COMPARATIVE STUDY ON ANGIOTENSIN CONVERTING ENZYME INHIBITORY ACTIVITY OF HYDROLYSATE OF MEAT PROTEIN OF INDONESIAN LOCAL LIVESTOCKS

Jamhari[1], L. M. Yusiati[1], E. Suryanto[1], M. N. Cahyanto[2],Y. Erwanto[1]and M. Muguruma[3]

[1]Faculty of Animal Science, Gadjah Mada University,
Jl. Fauna No.3, Bulaksumur, Yogyakarta 55281- Indonesia
[2]Faculty of Agricultural Technology, Gadjah Mada University,
Jl. Flora No.1 Bulaksumur, Yogyakarta 55281 - Indonesia
[3]Faculty of Agriculture, University of Miyazaki,
1-1 Gakuen Kibanadai Nishi, Miyazaki-shi, Miyazaki, 889-2192 Japan
Corresponding E-mail: jam_hari@ugm.ac.id

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ABSTRAK

Penelitian ini dilaksanakan dengan tujuan untuk mengetahui aktivitas angiotensin converting enzyme (ACE) inhibitor dari hidrolisat protein daging sapi Bali, kambing Kacang, ayam kampung dan itik lokal Indonesia. Daging sapi Bali, kambing Kacang, ayam kampung dan itik lokal digunakan dalam penelitian ini. Daging digiling dengan food processor dengan penambahan aquades untuk memperoleh ekstrak daging. Ekstrak daging dihidrolisis dengan enzim protease untuk memperoleh hidrolisat protein daging. Konsentrasi protein ekstrak daging dan hidrolisat protein daging ditentukan, dan dikonfirmasi dengan sodium dodecyl sulfate - poly acrylamide gel electrophoresis (SDS-PAGE). Aktivitas ACE inhibitor dari hidrolisat protein daging sapi Bali, kambing Kacang, ayam kampung dan itik lokal Indonesia juga ditentukan. Hasil penelitian menunjukkan bahwa konsentrasi protein hidrolisat protein daging sapi Bali, kambing Kacang, ayam kampung dan itik lokal Indonesia lebih tinggi dibandingkan dengan konsentrasi ekstrak daging. Analisis SDS-PAGE menunjukkan bahwa hidrolisat protein daging sapi Bali, kambing Kacang, ayam kampung dan itik lokal Indonesia mempunyai peptida dengan berat molekul lebih ringan, lebih banyak dibandingkan dengan ekstrak daging. Hidrolisat protein daging sapi Bali, kambing Kacang, ayam kampung dan itik lokal Indonesia mempunyai potensi yang kuat untuk menghambat aktivitas ACE, sehingga dapat berpotensi menurunkan tekanan darah.

Kata kunci: Aktivitas ACE inhibitor, Hidrolisat protein, Ternak lokal Indonesia.

ABSTRACT

The experiment was conducted to investigate the angiotensin converting enzyme (ACE) inhibitory activity of hydrolysate in meat protein of Bali cattle, Kacang goat, native chicken, and local duck. The meats of Bali cattle, Kacang goat, native chicken, and local duck were used in this study. The meats were ground using food processor added with aquadest to obtain meat extract. The meat extracts were then hydrolyzed using protease enzymes to obtain hydrolysate of meat protein. Protein concentration of meat extract and hydrolysate of meat protein were determined, and were confirmed by sodium dodecyl sulfate - poly acrylamide gel electrophoresis (SDS-PAGE). ACE inhibitory activity of hydrolysate of meat protein derived from Bali cattle, Kacang goat, native chicken, and local duck was also determined. The results showed that protein concentration of hydrolysate of meat protein of Bali cattle, Kacang goat, native chicken, and local duck meat was significantly higher than their meat extracts. SDS-PAGE analysis indicated that hydrolysate of meat protein of Bali cattle, Kacang goat, native chicken, and local duck had more peptides with lower molecular weight, compared to their meat extracts. Hydrolysate of meat protein of Bali cattle, Kacang goat, native chicken, and local duck had potencies in inhibiting ACE activity, so it will potentially reduce blood pressure.

