

## ANIMAL GROWTH STIMULATION BY ENZYMATIC PROTEIN HYDROLYSATE OF CHICKEN RESIDUES

W. Nawae and W. Suntornsuk\*

Department of Microbiology, Faculty of Science, King Mongkut's University of Technology Thonburi,  
Bangkok 10140, Thailand

\*Corresponding Author's E-mail: worapot.sun@kmutt.ac.th

### ABSTRACT

Frozen chicken process normally generates chicken residues as a by-product. The chicken residues contain high proteins of approximately 73%. To add a higher value to the residues, they could be hydrolysed by a protease to become peptides with functionality. The aim of this study was to produce protein hydrolysates from chicken residues to be applied in the animal feed industry as a feed ingredient for animal growth stimulatory purpose. The residues were hydrolysed by five commercial proteases: Alcalase, Neutrase, papain, pepsin and trypsin. During the hydrolysis, papain, pepsin and trypsin provided high degrees of hydrolysis which was found the highest at the end of hydrolysis, while Alcalase and Neutrase digested the residues poorly. However, cysteamine, a chemical responsible for animal growth promotion, was found at a maximum of 31 mg/ml in Alcalase hydrolysate at 6 h and Neutrase hydrolysate at 10 h. For animal growth experiments, chicken feeds supplemented with 6 h-Alcalase hydrolysate at cysteamine concentrations of 90 and 180 mg/kg diet gave significantly higher chicken growth than a controlled diet which was no hydrolysate added. Thus, chicken residue hydrolysate could have a potential use as a growth stimulant in animal feeds.

**Key words:** Chicken processing waste; Animal growth stimulant; Chicken protein hydrolysates; Enzymatic hydrolysis.

<https://doi.org/10.36899/JAPS.2021.2.0224>

Published online October 03, 2020

### INTRODUCTION

Thailand is a leading country as a global poultry producer for domestic consumption and exports. Over 450 million broilers were reported to be cultivated last year (Department of Livestock Development, 2019). They are undergone in chicken processing plants to yield edible parts. However, during processing steps, trimmed chicken residues were enormously generated as a by-product with a low-value. The by-product contains high quantity of protein and could become a good source of an effective protein hydrolysate.

Protein hydrolysate is a mixture of amino acids and short peptides obtained by acid, alkaline or enzymatic hydrolyses of animal and plant proteins. In recent years, protein hydrolysates obtained from enzymatic hydrolysis of various protein sources have been studied for their potential contribution on biological properties such as antioxidant, anticancer, antimicrobial as well as plant and animal growth promoter (Matsumiya *et al.* 2007; Tang *et al.* 2008; Centenaro *et al.* 2014; Onuh *et al.* 2014). The hydrolysates are reported to have a significant impact to animal nutrition (Hou *et al.* 2017)

Generally, the livestock industry requires high quality of animal feeds or ingredients to give a maximum of animal growth rate. At present, acidifiers, exogenous enzymes, herbs, plant extracts, chitosan, zinc oxide, prebiotics and probiotics have been mixed in animal feeds for growth stimulatory purpose instead of antibiotic

growth promoter not allowed to be used (Chattopadhyay, 2014). In addition, a bioactive peptide or protein hydrolysate might be an alternative for animal growth stimulation. Cysteamine, a derivative of amino acid cysteine, is classified in sulphhydryl compound group which affects to an endocrine system related to metabolism pathways of animal body (Yang *et al.* 2006). It is absorbed into a small intestine leading to inhibit somatostatin hormone (growth inhibitory hormone). This induces more growth hormone releasing into blood stream and eventually stimulating animal growth (Kataoka *et al.* 1994; Wang *et al.* 2015). This work, therefore, was aimed at producing protein hydrolysate containing cysteamine from enzymatic hydrolysis of chicken residues, wastes from a frozen chicken processing plant, and evaluating effects of the protein hydrolysate to animal growth stimulation.

### MATERIALS AND METHODS

**Raw material and commercial proteases:** Chicken residues, collected from a frozen chicken processing plant, were kindly provided by Betagro Public Company Limited, located at Lop Buri province, Thailand. Residue samples were minced uniformly and stored at -20 °C in polyethylene bags until use.

Five commercial proteases used in this study were Alcalase (EC 3.4.21.62, 7-15 U/g, from *Bacillus licheniformis* from Sigma-Aldrich (Copenhagen,

Denmark)), Neutrase (EC 3.4.24.28,  $\geq 0.8$  U/g, from *Bacillus amyloliquefaciens* from Sigma-Aldrich (Copenhagen, Denmark)), trypsin (10,000 U/mg, from bovine pancreas from Sigma-Aldrich (Copenhagen, Denmark)), papain (EC 3.4.22.2, 3,000 U/mg, from *Carica papaya* from Merck KGaA (Darmstadt, Germany)) and pepsin (EC 3.4.23.1, 0.7 U/g, from porcine gastric mucosa from Merck KGaA (Darmstadt, Germany)).

