vibrio* spp. in the hepatopancreas and intestines of pond-raised *Penaeus monodon*. Although the AHPND outbreak limited assessment of long-term growth and survival, the successful harvest from two SDP-fed ponds suggests that SDP may provide some degree of protection against disease under commercial farming conditions.

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