Keywords: ACE inhibitory activity, Protein hydrolysate, Indonesian local Livestock

INTRODUCTION

Angiotensin I-converting enzyme (ACE), which is a dipeptidylcarboxypeptidase, plays an important physiological role in regulating blood pressure (Skeggs *et al*., 1957). This enzyme catalyzes conversion of inactive angiotensin I to potent vasoconstrictor angiotensin II by cleaving the dipeptide from the C-terminal of angiotensin I, and it inactivates the vasodilator bradykinin (Astawan *et al*., 1995). For these reasons, specific inhibitors of ACE are useful for regulating physiological activities associated with ACE in the human body (Ondetti *et al*., 1977).

Some synthetic inhibitors of ACE, such as captopril and enalapril, have been proved to be useful as antihypertensive drugs (Ondetti *et al*., 1977; Astawan *et al*., 1995). However, the side effects of using those synthetic inhibitors, such as cough and angioneurotic edema associated with clinically used ACE inhibitors, have been addressed (Israili and Hall, 1992). Recently, food scientists are developing new angiotensin-I converting enzyme (ACE) inhibitors from natural foods (Murray and Fitzgerald 2007), with the purpose of reducing the side effects of using ACE inhibitors for the treatment of hypertension.

ACE inhibitory peptides are produced during protein hydrolysis by digestive enzymes such as trypsin, chymotrypsin or pepsin (Liepke *et al*., 2001). Thus, the peptides may also be generated by protein hydrolysis of foodstuffs during processing and digestion in the digestive tract (Yust *et al*., 2003). ACE inhibitory peptides have been found in enzymatic hydrolysates of many foodstuffs, such as casein (Karaki *et al*., 1990; Yamamoto *et al*., 1994), whey (Abubakar *et al*., 1998), dried bonito (Fujita *et al*., 2000), porcine skeletal muscle proteins (Arihara *et al*., 2001; Nakashima *et al*., 2002; Katayama *et al*., 2004; Katayama *et al*., 2007; Katayama *et al*., 2008). Moreover, these peptides have shown potent antihypertensive activity when administered orally to spontaneously hypertensive rats (SHR).

Many peptides derived from food proteins have been recommended to be ACE inhibitors. Some of those peptides have also been administered orally to SHR, and showed hypertensive effect, by reducing significantly blood pressure. In this study, ACE inhibitory activity of meat protein hydrolysate of Indonesian local Livestock will be studied. The objective of the study was to identify the ACE inhibitory activity of meat protein hydrolysate of Indonesian local Livestock.

MATERIALS AND METHODS

Materials used in the study were Bali cattle loin (*Longissimus dorsi*) obtained from Bali Island, Kacang goat meat loin (*Longissimus dorsi*) obtained from central Java area, local duck and native chicken meat (breast muscles) obtained from central Java area, pepsin (porcine stomach mucosa), trypsin, chymotrypsin obtained from Wako Pure Chemical Industry Ltd., Japan, ACE from rabbit lung obtained from Sigma Chemical Co. St. Louis, USA, Hippuryl-L-Histidyl-L-Leucine (HHL) free base obtained from Nacalai Tesque, Kyoto, Japan.

Sample Preparation

Fifty grams of meat with the addition of 100 ml water of were blended with a food processor (Panasonic) for 5 minutes. The meat extract was then homogenized with Polytron PT-MR2000 for 10 minutes. The meat extract was incubated into shaking water bath Taitec Personal-11 for 30 minutes at 70^{o}C, and then the extract was cooled in a room temperature.

Hydrolysis Protein by Pepsin

Meat extract was adjusted into pH 2.0 with 1 M of HCl. Pepsin (porcine stomach mucosa) was added into the meat extract at amount of 0.01g. After 2 hours of incubation at 37^{o}C, the hydrolysate pH was adjusted to 7.0 with 1 M of NaOH, and the hydrolysis by pepsin was terminated by heating at 95^{o}C for 10 min, followed by cooling in ice.

