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THE EVALUATION OF PROTEIN DIGESTIBILITY, SAPONIN AND TRYPSIN INHIBITOR CONTENT IN PACIFIC WHITE SHRIMP *(Litopenaeus vanamei)* FEED, DIGESTED WITH BROMELAIN CRUDE EXTRACT FROM PINEAPPLE WASTE

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Abstract: The present study aimed at investigating the effect of varying concentrations of bromelain crude extract from pineapple on percentage of protein digestibility and amount of saponin, and trypsin inhibitor in pacific white shrimp feed. The bromelain was extracted from the crown and peel of pineapple (Bhattavia strain) at dark green ripening stages. The trial was divided into two experiments; the first experiment determined the optimal condition for in vitro protein digestibility of pacific white shrimp feed which contains 38% of crude protein. It was digested by bromelain crude extract at different pH (6 – 9), hydrolysis time (5, 10, and 30 min), and temperature (25 and 30 °C). The second experiment investigated the saponin and trypsin inhibitor (TI) in white shrimp feed after being digested with different concentrations of bromelain crude extract at 0, 90, 170, and 250 ppt for 5, 10, and 30 min at 30 °C. The results presented that the optimal condition for protein digestion with bromelain was pH 6 at 25 °C for 5 and 30 min, the percentage of protein digestion was 63.15 and 70.66% ($P<0.05$). Besides, saponin content in the diet was varied after the bromelain levels and hydrolysis time, the highest level of saponin was found in digested diet with bromelain at 170 and 250 ppt for 30 min (1.84 and 1.88 mg/g feed) and the lowest saponin at 90 and 170 ppt for 5 min. (0.94 and 0.99 mg/g feed) ($P<0.05$). The TI content in the diet was varied inversely with the levels of bromelain at 5 – minute length which the lowest level of TI was shown in digested feed with bromelain at 250 ppt (0.008 mg/g feed) ($P<0.05$) whereas TI was varied with bromelain at 10 and 30 - minute lenght which the lowest level was shown at 0 ppt (0.0031 and 0.007mg/g feed) ($P<0.05$). This study showed that the optimal condition for protein digestion with bromelain was pH 6 at 25°C for 5 and 30 min, and the suitable level of bromelain and hydrolysis time to inactivate the saponin and trypsin inhibitor in shrimp feed were 250 ppt at 5 min.

Keywords: bromelain, *In vitro* digestibility, saponin, trypsin inhibitor, pacific white shrimp

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Introduction

From the past throughout the future, feed is still the main cost of animal production, especially in aquaculture industry. The high protein level in feed is the cause of the high feed price because the protein requirement for shrimp is also high. Furthermore, a high-quality protein source is fishmeal, which provides high-quality and quantity of essential amino acid, and is easy to digest, including the palatability attribute (Gasco *et al*., 2018). This reason made fish meal is still the most requirement as a protein source for all animal feed which opposite of quantity of global fish meal that line to decrease. On the contrary, it increases the price of fish meal and aquafeed. Thus, the aquafeed industry has been attempting to find the key in reducing feed cost and replacing fishmeal in feed formulation.

