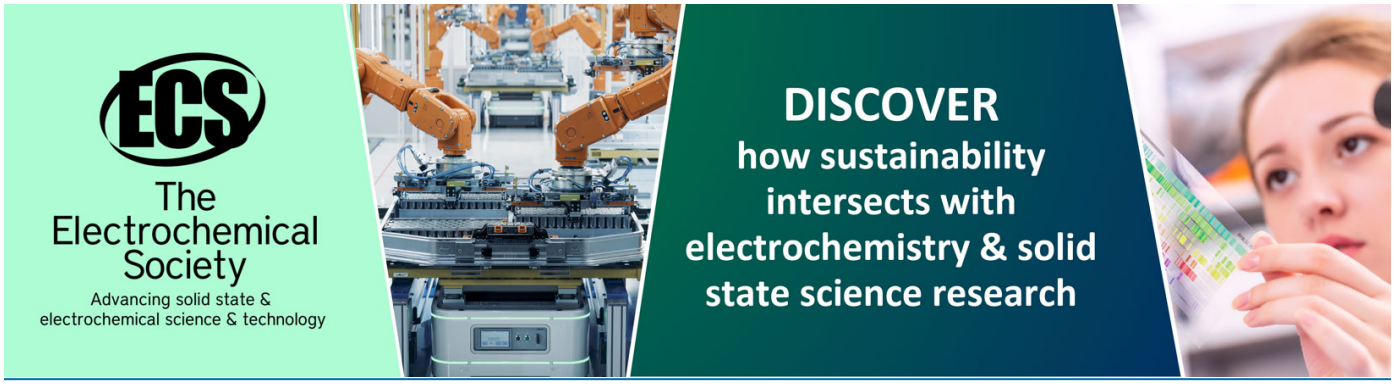
# Amino acids characterization of the Ongole crossbreed cattle edible head skin using HPLC





# Amino acids characterization of the Ongole crossbreed cattle edible head skin using HPLC

**E Susanto***, **A Fadlilah, M F Amin, Wahyuni, E Sutanto, I M Handayani, R Sholiha, G F Yolanda and N Khasanah**

Faculty of Animal Science, University of Islam Lamongan, Lamongan, Indonesia.

E-mail: *edysusanto@unisla.ac.id

**Abstract.** This research purpose was characterization of amino acids using HPLC at the edible head skin as edible by-product of ongole crossbreed cattle (OCC). The materials were the male and female head skin of OCC and the method was survey and exploration with analysis of laboratorium. The variables was amino acids profile with HPLC of edible head skin of OCC. The results of studies shown The edible head skin of OCC amino acids profile was the higher contents of *L-arginin, glysine,* and *L-proline* as essential category of amino acids and *L-glutamic acid* as non-essential amino acids. There were several defference between male and female amino acids profile of the OCC edible head skin, including: *L-glutamic acid, L-alanin, L-arginin, glysine, L-lisin, L-aspartid acid,* and *L-proline of* female OCC was the higher than male OCC. The peak absorption of mass spectofotometry between samples in males and females shows different.

## 1. Introduction

The Ongole crossbreed cattle (OCC) was a breed that quite widely raised in Indonesia and comes from the descendants of native Javanese cattle. The population of OCC were 1,6 million head in 2016 [1]. The OCC had a characteristic greyish-white colour, had a hump and was adaptively in dry climates so that it was suitable to be raise in Indonesia. The purposes of OCC producing was fulfil the needs of meat consumption. However, beef still tends to be expensive, so the by-products are still consumed by the Indonesian people, including head skin. The scalp of an animal has a high collagen content around 89%. However, it is important to remember that the use of bovine scalp in food must be done with care and care must be taken to ensure that the product was properly prepared and served to avoid any risk of contamination or illness.

The studies have been conducted related to the quality of meat and by product of local Indonesian livestock including chicken feet [2-5], Kacang goat [6]. There were several analyses used to characterize amino acids and bioactive peptides in foodstuffs, including using HPLC [7-9]. It has never been studied about the amino acids profile using HPLC in Indonesian indigenous edible by-product especially cattle head skin. So, research on amino acids on the scalp of cattle using the HPLC method really needs to be done.