**Proximate analysis:** Proximate analysis of the residues was carried out according to AOAC (AOAC, 2000).

**Preparation of protein hydrolysate from chicken residues:** Chicken protein hydrolysates (CPH) were prepared in a 250 ml-Erlenmeyer flask containing 75 g ground sample mixed with 100 units of commercial proteases under their optimum condition for a total mixture of 150 ml. The study condition of each enzymatic hydrolysis was as follows: Alcalase operated using 0.1 M Tris-HCl buffer pH 8.0 and 55 °C, Neutrase and papain carried out using 0.1 M sodium phosphate buffer pH 7.0 and 55 °C, pepsin incubated in 0.1 M citrate phosphate buffer pH 3.0 at 37 °C and trypsin operated using 0.1 M Tris-HCl buffer pH 8.0 and 37 °C. Samples were periodically taken during 10 h incubation. After hydrolysis, the hydrolysate was immediately heated to inactivate enzyme activities at 90 °C for 10 min and centrifuged at 12,000xg for 15 min to remove undigested material. Its supernatant was collected as protein hydrolysate for further analyses.

#### Analyses of chicken protein hydrolysate

**1. Determination of protein:** Soluble protein in the hydrolysates was determined according to Lowry assay (Lowry *et al.* 1951). Bovine serum albumin (BSA) was used as a protein standard.

**2. Determination of  $\alpha$ -amino acid and degree of hydrolysis:** Content of  $\alpha$ -amino acid in the protein hydrolysate samples was analyzed by the TNBS method (Adler-Nissen, 1979) based on the reaction of 2,4,6-trinitrobenzenesulfonic acid (TNBS) (Sigma-Aldrich, St. Louis, MO, USA) with primary amino groups under alkaline conditions. The  $\alpha$ -amino acid content was expressed in terms of L-leucine (Sigma-Aldrich, St. Louis, MO, USA) at the absorbance of 420 nm.

Degree of hydrolysis (DH) of the hydrolysates was determined according to the method of Adler-Nissen (1979). The DH (%) was calculated as  $[(AN_2 - AN_1)/N_{pb}] \times 100$ . The  $AN_1$  and  $AN_2$  (mg/g protein) are the amino nitrogen content of the sample before and after hydrolysis, respectively. The  $N_{pb}$  (mg/g protein) is the nitrogen content of the peptide bonds in the sample.

**3. Determination of cysteamine:** Cysteamine content in the hydrolysates was monitored by gas chromatography (GC, Perkin Elmer, Autosystem XL, USA) using the

method of Kataoka *et al.* (1994). GC was done by using a capillary column (30 m x 0.25 mm ID x 0.25  $\mu$ m, J&W Scientific Agilent DB-210, USA) under an operating condition of nitrogen flow rate at 5 ml/min, injection temperature of 130 °C and detector temperature of 260 °C. The analysis was carried out with a flame photometric detector (Perkin Elmer, USA).

**4. SDS-PAGE analysis:** Molecular weights of all protein hydrolysate samples were determined by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 10% gel according to Laemmli's method (1970). The standard protein used in this study was TriColor Protein Ladder containing 3.5-245 kDa protein molecular weights (biotechrabbit, Hennigsdorf, Germany). Electrophoresis was carried out at a constant voltage of 160 V. Protein bands on the gel were finally made visible by staining with Coomassie brilliant blue G-250.

**5. Determination of amino acids:** Samples of chicken residue suspended in sodium phosphate buffer (pH 8.0) without enzymatic digestion, the hydrolysate at 0 h (CPH0h) and the highest cysteamine-containing hydrolysate (CPH6h) were determined for amino acids (OJEC, 1998). The data were reported as milligrams of amino acid per 100 g protein.

**Study on chicken growth performance by protein hydrolysates:** Chicken growth performance was tested on effect of the protein hydrolysate to chicken growth. The chicken protein hydrolysate (CPH) was prepared from chicken residues hydrolysed by a protease producing the highest amount of cysteamine as described in the previous experiment. The growth experiments were designed under Completely Random Design (CRD) and carried out using 120 14-day old White Plymouth Rock live birds with all males and females mixed together divided into 4 groups (30 chickens/group): (1) Control (a commercial diet without the protein hydrolysate), (2) CPH0h (a commercial diet added with CPH obtained from 0 h hydrolysis), (3) CPH6h (a commercial diet formulated with the highest cysteamine CPH containing 90 mg cysteamine/kg diet as recommended by Yang *et al.* (2006), and (4) 2xCPH6h (a commercial diet formulated with the highest cysteamine CPH containing 180 mg cysteamine/kg diet). The commercial diet was composed of broken rice 36%, ground corn 35%, soybean meal 12%, rice bran 10%, fish meal 4%, soybean oil 1%, ground shell 0.5%, dicalcium phosphate 0.3%, minerals and vitamins 0.3%, sodium chloride 0.2% and DL-methionine 0.1% (Betagro Balance 900, Betagro Northern Company Limited, and Thailand). The experiments were carried out for 28 days at room temperature (30°C $\pm$ 2) with lights at night time. Birds were administered for Newcastle, and infectious bronchitis diseases at days 7, 14 and 21 and received a

Gumboro vaccination via water administration at 14 day of age. During a period of study, their health and death were monitored on a daily basis and their weights and consumption were weekly recorded. Body weight gain (BWG) was calculated by subtracting the initial bird weights from their monitoring weights as average per head. Average daily gain (ADG) was calculated by dividing BWG with a period of study. Feed intake (FI) was calculated by subtracting remaining feed weights from initial feed-added weights as average per head. Feed conversion ratio (FCR) was calculated by dividing FI with BWG.