Nowadays, plant protein is still popular to substitute fishmeal, especially soybean meal, which is used in the most proportion in feed formulas. Soybean meal is well known to provide the highest protein compared with other plant proteins and was produced in a tremendous volume (Dei, 2011). With the high protein and some amino acid contained more than other plant protein, that why soybean was used in a large amount. However, soy bean has more complicated protein digestion and less limiting amino acids (Lysine and Methionine) than fishmeal. This made the limitation for soybean to replacement fish meal. Crude aqueous extract from pineapple waste was extracted from peel and crown is the alternative key to solve the complicated digestion problem. Because the outstanding substance of crude extracted, bromelain, is well known that plant protease. Bromelain is a mixture of different thiol endopeptidase and other components. Bromelain is a mixture of different thiol endopeptidases and other components like phosphatase, glucosidase, peroxidase, cellulase, escharase, and several protease inhibitors (Bhattacharyya, 2008 and Chakraborty *et al*., 2021). Bromelain is a fusion of sulfur-containing enzymes. It contains other elements in smaller portions and is used as an anti-inflammatory agent, though scientists have also discovered its potential as an anticancer and antimicrobial agent. It has been reported as having positive effects on the respiratory, digestive, and circulatory systems and potentially on the immune system (Chakraborty *et al*., 2021). Thus, bromelain from pineapple waste crude extracted could resolve the complicated protein digestibility from plant protein in shrimp feed. In addition, the aqueous crude extracted consisted the other component such as saponin. Saponin was found in pineapple peel extracted by dissolved in ethanol and presented the maximum zone of inhibition against all the test pathogens. This guarantees that pineapple can be used as an antimicrobial agent to protect from selected plant and animal pathogens (Amalia *et al*., 2020). Furthermore, the active antimicrobial compounds in *Ananas comosus* L. Merr include saponin and bromelain, which work as antimicrobial through membranolytic properties (Zharfan *et al*., 2017). Moreover, both saponin and bromelain promote nutrient absorption, digestive capacity, and growth performance and provide the positive effects of saponins in the shrimp immune system and its resistance to pathogens (Acosta, 2019). Thus, they qualify both substances in aqueous crude extract from pineapple byproduct presented the positive effected on shrimp production. Nevertheless, the pineapple contained some anti–nutrition factors such as bromelain inhibitor VI from pineapple stem (BI-VI). The BI-VI in aqueous solution shared similar folding and disulfide bond connectivities not with cystatin superfamily inhibitors that inhibit the same cysteine proteinases but with the Bowman−Birk trypsin/chymotrypsin inhibitor from soybean (BBI-I). BBI-I is similar to BBI-I suggest that they have two independent inhibitory sites toward the serine proteinases trypsin and chymotrypsin (Hatano *et al*., 1996).

Furthermore, anti – nutrition factor is the primary factor for soybean utilization in aquafeed, especially trypsin inhibitor, although it was eliminated in producing process by heat. Soybean Trypsin Inhibitor (S.B.T.I.) has the function to the inhibitor of trypsin and chymotrypsin, this highly stable proteinaceous inhibitor can resist the heat treatment during the drying process feed fabrication. Therefore the molecule is often present in its active form in commercial shrimp feed (Rojo-Arreola *et al*., 2019; Lemos *et al*., 2000 and Maytorena-Verdugo *et al*., 2017). Soybean Trypsin Inhibitor (S.B.T.I.) binds both trypsins and chymotrypsins of *Litopenaeus vannamei* with 80–90% inhibition at 0.25 mM (Rojo-Arreola *et al*., 2019; Hernandez-Cortes *et al*., 1997; Sainz *et al*., 2004; Maytorena-Verdugo *et al*., 2017). However, shrimp respond to phenotypic plasticity in the digestive system of L. vannamei as an adaptive response to compensate the protein digestion capacity when some of the peptidase activities are reduced by the presence of protease inhibitors (Rojo-Arreola *et al*., **2019**). Therefore, this study investigated the feasibility and optimal condition of crude aqueous extract from pineapple byproducts action to improve the protein digestibility and to be a basic data for considering to modify soybean meal to be a perfect protein source.

Materials and Methods

Sample preparation

Shrimp feed (Inteqc® Samutsakhon, Thailand) contains 38% crude protein was ground which sieved past 200 mm and weighted. Defat the sample by Ether extraction with the Soxhlet extraction method (A.O.A.C. 2000) to decrease the interfere on protein digestion from lipid. The sample was dried with a hot air oven at 60oC for 30 min. Keep the sample in a zip lock bag at 4oC until use according to the Fageer *et al*. (2004) method.