Hydrolysis Protein by Trypsin and Chymotrypsin

The trypsin and chymotrypsin were added to the peptic hydrolysate at amount of 0.01g each. The peptic hydrolysate was then incubated at 37^{o}C for 2 hours. The hydrolysis by trypsin and chymotrypsin was terminated by heating at 95^{o}C for 10 min, followed by cooling in ice. Protein hydrolysate was then filtered by a 1 ml syringe set by a filter (Advantec Dismic-25ES, cellulose acetate 0.45 µM, Toyo Roshi Co., Japan). Filtrate of meat extract was collected for future experiments.

Protein Concentration

Protein concentration was analyzed spectrophotometically using Biuret method at the

wave length of 540 nm (Owasu-Apeten, 2002). Protein concentration was obtained by comparing the absorbance of sample and the absorbance of bovine serum albumin (BSA).

***Sodium Dodecyl Sulfate*-Polyacrylamide Gel Electrophoresis (SDS-PACE)**

A 500 mL of protein hydrolysate was added by 500 ml of *sodium dodecyl sulfate* (SDS) buffer, and then was boiled for 5 min at 100°C. SDS-PAGE analysis used separating gels with the concentration of acrylamide of 12 and 17%. Molecule weights of proteins were estimated by using a protein marker.

ACE Inhibitory Activity

ACE inhibitory activity was determined using the method of Cushman and Cheung (1971). A sample solution of synthetic peptide at the amount of 6 ml of a particular concentration was mixed with 50 mL of 7.6 mM HHL containing 100 mM borate buffer (pH 8.3) and 608 mM NaCl. Before reacting with ACE, sample was pre-incubated for 5 min at 37°C in a water bath. The reaction was initiated by the addition of 20 mL of 60 mU/mL ACE dissolved in borate buffer (pH 8.3) containing 200 mM boric acid and 50 mM sodium tetraborate, and the mixture was incubated for 30 minutes at 37°C. The reaction was stopped by adding 554 mL of 0.1 M HCl, except for the blank which had already added by 554 mL of 0.1 M HCl before the incubation. The product (hippuric acid) of the reaction was extracted by addition of 1.5 mL of ethyl acetate and vigorous mixing, and then the mixture was centrifuged at 2500 rpm (1170 g) for 15 minutes. One mL of supernatant was collected into another tube and was dried at 100°C for 10 minutes. The tube was cooled at room temperature for 10 minutes and then 1 mL of 1 M NaCl was added into it. It was also stirred with a vortex mixer for 30 seconds. The hippuric acid liberated by ACE was photometrically determined at 228 nm. The ACE inhibitory activity was calculated with the following formula:

Inhibition = [(Ec - Es)/(Ec - Eb)] x 100%

Wherte:

Ec : Control absorbance

Es : Sample absorbance

Eb: Blank absorbance

The concentration of ACE inhibitors needed to inhibit 50% of ACE activity was defined as the IC value (IC_{50}).

RESULTS AND DISCUSSION

Protein Concentration

Protein concentrations of Bali cattle, Kacang goat, Indonesian native chicken, and local duck were illustrated in Figure 1. The results showed that the protein concentration of Bali cattle, Kacang goat, Indonesian native chicken, local duck increased significantly after being hydrolyzed by pepsin, trypsin, and chymotrypsin. The protein concentration of meat extract of Bali cattle, Kacang goat, Indonesian native chicken, and local duck before being hydrolyzed were 14.00; 17.13; 18.20; and 12.07 mg/mL, respectively, whereas the protein concentration of protein hydrolysate were 32.44; 31.57; 35.57; and