**Statistical analysis:** Statistical data of protein hydrolyses, bird's body weight and bird's feed intake were analyzed by the Analysis of Variance (ANOVA). Means of each parameter as hydrolysis time, type of enzyme, period of growth study and amount of hydrolysate in diets were compared by Duncan's New Multiple Range Test (DMRT) using the SPSS software version 16 (SPSS, Chicago, Illinois, USA). Differences were considered statistically significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

**Properties of chicken residues and chicken protein hydrolysates:** Chicken residues contained 72.9% (w/w, dry weight) protein, 7.5% (w/w, dry weight) lipid, 4.3% (w/w, dry weight) ash, 1.1% (w/w, dry weight) fiber and 14.3% (w/w, dry weight) carbohydrate. Their contents are comparable to those in chicken parts previously reported as 83.2-89.6% protein, 4.7-12.2% lipid and 4.6-5.7% ash (w/w, dry weight) (Badr, 2005). They are good sources of protein for consumption and protein hydrolysis.

Protein hydrolysis can be quantified by the degree of hydrolysis which refers to the percentage of peptide bonds cleaved. The effect of protease hydrolyses on chicken residues is shown in Fig. 1. Enzymatic hydrolysis profiles in this study were different depending on hydrolysis time and enzyme type. At the initial hydrolysis, the rate of hydrolysis increased rapidly especially papain, trypsin and pepsin and it then was moderately increased except for Neutrase and Alcalase. The DH rate was high at the initial period of hydrolysis since a large number of peptide bonds in protein molecules were cleaved, resulting in increased soluble peptides in the reaction mixture. The released soluble peptides might be substrate competitors to compete with undigested or partially digested proteins, leading to the steady or lower DH at the end of hydrolysis time.

In this study, papain, trypsin and pepsin digested the chicken samples well (16-18% DH) and gave significantly higher DH than Neutrase and Alcalase (1-3% DH) (Fig. 1). High DH was also observed by hydrolysis of chicken muscle proteins with papain,

trypsin and pepsin. Extensive hydrolysis of those proteases may be because the enzymes have broader specificities to the structure and conformation of chicken muscle proteins (Maruyama *et al.* 1981; Ménard and Starer, 2004). In general, DH is affected by enzyme specificity, physical state and chemical characteristics of intact protein, and hydrolysis conditions (Karamać and Rybarczyk, 2008).

Free amino acids released from the protein isolate by enzymatic hydrolyses at various hydrolysis times were similar to DH profiles (data not shown). Extended hydrolysis time led to enhance interaction among enzymes and chicken proteins and cleaved peptide bonds to release free amino acids. The treatments of chicken proteins with papain, trypsin and pepsin gave a dramatic increase in amino acids during the first few hours of hydrolysis. Papain and trypsin provided the highest amount of amino acids released with approximately 18.7 mg/ml and 19.9 mg/ml, respectively, at 10 h of hydrolysis. The amino acid released by Neutrase and Alcalase gave small amounts of amino acids. Different protein digestibility may result from the enzyme specificity to peptide bonds at different amino acid sequences in chicken proteins.

During hydrolysis of chicken residues, soluble protein increased over time especially pepsin and papain hydrolysis and reached the maximal of 133.5 mg/ml and 108.0 mg/ml, respectively, at 10 h of hydrolysis (data not shown). Pepsin is an endopeptidase which is efficient to cleave the bonds at aromatic amino acids or non-polar amino acid such as phenylalanine, tryptophan and tyrosine (Centenaro *et al.* 2014), while papain, a plant cysteine protease, has been reported to express several proteolytic activities such as endopeptidase, amidase and esterase activities and to cleave peptide bonds at amino acid positions of arginine, lysine and phenylalanine (Ménard and Starer, 2004; Amri and Mamboya, 2012). Neutrase, Alcalase and trypsin hydrolysis gave little soluble protein and its amount remained constant.

SDS-PAGE profiles of the protein hydrolysates obtained by all proteolytic digestions at various hydrolysis times and undigested chicken protein (without enzyme) as a control were monitored (data not shown). The difference in electrophoresis patterns was observed among the control and the protein hydrolysate samples. Protein bands of the control and the protein hydrolyses at 0 h of hydrolysis had bands at an estimated size of 35 kDa, due to chicken muscle contains actin and myosin filaments consisting of tropomyosin which is build up by 2 polypeptide chains, estimated molecular weight of 35 kDa (Phetploy, 2005). Increased hydrolysis time by papain, pepsin and trypsin provided small protein bands at less than 5 kDa. It is expected that almost complete hydrolysis was reached. However, samples of 10 h-Alcalase hydrolysate and 10 h-Neutrase hydrolysate had bands at various sizes demonstrating incomplete

digestion by these enzymes. These are in agreement with results of their degree of hydrolysis.