Enzyme preparation and activity assay

Bromelain crude extract from pineapple was used crown and peel of pineapple (Batavia strain). The pineapple (*Ananus comosus* L.) ("Batavia") was collected from a plantation in the Petchaburi province, Thailand. The crown and peel were separated and weighed before being chopped into small pieces. The fruit was cleaned after that, air-dried and weighted. The sample of crown and peel were blended with a multipurpose blender with cold distilled water at a 0.5:1 ratio for 5 min, and the blended liquid was filtered through a cloth sheet and then centrifuged at 10,000 x g at 4 oC for 20 minutes followed by Ketnawa *et al*. (2012). The supernatant (crude extract) will be collected to determine the in vitro protein digestibility according to the Fageer *et al*. (2004) method and specific activity assay followed the method according to Ketnawa *et al*. (2012).

The in vitro protein digestibility

The in vitro protein digestibility was determined according to the Fageer *et al*. (2004) method with slight modification. The samples **(**shrimp feed No.1, 2 and 3 with different sizes were 1, 1.2 and 1.5 mm) were digested with bromelain crude extracted from crown and peel of pineapple (Batavia strain) at pH 6 – 9 and temperature at 25, 30 and 40oC for 24 hours. The dissolved protein before and after digestion was determined with the Bradford assay (Bradford, 1976). The in vitro protein digestibility value is expressed in percentage on protein digestibility. The data were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range tests. A significance level of $P<0.05$ was used.

Saponin and Trypsin inhibitor assay

The shrimp feed was mixed with all No. (1, 2, and 3) at the same ratio (1:1:1) and grind to passes the sieve at 200 µm. The smallest shrimp feed was prepared to detect saponin and trypsin inhibitor contained by weight and digested with bromelain crude extracted at 0, 90, 170, and 250 ppt for 5, 10, 30, and 1 hour. Sample was dried after the finish each time at 80oC for 1 hour. Saponin and trypsin inhibitor was detected followed the method according to Liu (2019) and Chen *et al*. (2014), respectively.

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests at a significance level of $P<0.05$ was used.

Results and Discussion

In vitro protein digestibility results in white shrimp feed with bromelain crude extracted from pineapple showed in Table 1. The protein digestibility showed significant difference ($P<0.05$) among groups. The optimal temperature and pH for bromelain crude extracted digested shrimp feed No.1 was pH 6 at 25^{o}C for 30 minutes and 24 hours were 70.66 and 72.16%. On the contrary, shrimp feed No.2 provides the optimal temperature and pH at 40^{o}C and pH 9 for 24 hours was 65.65%, while the percentage of shrimp feed No.3 was highest at 25 and 30^{o}C at pH 9 for 24 hours were 63.22 and 64.03 % respectively. The ability of digestion of bromelain crude extracted was higher than 50 % and reached 72% means that it can promote protein digestion in shrimp and increase the feed utilization in shrimp. The results pointed out that each No. of shrimp feed is specific to the optimal condition of bromelain crude extracted action. The specific condition results from the various type of feedstuff in shrimp feed, especially protein sources in feed. Because of the different types of protein souse affected the protein digestibility of feed also. The protein source such as fishmeal is perfect protein source because it was easier to digest more than protein source from the animal such as meat and bone powder or plant protein such as soybean meal etc. this results according to Sousa *et al*. (2020), who found that the different of feedstuff effected on difficulty protein digestibility. Furthermore this result similar with the data which found in crustacean from the report of Gimenez *et al*. (2009) that protein digestibility percentage difference in differentiating of various types of feedstuff especially in protein source by the protein digestibility was highest to 92% in feed contained fish meal while it decreases to 83% for feed contain meat and bone as a protein source. In addition, feed contained plant protein such as soybean meal as protein source provides the protein digestibility lowest 63%.

In general, aquafeed contained different feedstuff, including protein sources, which affected protein digestibility and enzyme activity, including the varied carbohydrate and lipid sources from many kinds of feedstuff (Robinson, 2015). According to Sangpradub *et al*. (2013), these data reported that in vivo protein digestibility of fish meal was better than feedstuff from plant or carbohydrate in white shrimp. Excluding from feedstuff, the extraction and purification process of bromelain from pineapple (*Ananas comosus*), which is established in many methods, is one of the factors that affect the different abilities in protein digestion.

