Amino Acids Profile of the Indonesian Endogenous Meats Antioxidant Peptides

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Abstract. This study conducted to characterization amino acids of the antioxidant bioactive peptides from Indonesian endogenous meats among them “ongole cross breed” beef, “Kacang” goat meat, “Mojosari” duck meat and native chicken meat. The research was conducted in the Lamongan district of East Java. The method was laboratory exploration. The variables observed included antioxidant activity, crude protein and amino acids profile with LC-MS/MS. The results of studies appears there are variation in antioxidant activity of various endogenous meats in Indonesia. The highest crude protein was obtained native chicken meat 23.88 ±0.65%. Furthermore the highest antioxidant activity was obtained “Kacang” goat meat 12.085±0.22 %. The amino acids profile also exhibit diversity with each other. Amino acids obtained are distributed evenly to the types of essential and non essential amino acids. Ongole cross breed beef content a better amino acids profile than others. The beef appears to have higher contents of leucine, lysine and arginine of essential category.

Keywords: amino acids, Indonesian endogenous, antioxidant bioactive peptides

1. Introduction
Indonesia is a country that has a variety of genetic resources, especially in its endogenous livestock. “Ongole cross breed” cattle, “Kacang” Goats, “Mojosari” Ducks and native Chickens are Endogenous animals that have existed for a long time. The population of “ongole cross breed” cattle 1.6 million head, “Kacang” goats 1.96 million head, “Mojosari” ducks 23.18 million head and native chickens 298,673 million head in 2016 [1].

There is a decrease in interest in raising endogenous animals in Indonesia, exploration of the advantages of endogenous livestock is needed to increase the motivation of farmers, one of which is through the exploration of the superiority of meat quality characteristics. the endogenous livestock raising system in Indonesia is still done non-intensively by grazing, so that the potential of the biochemical content of the meat has advantages.
Researchers in various countries study the bioactive content specially peptides in meat, this is done in line with human needs for healthy and good food. Pollution of environmental causes exposure to free radicals, so it takes a good source of antioxidants, especially from natural ingredients as well as a source of amino acids. They were assumed that antioxidant compounds from natural ingredients are very important. Antioxidant peptides can be bind of metals and potential hydrogen donor to stop the chain of free radical reactions in the body [2]. It has never been studied about the antioxidant activity associated with amino acids profile in Indonesian endogenous meat.

2. Methodology
Sample of “ongole cross breed” beef, “Kacang” goat meat, “Mojosari” duck meat and native chicken meat obtained from Slaughterhouse at Lamongan District of East Java Indonesia. (NH₄)₂CO₃ (Merck), Ethanol 40% (Merck), petroleum ether (Merck), NaSO₄ anhidrat (Merck), CuSO₄ (Merck), H₂SO₄ (Merck), Zn (Merck), NaOH 40% (Merck), HCL 0,1 N (Merck), indicator of methyl red 15 (Merck), NaOH 0,1 N (Merck), phosphate buffer (0,2 mmol/L, pH 7,2) (Merck), aquadest (Merck), Bradford reagent (Sigma), Bovine Serum Albumine (BSA) (Sigma), Asam asetat glasial (Merck), and DPPH 0,1 mM (Sigma).

Analitical balance (Mettler PM 200 Switzerland), Vortex (Janke 43480), refrigerated (Panasonic), magnetic stirrer 3,5 cm (Labinco), centrifuge Refrigerated Mikro 22 R (Hettich), dan pH meter CG.818T (Schoot Gerate), Water bath digital tipe J.26, Shaker (Hettich), Oven (Hettich), eppendorf, micropipett 10µl - 1000 µl (Hamilton syringe), beaker glass (Pyrex), blue tip, yellow tip, micropipett 10µl - 1000 µl (Hamilton syringe), kuvet, spektrofotometer UV-2100 (Unico), SPE atau μSPE plate OASIS HLB 60 mg (Waters Corp, Milford, MA, sistem kromatografi LC-MS/MS), waters biner pompa (Waters, USA). Coloum ACQUITY UPLC ™ BEH HILIC (100 mm x 2,1 mm id, 1,7 mm), PDA detektor ACQUITY UPLC ™ and spectrometer massa TQD ™ (Waters, USA), electrospray (ESI), mortar agate, hidrolic vacuum (Hettich).

Crude Protein Analysis
Total crude protein content measured by AOAC methode (2005: 992.15).

Peptides Extraction
The peptides extraction used a combination of methods of [3], [4] and [5].