The amount of cysteamine was found to be high in the protein hydrolysates obtained from 6 h-Alcalase and 10 h-Neutrase hydrolysates at approximately 31 mg/ml (Fig. 2). The high values of cysteamine were opposite to the amount of soluble protein content and free amino acid content obtained by both enzymes. However, cysteamine content decreased as degree of hydrolysis increased. Therefore, due to the shorter hydrolysis time, 6 h-Alcalase hydrolysate was selected to test on animal growth performance.

**Animal growth performance:** The protein hydrolysate obtained from 6 h-Alcalase hydrolysis of the residue having the maximal value of cysteamine was added into a chicken diet and was studied for chicken growth performance compared to the diets without the protein hydrolysate and the protein hydrolysate obtained at 0 h as controls. Growth performance of broilers fed by 4 formulas of diet was shown in Fig. 3. Average chicken weights at the end of experimental period of the control, CPH0h, CPH6h (90 mg cysteamine/kg diet) and 2xCPH6h (180 mg cysteamine/kg diet) were 2.16, 2.28, 2.85 and 2.92 kg, respectively. It was also observed that no significant difference in weekly and total chicken diet consumption (Fig. 4 and Table 1), however, after 28 days of experimental period, significantly increase in chicken weight by the diets mixed with CPH6h and 2xCPH6h might be due to the presence of cysteamine and its concentration in the diet. According to the study of Chattopadhyay (2014), chicken fed with 90 mg cysteamine/kg diet improved the growth performance as well as improved the activities of protease, amylase and lipase in digestive tract of broilers. Due to the mechanism of cysteamine affecting somatostatin hormone (SS) which is a growth hormone (GH) inhibitor via thiol group of cysteamine (Wang *et al.* 2015), so cysteamine has reported to be a high potential to promote growth rate of broilers. In addition, cysteamine supplementation in the diet may enhance protein metabolisms of animal muscle and decrease fat deposition in animal body (Liu *et al.* 2009). As observed by chicken carcass samples, firm

muscle and low fat deposition appeared in chicken carcass fed by 90 and 180 mg cysteamine containing diets. Moreover, average daily gain (ADG) of chicken treated by 90 and 180 mg cysteamine/ kg diet were 77.82 and 80.57 g/chicken/day, respectively (Table 1). All healthy chicken with no death and normal consumption behavior were observed along 28-day experimental period. Body weight gain of chicken broilers significantly increased by fed diets mixed with protein hydrolysate of 6 h-Alcalase hydrolysis at 90 and 180 mg/kg (Table 1). Chicken broilers in each group ate the diet in a similar manner and quantity of approximately 3,003-3,034 g/chicken (Table 1). At the first week, they in each group consumed the feed around 60-64 g/chicken/day, until the fourth week, they ate at 150-157 g/chicken/day (Fig 4). No significant difference in average daily chicken consumption was found (Fig 4).

**Amino acids in protein hydrolysates:** Amino acid composition in samples (the residue without enzyme as a control, Alcalase hydrolysate at 0 h and Alcalase hydrolysate at 6 h) is shown in Table 2. It is indicated that histidine and glutamic acid were major amino acids in the samples and an amount of amino acids in the control and 0 h-Alcalase hydrolysate was similar, while 6 h-Alcalase hydrolysate had generally higher amino acid contents than the other samples. The results suggest that increased hydrolysis time led to increased more amino acids as previously described by Onuh *et al.* (2014). It is also observed that hydrophobic amino acids such as alanine, leucine, tryptophan and phenylalanine and negative charged amino acids such as aspartic acid and glutamic acid were significantly increased in 6 h-Alcalase hydrolysate. Histidine plays an important role on a free radical scavenging activity by its imidazole ring and hydrophobic amino acid can be a strong antioxidant (Alashi *et al.* 2014; Centenaro *et al.* 2014). Negative charged amino acids also performed as antioxidants by transferring the excess electrons to stabilize free radicals which can inhibit oxidative stress and prevent cell damage (Yang *et al.* 2006; Centenaro *et al.* 2014; Onuh *et al.* 2014). These could be an additional effect to enhance the growth and health benefit of animal.