Table 1: The comparison of protein digestibility in different shrimp feed No. with bromelain crude extract from pineapple at various pH, temperature, and time.

Temperature/ Feed No.	Time	pH			
		6	7	8	9
No.1 at 25ºC	5 m.	63.15±4.84 ^{a}	37.83±0.71 ^{de}	33.16±1.90 ^{e}	36.09±1.07 ^{de}
	10 m.	58.38±4.08 ^{a}	35.30±3.23 ^{d}	29.55±0.92 ^{ef}	30.55±0.37 ^{def}
	30 min	70.66±5.14 ^{a}	30.03±14.49 ^{de}	37.83±1.05 ^{cd}	34.43±0.10 ^{d}
	1 H.	66.52±2.84 ^{a}	34.77±1.94 ^{e}	42.57±0.48 ^{d}	38.89±1.20 ^{de}
	24 H.	72.16±0.40 ^{a}	44.06±2.22 ^{e}	54.76±2.25 ^{d}	33.25±2.24 ^{g}
No.1 at 30ºC	5 m.	33.85±2.71 ^{de}	17.37±0.74 ^{g}	16.60±1.36 ^{g}	37.64±0.84 ^{de}
	10 m.	34.79±1.38 ^{de}	17.55±1.85 ^{g}	16.24±0.56 ^{g}	34.92±0.56 ^{de}
	30 min	33.69±2.35 ^{de}	14.36±2.71 ^{g}	17.43±2.52 ^{fg}	36.82±0.96 ^{cd}
	1 H.	31.95±1.89 ^{f}	14.27±2.62 ^{h}	11.13±0.88 ^{h}	35.67±1.02 ^{ed}
	24 H.	38.22±3.18 ^{f}	14.25±0.07 ^{i}	24.80±2.04 ^{h}	60.40±0.67 ^{b}
No.1 at 40ºC	5 m.	54.29±4.33 ^{b}	24.55±3.81 ^{f}	39.23±3.12 ^{d}	47.48±2.23 ^{c}
	10 m.	56.32±4.40 ^{a}	28.71±3.14 ^{f}	42.19±4.52 ^{c}	49.21±1.21 ^{b}
	30 min	54.76±4.63 ^{b}	24.91±2.64 ^{ef}	36.83±3.91 ^{cd}	45.48±0.91 ^{c}
	1 H.	54.37±4.30 ^{b}	25.39±2.87 ^{g}	31.99±2.46 ^{f}	47.28±1.56 ^{c}
	24 H.	58.90±0.14 ^{bc}	36.24±0.01 ^{fg}	52.88±2.77 ^{d}	56.18±0.35 ^{cd}
No.2 at 25ºC	5 m.	45.53±2.34 ^{b}	39.65±1.16 ^{c}	29.26±1.01 ^{e}	42.49±0.51 ^{bc}
	10 m.	42.07±2.84 ^{bc}	39.53±0.43 ^{bc}	31.36±4.54 ^{e}	38.68±2.96 ^{cd}
	30 min	47.46±3.30 ^{ab}	43.12±1.52 ^{bc}	32.24±3.72 ^{d}	49.50±0.22 ^{a}
	1 H.	49.26±2.29 ^{ab}	44.43±0.84 ^{c}	26.64±2.53 ^{e}	51.98±1.83 ^{a}
	24 H.	41.77±3.52 ^{d}	34.97±1.16 ^{e}	30.86±0.66 ^{f}	62.49±1.56 ^{ab}
No.2 at 30ºC	5 m.	36.22±1.58 ^{d}	15.02±0.26 ^{h}	16.55±0.74 ^{gh}	39.77±0.53 ^{c}
	10 m.	35.16±1.39 ^{de}	12.45±1.44 ^{h}	19.04±1.90 ^{g}	41.26±0.38 ^{bc}
	30 min	36.48±2.29 ^{d}	14.20±0.01 ^{f}	14.58±1.27 ^{f}	42.28±1.53 ^{d}
	1 H.	38.03±1.48 ^{d}	7.29±1.02 ^{h}	13.63±0.86 ^{fg}	39.27±1.01 ^{d}
	24 H.	38.02±1.45 ^{e}	12.07±0.57 ^{h}	13.42±0.52 ^{h}	59.54±1.71 ^{b}
No.2 at 40ºC	5 m.	53.86±4.00^{a}	18.58±0.33 ^{fg}	21.24±0.77 ^{f}	44.64±2.84 ^{b}