Measurement of Antioxidant Activity
Analysis of antioxidant activity was performed by DPPH method [6] the sample was taken as 0.5 mL, Added 2.7 mL DPPH 20 ppm then shaken, incubated in dark conditions for 30 min, Measured absorbance at 517 nm wavelength, DPPH as negative control measured its absorbance at 517 nm wavelength, Calculated % Antioxidant activity with % AA = Abs. DPPH - Abs. Sample x 100%

Amino Acids Analysis
Amino acids profile of Indonesian Endogenous meat measured by LC MS/MS [7].

3. Results and Discussion
Crude Protein
The highest crude protein was obtained native chicken meat 23.88 ±0.65%. The research was still in progress for the better understanding of the probable differences among the nutritional value of different meat cuts, variant animal species and breeds. It is quite evident from the previous research that the meat having lesser connective tissues is likely to have low scores of digestion and absorption. Moreover, the meat having more connective tissues are supposed to have less contents of essential amino acids, which make them less nutritious as compared to the meat piece having lesser connective tissues and results in more digestibility and nutritional value [8].
Based on the results of crude protein analysis of Indonesian endogenous meat are showed in the Table 1.

### Table 1. Crude protein of indonesian endogenous meat

| Sex of Animals | Muscle Location | Sample | “Ongole Cross breed” beef | “Kacang” goat meat | “Mojosari” duck meat | Native chickens meat |
|----------------|----------------|--------|---------------------------|--------------------|----------------------|----------------------|
| Male           | Breast, Loin, raw | 1      | 21.45                     | 20.19              | 19.20                | 24.10                |
|                |                 | 2      | 21.61                     | 19.88              | 20.03                | 22.80                |
|                |                 | 3      | 20.50                     | 20.04              | 19.17                | 24.20                |
|                |                 | 4      | 21.15                     | 19.96              | 19.11                | 23.81                |
|                |                 | 5      | 20.81                     | 20.35              | 20.01                | 24.50                |
|                |                 | Average±STDev | 21.10±0.46             | 20.08±0.19         | 19.50±0.47           | 23.88±0.65           |
| Female         | Breast, Loin, raw | 1      | 19.21                     | 18.55              | 20.15                | 22.30                |
|                |                 | 2      | 18.97                     | 19.28              | 21.1                 | 23.10                |
|                |                 | 3      | 19.46                     | 18.98              | 20.16                | 22.40                |
|                |                 | 4      | 19.89                     | 19.89              | 20.05                | 22.90                |
|                |                 | 5      | 20.02                     | 18.11              | 19.56                | 22.80                |
|                |                 | Average±STDev | 19.51±0.44             | 18.96±0.68         | 20.20±0.56           | 22.70±0.34           |
| Female         | Thigh, Shank, round, raw | 1      | 18.64                     | 18.31              | 19.87                | 22.70                |
|                |                 | 2      | 18.90                     | 18.54              | 21.16                | 21.20                |
|                |                 | 3      | 19.53                     | 19.11              | 20.55                | 21.50                |
|                |                 | 4      | 19.69                     | 18.77              | 19.75                | 21.10                |
|                |                 | 5      | 18.08                     | 18.28              | 19.7                | 22.80                |
|                |                 | Average±STDev | 18.97±0.66             | 18.60±0.35         | 20.21±0.63           | 21.86±0.83           |

### Antioxidant Activity

The following is the result of an analysis of antioxidant activity were showed in Table 2.

### Table 2. Antioxidant Activity of Indonesian Endogenous Meat

| Sex of Animals | Muscle Location | Sample | Average±STDev Antioxidant Activity (%) |
|----------------|----------------|--------|----------------------------------------|
| Male           | Breast, Loin, raw | 1      | 11.04±0.35 11.96±0.26 9.629±0.21 8.027±0.18 |
|                | Thigh, Shank, round, raw | 1      | 11.60±0.40 12.08±0.22 10.165±0.41 8.266±0.30 |
| Female         | Breast, Loin, raw | 1      | 10.429±0.37 12.299±0.19 8.976±0.25 7.721±0.25 |
|                | Thigh, Shank, round, raw | 1      | 10.744±0.18 12.651±0.21 9.199±0.38 7.569±0.13 |

Studies appears there are variation in antioxidant activity of various endogenous meats in Indonesia. The highest antioxidant activity was obtained “Kacang” goat meat 12.085±0.22%. According previous research that antioxidant activity of indonesian endogenous duck meat is 9.652% [9]. The value is not difference with the results of this study. The diversity of amino acid composition in each local animal causes a difference in the value of antioxidant activity. The amino acid is able to donate hydrogen ions to reduce the nitrite group at DPPH [10].