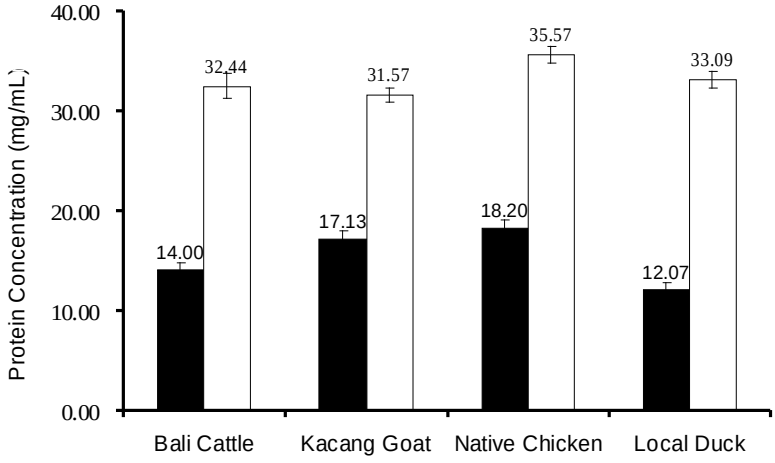

Figure 1. Mean of Protein Concentration of Meat Extract (■) and Hydrolysate of Meat Protein (□) of Bali Cattle, Kacang goat, Native Chicken, and Local Duck

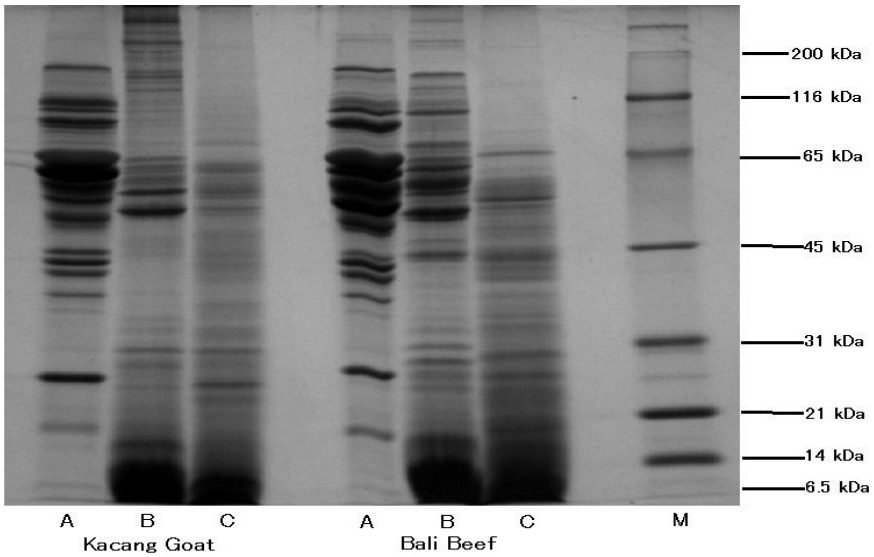

Figure 2. SDS-PAGE Analysis of Protein of Bali Cattle and Kacang Goat Meat Before Hydrolysis (A) and After Hydrolysis by Pepsin (B), Trypsin and Chymotrypsin (C), and Marker (M).