	10 m.	51.58±1.69 ^{a}	22.16±0.09 ^{fg}	25.29±0.73 ^{f}	43.63±1.27 ^{b}
	30 min	51.30±5.27 ^{a}	15.49±1.58 ^{f}	24.34±1.86 ^{e}	44.48±0.99 ^{bc}
	1 H.	48.81±0.97 ^{b}	12.89±0.70 ^{g}	16.38±0.22 ^{f}	43.53±3.66 ^{c}
	24 H.	46.80±0.26 ^{c}	16.63±1.04 ^{g}	28.28±1.69 ^{f}	65.65±1.54 ^{a}
No.3 at 25ºC	5 m.	29.68±0.44 ^{bcd}	34.56±0.83 ^{bc}	36.37±16.17 ^{b}	51.04±0.13 ^{a}
	10 m.	26.64±1.92 ^{bcd}	34.17±1.54 ^{b}	27.98±1.16 ^{bc}	44.85±0.76 ^{a}
	30 min	30.38±1.15 ^{de}	40.19±0.98 ^{bc}	28.06±2.32 ^{de}	56.21±1.34 ^{a}
	1 H.	31.66±0.94 ^{de}	40.19±2.01 ^{bc}	26.41±1.43 ^{ef}	60.22±1.31 ^{a}
	24 H.	22.95±0.04 ^{de}	34.12±0.51 ^{de}	30.50±1.15 ^{de}	63.22±1.58 ^{b}
No.3 at 30ºC	5 m.	26.78±1.18 ^{bcd}	17.99±1.00 ^{d}	26.05±1.15 ^{bcd}	31.82±2.40 ^{bc}
	10 m.	27.69±1.32 ^{bc}	18.67±2.36 ^{d}	26.36±0.90 ^{bcd}	33.51±2.97 ^{bc}
	30 min	27.04±1.31 ^{de}	15.94±0.03 ^{f}	23.66±1.02 ^{ef}	34.10±0.33 ^{cd}
	1 H.	27.63±1.18 ^{def}	11.92±1.13 ^{g}	22.30±1.39 ^{f}	35.10±1.94 ^{cd}
	24 H.	25.78±0.79 ^{de}	4.27±0.09 ^{f}	34.77±2.29 ^{d}	64.03±2.54 ^{b}
No.3 at 40ºC	5 m.	48.45±0.36 ^{a}	31.76±0.12 ^{bc}	33.44±16.31 ^{bc}	22.22±2.12 ^{cd}
	10 m.	50.64±0.70 ^{a}	31.03±1.71 ^{bc}	34.66±17.55 ^{b}	24.67±1.35 ^{cd}
	30 min	47.32±3.25 ^{b}	26.52±3.52 ^{de}	29.34±18.03 ^{de}	26.81±2.05 ^{de}
	1 H.	45.45±1.26 ^{b}	24.92±0.20 ^{ef}	28.50±16.36 ^{def}	28.70±2.41 ^{def}
	24 H.	50.23±1.93 ^{c}	24.38±2.38 ^{de}	58.58±2.08 ^{a}	22.47±1.30 ^{e}

Note: ^{a,b,c} Means within a column with common superscript are significantly different (P<0.05)

The novel method affected the protein digestibility and provided the variant condition of enzyme action. Wherewith temperature and pH were the primary factors in enzyme action. This data supported by the research of bromelain extracted and purification by PEG4000/Phosphate A.T.P.S. method exhibited the optimal pH and temperature at pH 7 and 30-40°C (Ferreira *et al*. 2011), which showed only optimal pH but the wide range of temperature. The data from this study compared to bromelain extract and purify from the waste of pineapple which residue from pineapple can industry with ethanol factional showed the optimal pH was in range pH 7.0 to 8.0 at 50 ^{o}C (Martins *et al*. 2014).