### Amino Acids Profile

Amino acid Composotions testing is done by LC MS / MS or high performance liquid chromatography with triple quadrupole tandem mass spectrometry detector. The results of calibration
are done using the Single Point Matrix Based Calibration at RL (Reporting Limit) method. Tests were carried out on 3 selected samples in each stage of the study. The test results are presented in Table 3.

| Category       | Amino Acid       | Unit | RL | “Ongole Cross breed beef” | “Kacang goat meat” | “Mojosari duck meat” | Native chickens meat |
|----------------|------------------|------|----|--------------------------|-------------------|----------------------|---------------------|
| Essential      | Threonine        | mg/kg| 20.0| 42.8                     | 56.2              | 18.5                 | 20.9                |
|                | Leucine          | mg/kg| 20.0| 86.6*                    | 75.8*             | 74.8                 | 80.1                |
|                | Valine           | mg/kg| 20.0| 55.1                      | 47.2              | 55.3                 | 32.9                |
|                | Isoleucine       | mg/kg| 20.0| 50.7                      | 36.5              | 31.7                 | 40.6                |
|                | Histidine        | mg/kg| 20.0| 27.2                      | 29.6              | 22.4                 | 12.1                |
|                | Lysine           | mg/kg| 20.0| 82.1                      | 66.2              | 56.2                 | 71.8                |
|                | Tryptophan       | mg/kg| 20.0| 14.6                      | 21.4              | 27.4                 | 22.5                |
|                | Arginine         | mg/kg| 20.0| 64.9                      | 62.1              | 71.4                 | 89.1                |
|                | Phenylalanine    | mg/kg| 20.0| 41.3                      | 29.6              | 42.0                 | 36.3                |
|                | Cystine          | mg/kg| 20.0| 15.7                      | 22.3              | 14.8                 | 16.2                |
|                | Methionine       | mg/kg| 20.0| 22.4                      | 20.1              | 41.2                 | 23.8                |
| Non essential  | Glutamic Acid    | mg/kg| 20.0| 152.1*                    | 162.5*            | 149.7                | 172.5               |
|                | Hidroxyproline   | mg/kg| 20.0| 84.4                      | 29.1              | 64.1                 | 70.8                |
|                | Glutamine        | mg/kg| 20.0| 42.8                      | 38.3              | 51.0                 | 43.6                |
|                | Aspartic Acid    | mg/kg| 20.0| 89.2                      | 41.5              | 113.3                | 113.3               |
|                | Proline          | mg/kg| 20.0| 52.7                      | 52.4              | 42.3                 | 42.3                |
|                | Tyrosine         | mg/kg| 20.0| 33.2                      | 32.9              | 32.7                 | 20.9                |
|                | Alanine          | mg/kg| 20.0| 63.6                      | 31.1              | 32.1                 | 57.3                |
|                | Serine           | mg/kg| 20.0| 39.0                      | ND                | 34.8                 | 22.6                |
|                | Glycine          | mg/kg| 20.0| 72.9                      | 54.7              | 57.9                 | 61.5                |
|                | Asparagine       | mg/kg| 20.0| ND                        | 41.7              | ND                   | ND                  |
|                | Trans-4-proline  | mg/kg| 20.0| 22.5                      | 35.6              | 22.9                 | 18.4                |

RL : Reporting Limit; ND : Not detected, bellow RL; *: shows the highest value compared to the others

The beef meat appears to have higher contents of leucine, lysine and arginine of essential category. Studies have revealed that main reason of the difference in essential amino acids proportion lies with the breed, animal age, and muscle location [8]. Amino acid profile of “Kacang” goat meat is also identical to previous studies on “Saanen” goat meat [11].

4. Conclusions

Studies appears there are variation in antioxidant activity of various endogenous meats in Indonesia. The highest crude protein was obtained native chicken meat 23.88 ±0.65 % Futhermore the highest antioxidant activity was obtained “Kacang” goat meat 12.085±0.22 %. The amino acids profile also exhibit diversity with each other. Amino acids obtained are distributed evenly to the types of essential and non essential amino acids. Ongole cross breed beef content a better amino acids profile than others. The beef appears to have higher contents of of leucine, lysine and arginine of essential category.

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