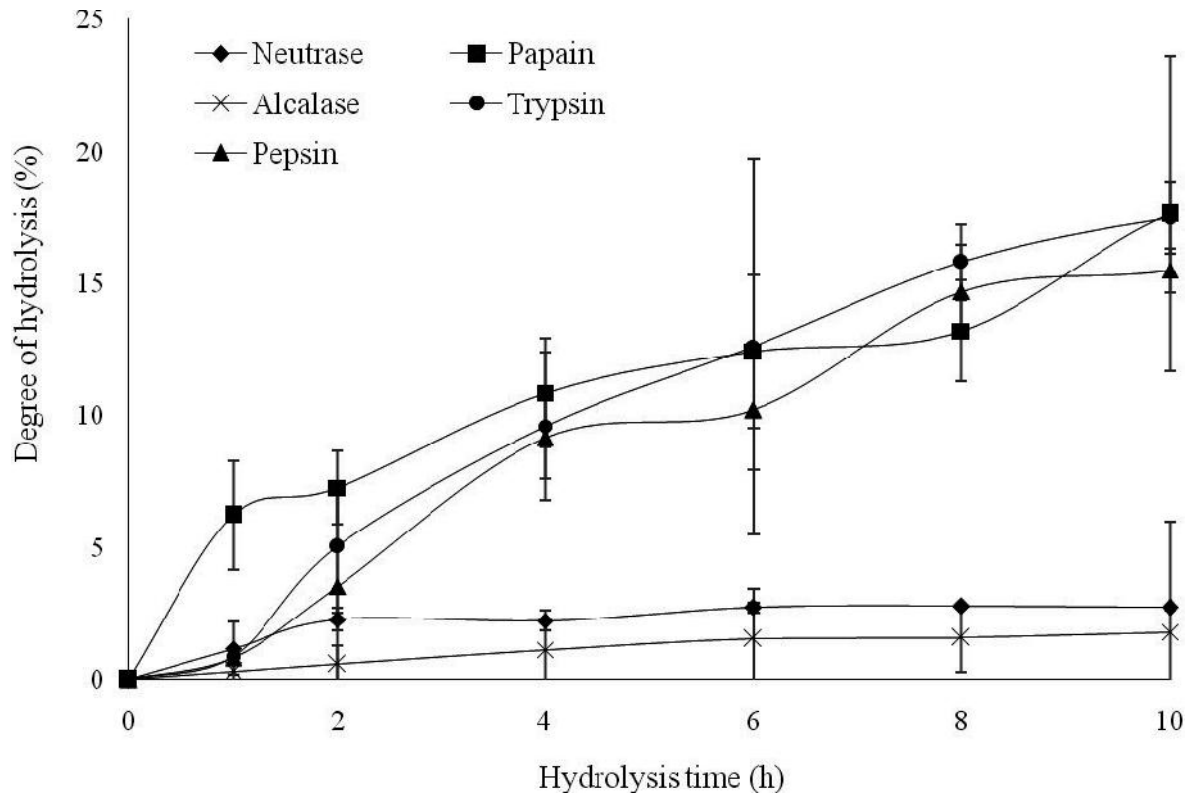


Fig 1. Degree of hydrolysis (DH) of chicken residues hydrolysed by different proteases