Furthermore, Martins *et al*. (2014) pointed that the optimal temperature and pH for bromelain digestion were 50°C at pH 7.0 while the optimal conditions for bromelain digestion before purification are pH 6 at 45°C, but after purification, it changes to pH 6 - 7 and temperature 50°C. (Sangkharak *et al*., 2016). Therefore, the type of food, extraction, and purification methods also differed in the activity conditions of the bromelain enzyme. However, the optimal conditions for enzyme operating were in the same range. The enzyme bromelain extracted from pineapple peels and crowns can be used at pH 7 at ambient temperature 25^{o}C, and pH 9 at 30 - 40 ^{o}C are required to achieve maximum digestibility optimum condition for the bromelain activity is at pH 4.5 – 9.8 (Bhattacharyya, 2008). The percentage of protein digestion provided the highest in shrimp feed No.3, which is affected by the differentiation of protein source in each feed No. although they have the same protein level. Although protein levels are the same in shrimp diets to use in shrimp of different ages and raw materials, to reduce feed production costs, raw materials in fish feed or large shrimp feed will use feedstuff, especially protein sources that are more difficult to digest such as animal or plant proteins. Apart from the different feedstuff, especially protein sources affected on protein digestion, pellet size and feed type also affect the digestibility of proteins **(**Halver and Hardy, 2002). The saponin contained in shrimp feed predigested with bromelain crude extracted was showed the significant difference data ($P<0.05$) in Table 2. The results exhibited that levels of saponin varied followed the bromelain level and increasing time. Thus, the level of saponin in shrimp feed predigested with bromelain crude extracted at 170 and 250 ppt for 30 min was highest were in ranged 1.84 – 1.88 mg/g.

Table 2: The amount of saponin (mg/g) in shrimp feed digested with bromelain crude extracted at various concentration and times.

Time (minute)	Bromelain crude extract (ppt) supplemented			
	0	90	170	250
Initial	0.95 0.08^{f}	0.95 0.08^{f}	0.95 0.08^{f}	0.95 0.08^{f}
5	1.014 ± 0.05^{ef}	0.94 ± 0.06^{f}	0.99 ± 0.05^{f}	1.24 ± 0.04^{cde}
10	1.09 ± 0.10^{def}	1.15 ± 0.15^{def}	1.29 ± 0.22^{cd}	1.25 ± 0.05^{cd}
30	1.44 ± 0.17^{bc}	1.51 ± 0.09^{b}	1.84 ± 0.1^{a}	1.88 ± 0.08^{a}

Note: ^{a,b,c} Means within a column with common superscript are significantly different ($P<0.05$).

The saponin was found in feed because it is already present in the feed from feedstuff was plant-based protein sources such as soybean meal. Nevertheless, the increase in the bromelain crude extract predigestion is a crude extract that is not purified. Therefore, a portion of saponin in the pineapple peel can be transferred to feed, followed by the time and line up of bromelain crude extracted.

According to Amalia *et al*. (2020), it found saponin was contained in pineapple, which saponin is a triterpenoid found in the plant. Saponin is nontoxic with crustaceans such as shrimp and crab, but it is toxic with fish.