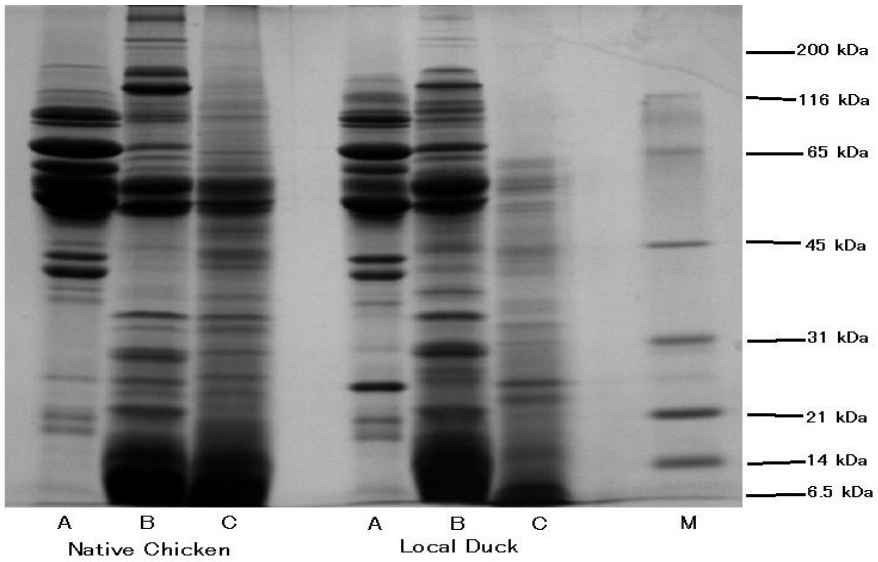

Figure 3. SDS-PAGE Analysis of Protein of Native Chicken and Local Duck Meat before Hydrolysis (A) and After Hydrolysis by Pepsin (B), Trypsin and Chymotrypsin (C), and Marker (M).

33.09 mg/mL, respectively. The increase of protein concentration mainly doubled after being hydrolyzed by pepsin, trypsin, and chymotrypsin. Hydrolysis of protein produced simpler peptides that are more soluble in water. Thus, the protein concentration of protein hydrolysate was higher than the protein concentration of the meat extract. It showed that proteases (pepsin, trypsin, and chymotrypsin) hydrolyzed protein into more peptides, compared to before being hydrolyzed by proteases. The amount of peptides of protein hydrolysate was higher so that the water-soluble protein of hydrolysate was also higher. Beck et al. (1973) reported that trypsin hydrolyzed protein into peptides by cleaving peptide bonds on carboxyl groups of arginine or lysine, while chymotrypsin hydrolyzed protein into peptides by cleaving peptide bonds on carboxyl groups of tyrosine, tryptophan and phenylalanine.

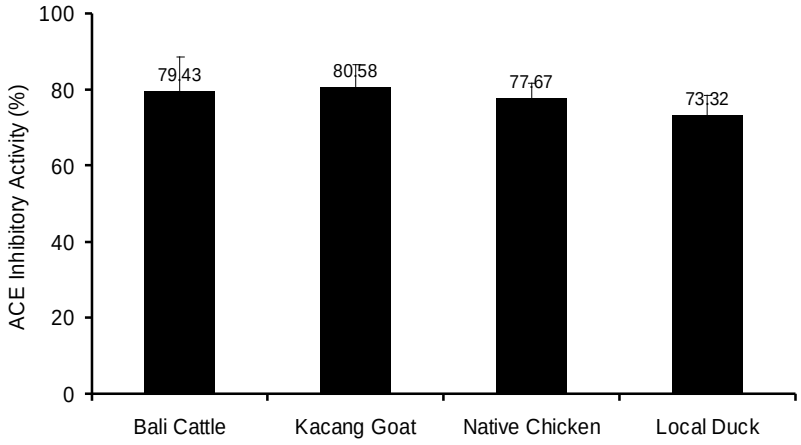

Figure 4. ACE Inhibitory Activity (%) of Hydrolysate of Meat Protein of Bali Cattle, Kacang Goat, Native Chicken, and Local Duck.

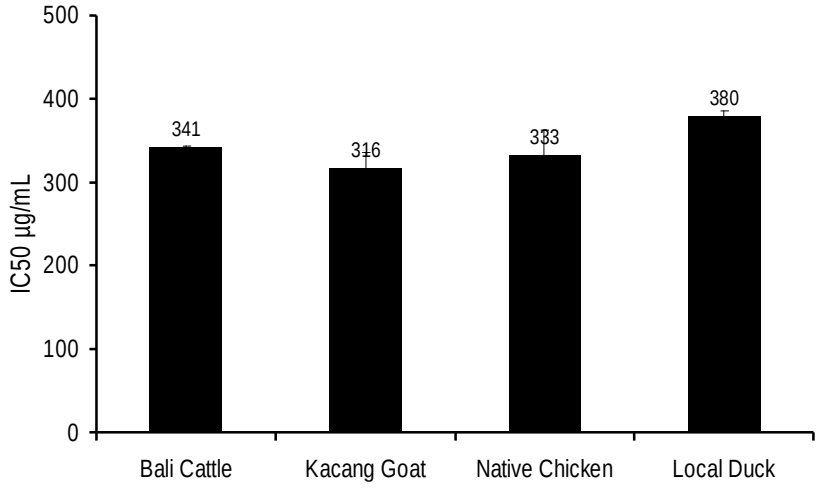

Figure 5. ACE Inhibitory Activity (IC_{50}) of Hydrolysate of Meat Protein of Bali Cattle, Kacang Goat, Native Chicken, and Local Duck.