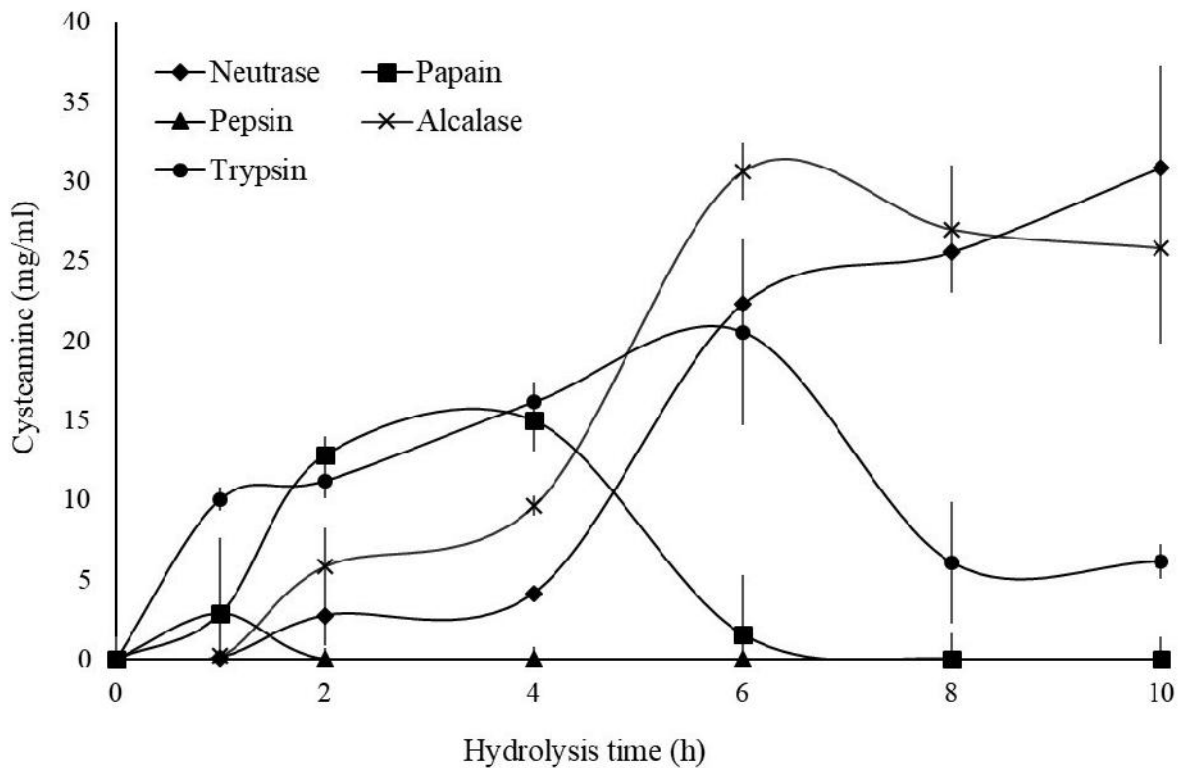
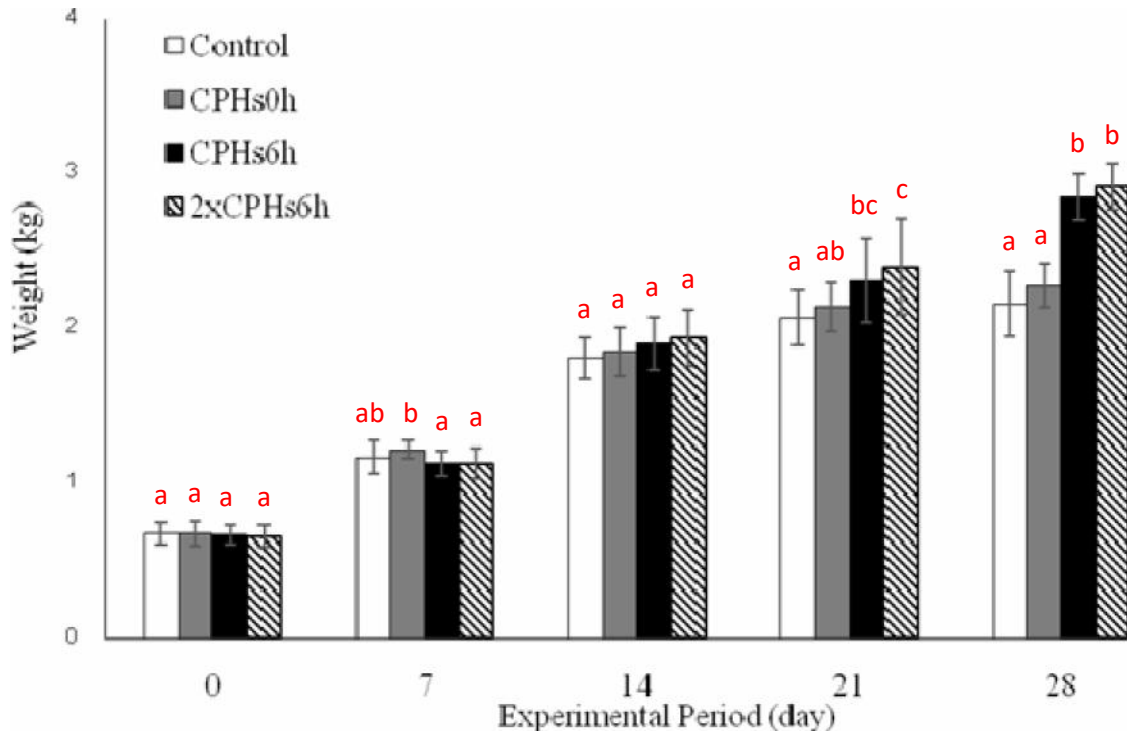
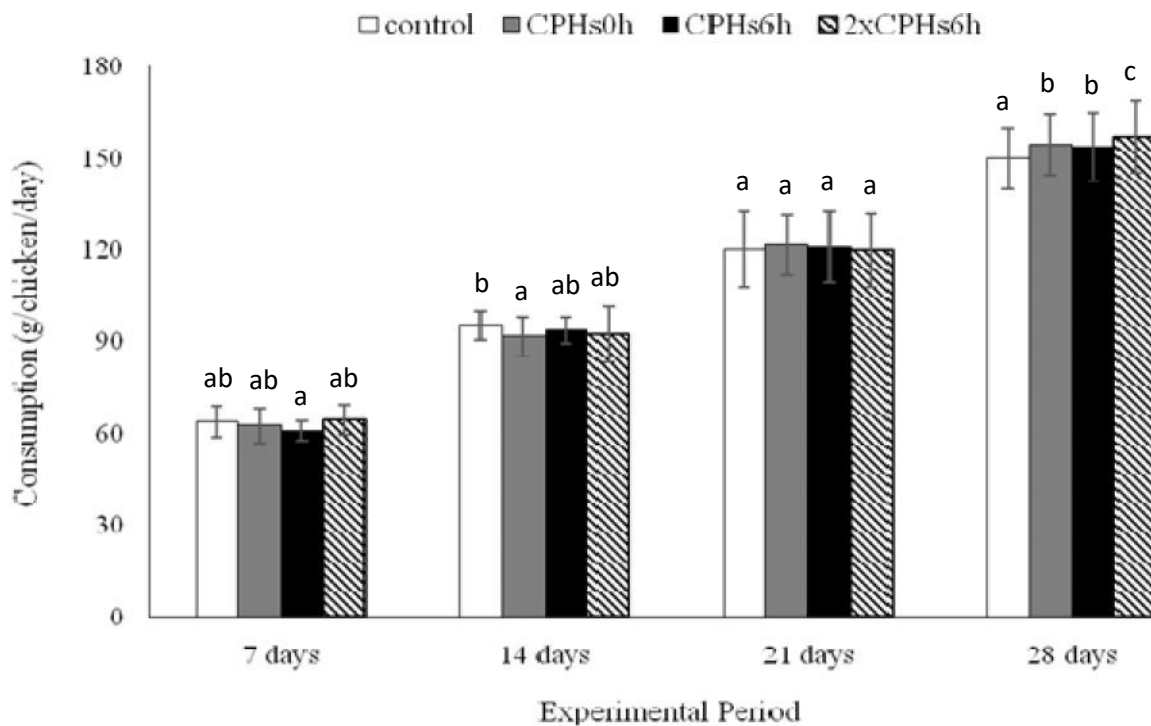