Furthermore, Emmanuel and Deborah (2018) reported that saponin contained in pineapple was 0.41 ± 0.03 %, which is higher than the data from this study. Thus, this study's increasing level of saponin found in feed has no negative feedback on shrimp's health and growth rate. In contrast, the increasing saponin supported and promoted growth rate, feed digestibility, and shrimp immunity. Because of this, the saponin role was promoted nutrient absorption, the immune system, and resistance to pathogens. Moreover, saponin also helps to reduce waste caused by shrimp excretion. **(**Acosta *et al*. 2019)

The data of trypsin inhibitor presented in table 3 provide a significant difference among the group ($P<0.05$). The trend of this data was slightly different from the saponin result. The trypsin inhibitor sharp decrease in the lowest level in shrimp feed was digested at 250 ppt for 5 minutes, and a sharp increase in 10 followed to 30 minutes. Moreover, the other groups of shrimp feed were digested with bromelain crude extracted at 90, and 170 ppt slowly decreased in 0 – 10 minutes and increased to a high level at 30 minutes in the lower than 250 ppt group. In comparison, the level of trypsin inhibitor in the control group was immensely fluctuated with the high level at the first 5 minutes and decrease in 10 minutes, and increased again in 30 minutes. These results indicated that the trypsin inhibitor level decreases in the first period of bromelain crude extract concentrate and increases in the second time. Furthermore, the increasing level was upon the concentration of bromelain crude extracted.

Table 3: The amount of Trypsin inhibitor (mg/g) in shrimp feed digested with bromelain crude extracted at various concentrations and times.

Time (minute)	Bromelain crude extract (ppt) supplemented			
	0	90	170	250
	0.0042 ± 0.0002^{ab}	0.0042 ± 0.0002^{ab}	0.0042 ± 0.0002^{ab}	0.0042 ± 0.0002^{ab}
5	0.0045 ± 0.0001^{a}	0.0039 ± 0.0002^{cd}	0.0038 ± 0.0001^{de}	0.0028 ± 0.0000^{h}
10	0.0031 ± 0.0000^{g}	0.0034 ± 0.0000^{f}	0.0035 ± 0.0002^{ef}	0.0037 ± 0.0002^{def}
30	0.0037 ± 0.0002^{def}	0.0043 ± 0.0000^{ab}	0.0041 ± 0.0001^{bc}	0.0045 ± 0.0001^{a}

Note: ^{a,b,c} Means within a column with common superscript are significantly different ($P<0.05$).

Trypsin inhibitor levels in white shrimp feed were highest in undigested bromelain at 5 minutes and bromelain predigested with bromelain crude extracted at 250 ppt for 5 minutes. While the group found the lowest Trypsin inhibitor contained was bromelain crude extracted predigested at 250 ppt for 5 minutes. These results indicated that bromelain crude extracted and period have no effect of decreasing the trypsin inhibitor contained in the feed, although the molecular structure of trypsin inhibitor is protein but cannot destroy from bromelain. Because of this, the attribute of this anti-nutritional factor was to inhibit the action of trypsin and chymotrypsin (Norton, 1991), which bromelain is an action like trypsin, so it has not affected to destroy the trypsin inhibitor. In addition, pineapple also contained the trypsin inhibitor, which from the report of Emmanuel and Deborah (2018) pointed that the level of trypsin inhibitor in pineapple was 1.06 TIU/mg. Thus, it exhibited that bromelain crude extracted supplemented in shrimp feed did not decrease trypsin inhibitor. However,

the recommended bromelain crude extract optimal for adding in shrimp feed was 250 ppt for 5 minutes.

Conclusion

The optimal condition for protein digestion from pineapple waste crude extracted was pH 6 for the smallest shrimp feed and pH 9 for more extensive shrimp feed at 25^{o}C for all size feed. While the saponin contained in shrimp feed varied, followed by crude extracted from pineapple waste supplemented and increasing time, and the optimal condition were 250 ppt concentrate at 30 minutes. The optimal condition to decrease trypsin inhibitor was 250 ppt at 10 minutes. Finally, the supplementation of crude extracted from pineapple waste promotes protein digestibility over 50 percent and can provide saponin and decrease the trypsin inhibitor in shrimp feed.

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Declaration of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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