Protein Confirmation by SDS-PAGE

Protein confirmation by SDS-PAGE of meat extract and hydrolysate protein by pepsin, trypsin and chymotrypsin of Bali cattle and Kacang goat was illustrated in Figure 2. While the protein confirmation by SDS-PAGE of meat extract and hydrolysate protein by pepsin, trypsin and chymotrypsin of Native chicken and local duck was illustrated in Figure 3. SDS-PAGE analysis of those meat showed that hydrolyzing protein produced many smaller sizes of peptide compared to peptide of meat extract protein, as being showed that bands of the peptides of protein.

SDS-PAGE analysis also showed that hydrolysis of protein by pepsin, trypsin and chymotrypsin produced many simpler peptides compared to meat extract. It was showed that bands with higher molecular weight (MW) were not obtained in the hydrolysate protein. Based on the marker, the molecular weight of the meat extract of Bali cattle, Kacang goat, native chicken, and local duck ranged from 21 to 200 kDa and almost at 45 to 65 kDa. Whereas, the molecular weight of protein in Bali cattle, Kacang goat, native chicken, and local duck after being hydrolyzed by pepsin, trypsin and chymotrypsin ranged from 6.5 to 65 kDa, and almost at 6.5 to 21 kDa.

ACE Inhibitory Activity

Meat extract of Bali cattle, Kacang goat, native chicken, and local duck did not show ACE inhibitory activity. ACE inhibitory activity was supported by peptides resulted by hydrolysis of protein by protease. Meat extract did not contain any peptides supporting ACE inhibitory activity. Korhonen and Pihlanto (2006) said that such peptides are inactive within the sequence of the parent protein and can be released in three ways: (a) through hydrolysis by digestive enzymes, (b) through hydrolysis by proteolytic microorganisms and (c) through the action of proteolytic enzymes derived from microorganisms or plants.

ACE inhibitory activity of hydrolysate protein of Bali cattle, Kacang goat, native chicken, and local duck meat was illustrated at Figure 4 and Figure 5. ACE inhibitory activity of protein hydrolysate of Bali cattle, Kacang goat, native chicken, and local duck meat in percentage was 79.43, 80.58, 77.67, and 73.32%, respectively (Figure 4). While the ACE inhibitory activity of protein hydrolysate of Bali cattle, Kacang goat, native chicken, and local duck meat in IC_{50} was 341, 316, 333, and 380 µg/mL, respectively (Figure 5). Hydrolysate of protein of Bali cattle, Kacang goat, native chicken, and local duck meat hydrolyzed by pepsin, trypsin, and chymotrypsin had a high potential as an ACE inhibitor.

ACE inhibitory activity of hydrolysate of protein of Bali cattle, Kacang goat, native chicken, and local duck meat was resulted from simpler peptides inhibiting ACE in the process of liberating hippuric acid from hippuryl-L-hystidyl-L-leucine (HHL). Hydrolysis of meat protein by pepsin, trypsin, and chymotrypsin resulted many peptides. They played roles in inhibiting the ACE activity. ACE inhibitory activity of hydrolysate of protein of Bali cattle, Kacang goat, native chicken, and local duck meat was relatively high. Thus, the hydrolysate of protein of Bali cattle, Kacang goat, native chicken, and local duck meat had a high potential as an antihypertensive agent.

CONCLUSION

Hydrolysate of protein of Bali cattle, Kacang goat, native chicken, and local duck meat had a high potential in inhibiting ACE activity, so it will be potential in reducing blood pressure. Purification of peptides was needed to investigate the peptide sequence of ACE inhibitory peptides, and where the peptide sequences take place.

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