Fig 2 Cysteamine generated in protein hydrolysates during hydrolysis of chicken residues by different proteases



**Fig 3. Chicken weight fed by formulated diets with different amounts of protein hydrolysate derived from Alcalase digestion of chicken residue for 6 h**  
 (Values with different letters (a, b, c) for each treatment at a period of time indicate significant differences at  $p < 0.05$ )



**Fig 4. Weekly individual chicken consumption of formulated diets with different amounts of protein hydrolysate derived from Alcalase digestion of chicken residue for 6 h**  
 (Values with different letters (a, b, c) for each treatment at a period of time indicate significant differences at  $p < 0.05$ )

**Table 1. Growth performance of chicken broilers after fed by different diets with and without protein hydrolysates for 28 days.**

Growth performance	Chicken broiler group			
	Control	CPH0h	CPH6h	2xCPH6h
Body weight gain (g/chicken)	1,480 ± 190 <sup>a</sup>	1,600 ± 190 <sup>a</sup>	2,180 ± 110 <sup>b</sup>	2,260 ± 190 <sup>b</sup>
Average daily gain, ADG (g/chicken/day)	53.00 ± 6.89 <sup>a</sup>	57.00 ± 6.77 <sup>a</sup>	77.82 ± 3.88 <sup>b</sup>	80.57 ± 6.65 <sup>b</sup>
Feed intake, FI (g/chicken)	3,003.00 ± 33.22 <sup>a</sup>	3,010.00 ± 35.67 <sup>a</sup>	3,004.40 ± 35.73 <sup>a</sup>	3,033.80 ± 36.08 <sup>ab</sup>
Feed conversion ratio, FCR	2.03	1.88	1.38	1.34

(Values with different letters (a, b) at the same row indicate significant differences at  $p < 0.05$ )

**Table 2. Free amino acid profile of chicken residue suspended in buffer and chicken protein hydrolysates obtained from Alcalase digestion at 0 h (CPH0h) and at 6 h (CPH6h).**

Free amino acids (mg/100 g)	Chicken	CPH0h	CPH6h
Alanine	3.65	3.76	5.89
Arginine	1.97	2.56	4.82
Aspartic acid	2.29	2.12	3.74
Cystine	0.93	0.96	1.03
Glutamic acid	5.74	4.48	7.94
Glycine	2.26	2.25	3.44
Histidine	10.76	14.57	19.4
Hydroxylysine	ND	ND	ND
Hydroxyproline	ND	ND	ND
Isoleucine	1.73	1.46	2.93
Leucine	2.80	2.74	6.09
Lysine	3.67	3.50	5.7
Methionine	1.35	1.19	2.42
Phenylalanine	1.57	1.87	3.78
Proline	1.84	1.69	2.97
Serine	4.60	3.67	5.29
Threonine	1.67	1.54	2.61
Tryptophan	0.50	0.11	0.98
Tyrosine	0.71	1.68	2.93
Valine	2.32	2.06	3.61

ND: not detected.

\* Indicated that the Mercury content is below the detection limit of machine.

**Conclusions:** The study on chicken growth stimulation was successfully achieved in a small-scale husbandry by using protease hydrolysate from Alcalase hydrolysis of chicken residues digested for 6 h. The hydrolysate could be a low-cost diet supplement derived from protein waste from the poultry processing industry and would be commercially applied for animal growth stimulation. Cysteamine and certain amino acids in the hydrolysate would play an important role for the growth promotion

by the suppression of growth inhibitory hormone and the expression of antioxidant activities.

**Acknowledgements:** This work was supported by the Thailand Research Fund (TRF) and the program of Research and Researchers for Industries (RRI) for a research budget (Grant No. MSD57I0028). A scholarship supported by Faculty of Science, KMUTT, to Ms. Nawae is also acknowledged. The authors are thankful to Betagro Public Company Limited for financial and material supports of this work.

## REFERENCES

- Adler-Nissen, J. (1979) Determination of the degree of hydrolysis of food proteins hydrolysates by trinitrobenzene sulfonic acid. *J. Agric. Food Chem.* 27(6): 1256-1262.
- Alashi, A.M., C.L. Blanchard, R.J. Mailer, S.O. Agboola, A.J. Mawson, R. He, A. Girgih, and R.E. Aluko (2014) Antioxidant properties of Australian canola meal protein hydrolysates. *J. Food Chem.* 146: 500-506.
- Amri, E. and F. Mamboya (2012) Papain, a plant enzyme of biological importance: A review. *Biochem. Biotech.* 8(2): 99-104.
- AOAC (2000). Official methods of analysis of the association of official analytical chemistry, 17<sup>th</sup> ed. AOAC International, Virginia, USA.
- Badr, H.M. (2005) Chemical properties of chicken muscles and skin as affected by gamma irradiation and refrigerated storage. *J. Food Technol.* 3(1): 1-9.
- Centenaro, G., M. Salas-Mellado, C. Pires, I. Batista, M. Nunes and C. Prentice (2014) Fractionation of protein hydrolysates of fish and chicken using membrane ultrafiltration: Investigation of antioxidant activity. *Appl. Biochem. Biotechnol.* 172(6): 2877-2893.
- Chattopadhyay, M. K. (2014) Use of antibiotics as feed additives: a burning question. *Front. Microbiol.* 5: 334.

- Department of Livestock Development (2019) [http://ict.dld.go.th/webnew/images/stories/stat\\_web/yearly/2562/T6-1-chicken.pdf](http://ict.dld.go.th/webnew/images/stories/stat_web/yearly/2562/T6-1-chicken.pdf) (accessed 14.03.2020)
- Hou, Y.G., Z. L. Wu, Z.L. Dai, G.H. Wang and G.Y. Wu (2017) Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. *J. Anim. Sci. Biotechnol.* 8:24 Doi 10.1186/s40104-017-0153-9
- Karamać, M. and A. Rybarczyk (2008) Chymotryptic hydrolysis of lentil meal proteins and characteristics of the resulting hydrolysates. *Pol. J. Food Nutr. Sci.* 58(3): 351–357.
- Kataoka, H., H. Tanaka, and M. Makita (1994) Determination of total cysteamine in urine and plasma samples by gas chromatography with flame photometric detection. *J. Chromatogr. B. Biomed. Appl.* 657(1): 9-13.
- Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature.* 227(5259): 680–685.
- Liu, G., Z. Wang, D. Wu, A. Zhou, and G. Liu (2009) Effects of dietary cysteamine supplementation on growth performance and whole-body protein turnover in finishing pigs. *Livest. Sci.* 122(1): 86-89.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Pandall (1951) Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193(1): 265-275.
- Matsumiya, Y., S. Sumiyoshi, T. Matsukura, and M. Kubo (2007) Effect on epidermal cell of soybean protein-degraded products and structural determination of the root hair promoting peptide. *Appl. Microbiol. Biotechnol.* 77(1): 37–43.
- Ménard, R. and A.C. Starer (2004) Papain. In: Handbook of proteolytic enzymes, 2<sup>th</sup> ed., Barrett, A.J., Rawlings, N.D. and Woessner, J.F., (Eds.), Elsevier Academic Press; (Netherlands). 1125-1128p.
- Maruyama, K., M. Kimura, S. Kimura, K. Ohashi, K. Suzuki, and N. Katunuma (1981) Connectin, an elastic protein of muscle. Effects of proteolytic enzymes. *J. Biochem.* 89 (3): 711-715.
- OJEC (1998) Official J. The European Communities (OJ L257/16) Official J. The European Union. 14-28p.
- Onuh, J.O., A.T. Girgih, R.E. Aluko, and M. Aliani (2014) *In vitro* antioxidant properties of chicken skin enzymatic protein hydrolysates and membrane fractions. *J. Food Chem.* 150: 366-373.
- Phetploy, J. (2005) Frozen stability of muscle protein from freshwater. Ms. Thesis, Suranaree University of Technology, Thailand. 6-9p.
- Tang, H.G., T.X. Wu, Z.Y. Zhao, and X.D. Pan (2008) Effects of fish protein hydrolysate on growth performance and humoral immune response in large yellow croaker (*Pseudosciaena crocea* R.). *J. Zhejiang Uni. Sci. B.* 9(9): 684-690.
- Wang, C., C. J. Dong, Z.Q. Wang, F. Yang, H.L. Mao, Z. Wu, Q. Zhou, and H.F. Wang (2015). Effect of cysteamine hydrochloride supplementation on the milk performance of dairy cow. *Livest. Sci.* 178(3): 94-99.
- Yang, C.M., A.G. Chen, Q.H. Hong, J.X. Liu, and J.S. Liu (2006) Effects of cysteamine on growth performance, digestive enzyme activities, and metabolic hormones in broilers. *Poult. Sci.* 85(11): 1912-1916.