## 2. Materials and methods

### *2.1. Materials*

Sample of “Ongole cross breed” head skin from male and female of Slaughterhouse at Lamongan

District of East Java Indonesia. The materials used in this study were: standard amino acids namely: L-aspartic acid, L-glutamic acid, L-histidine, L-serine, L-arginine, L-threonine, glycine, L-alanine, L-proline,L- cysteine, L- tyrosine, L-valine, L-methionine, L- lysine, L- isoleucine, L-phenylalanine, L-leucine, tryptophan, bovine gelatine standard (sigma aldrich), porcine gelatine standard (sigma) Aldrich), internal standard AABA (alpha amino butyric acid). Accq-fluor borate, fluorine reagent A, HPLC grade acetonitrile HCL, and aquabidest.

*2.2. Equipment*

The tools used in this study were a set of High Performance Liquid Chromatography (Waters) devices, a refrigerator, an oven, an analytical balance, a homogenizer, a hot plate, a measuring flask, an Erlenmeyer flask, a watch glass, a test tube with a lid, a micro pipette and tip, spatula, measuring cup, beaker glass, vortex, incubator, pipette, tweezers, syringe filter, thermometer, filter membrane 0.45 um vial, porcelain cup, stir bar

*2.3. Amino acids analysis*

Analysis of amino acids profile of OCC edible head skin measured by HPLC [10] in modification by laboratory of PT. Saraswanti Indo Genetech.

## 3. Results and discussion

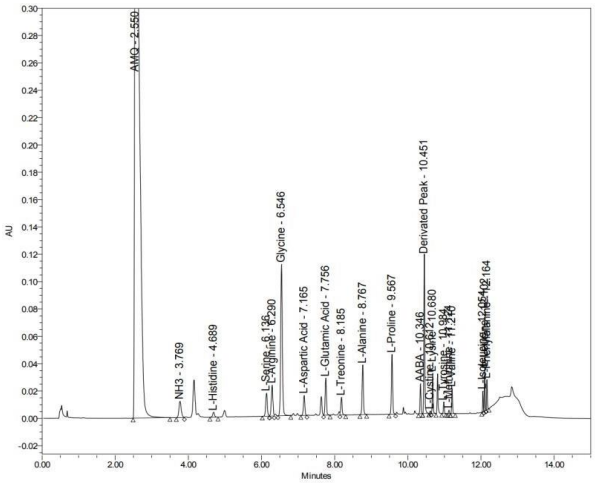
The amino acid profile omposition testing was performed by HPLC or high-performance liquid chromatography with a double quadrupled tandem mass spectrometry detector. The head skin of male and female OCC was tested on 2 scalp samples selected at each stage. The results of the amino acid profile of OCC head skin were in Table 1.

**Table 1.** The Amino acids profile of male and female OCC head skin.

| No | Parameter | Unit | Male | | Female | | Limit of detection | Method |
|---|---|---|---|---|---|---|---|---|
| | | | Simplo | Duplo | Simplo | Duplo | | |
| 1 | L-Serine | mg / kg | 26783.15 | 26928.64 | 9497.98 | 9539.66 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |
| 2 | L-Glutamic Acid | mg / kg | 39479.37 | 39651.04 | 30646.03 | 30778.23 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |
| 3 | L-Fenilalanine | mg / kg | 10774.64 | 10749.36 | 8566.4 | 8607.45 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |
| 4 | L-Isoleucine | mg / kg | 8928.83 | 8958.46 | 8554.77 | 8560.97 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |
| 5 | L-Valine | mg / kg | 14599.52 | 14693.37 | 9490.26 | 9513.81 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |
| 6 | L-Alanine | mg / kg | 19362.94 | 19497.81 | 12318.96 | 12400.45 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |
| 7 | L-Arginine | mg / kg | 35410.35 | 35555.69 | 14290.1 | 14350.36 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |
| 8 | Glycine | mg / kg | 45435.09 | 45709.34 | 12703.59 | 12748.24 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |
| 9 | L-Lycine | mg / kg | 18321.22 | 18432.74 | 16885.6 | 16966.15 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |
| 10 | L-Aspartic Acid | mg / kg | 17814 | 17921.53 | 16829.86 | 16906.65 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |
| 11 | L-Leucine | mg / kg | 20985.35 | 21095.35 | 16794.91 | 16873.61 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |

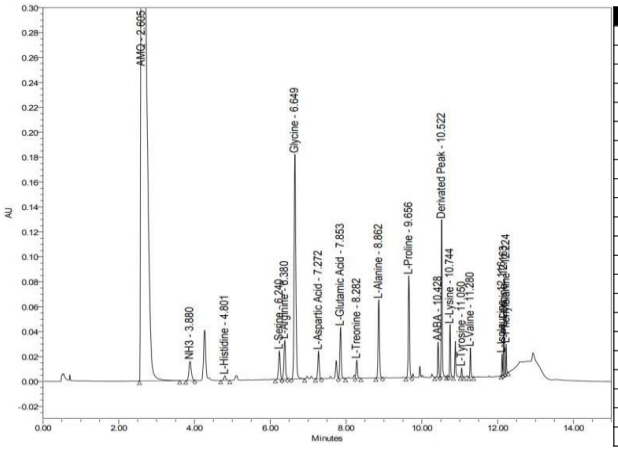
| No | Parameter | Unit | Male | | Female | | Limit of detection | Method |
|---|---|---|---|---|---|---|---|---|
| | | | Simplo | Duplo | Simplo | Duplo | | |
| 12 | L-Tyrosine | mg / kg | 9399.95 | 9456.65 | 6694.66 | 6713.21 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |
| 13 | L-Proline | mg / kg | 30790.08 | 30979.56 | 9456.72 | 9498.65 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |
| 14 | L-Threonine | mg / kg | 17198.2 | 17282.36 | 10176.44 | 10219.38 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |
| 15 | L-Histidine | mg / kg | 4466.88 | 4479.66 | 4629.13 | 4625.29 | - | 18-5-17/MU/SMM-SIG(HPLC-PDA) |

There were two types of amino acids that consist of essential amino acids and non-essential amino acids. The non-essential amino acids were amino acids that could be made in the body were also known as endogenous amino acids. Whereas essential amino acids were amino acids that could not be make in the body and could only be obtained by consuming food that containing protein [11]. Amino acids consists of 20 types which include **8** essential amino acids for adult consists of lysine, isoleucine, phenylalanine, leucine, threonine, methionine, tryptophan, and valine. **2** essential amino acid were arginine and histidine acids for children, and **10** non-essential amino acids consisting of alanine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, tyrosine, proline, glutamine and serine [12]. The head skin of OCC amino acids profiles was the higher contents of *L- arginine, L-proline* and *L-glycine,* as essential category of amino acids and *L-glutamic acid* as non-essential type of amino acids. *L-arginine* was an essential amino acid that plays a role in protein formation, and could be converted into nitric oxide which useful in dilating blood vessels and increasing blood flow. Glycine was a non-essential amino acid that important for collagen synthesis and the formation of body tissues. *L-proline* was a non-essential amino acid involved in collagen synthesis and the formation of body tissues. Studies had shown that head skin of OCC contains many essential and non-essential amino acids, including L-arginine, glycine, and L-proline. The content of amino acids in OCC beef scalp could vary depending on the processing and storage methods. However, OCC bovine scalp was a good source of important amino acids, especially for people who eat a meat-based diet. There were several difference between male and female amino acids profile of the OCC head skin, including *: L-glutamic acid, L-alanine, L-arginine, glycine, L-lysine, L-aspartid acid, and L-proline* from female OCC higher than male OCC. Studies had shown that the main reason for the difference in the ratio of essential amino acids was the age of the animals of the breed and the location of the muscles [13]. Laboratory analysis using HPLC showing chromatogram data as Figure 1-2.




| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | AMQ | 2.550 | 4174245.67 | 76.57 | 519440 |
| 2 | NH3 | 3.769 | 52077.33 | 0.96 | 11818 |
| 3 | L-Histidine | 4.689 | 12843.23 | 0.24 | 3625 |
| 4 | L-Serine | 6.136 | 52245.43 | 0.96 | 16769 |
| 5 | L-Arginine | 6.290 | 67448.44 | 1.24 | 22480 |
| 6 | Glycine | 6.546 | 348857.11 | 6.40 | 110766 |
| 7 | L-Aspartic Acid | 7.165 | 39700.68 | 0.73 | 14574 |
| 8 | L-Glutamic Acid | 7.756 | 67268.43 | 1.23 | 26936 |
| 9 | L-Treonine | 8.185 | 32125.62 | 0.59 | 12817 |
| 10 | L-Alanine | 8.767 | 89395.43 | 1.64 | 36250 |
| 11 | L-Proline | 9.567 | 99826.58 | 1.83 | 43803 |
| 12 | AABA | 10.346 | 40653.85 | 0.75 | 21876 |
| 13 | Derivated Peak | 10.451 | 193578.63 | 3.55 | 116875 |
| 14 | L-Cystine | 10.612 | 2604.69 | 0.05 | 1663 |
| 15 | L-Lysine | 10.680 | 45470.08 | 0.83 | 29225 |
| 16 | L-Tyrosine | 10.984 | 13334.06 | 0.24 | 8454 |
| 17 | L-Methionine | 11.124 | 4079.52 | 0.07 | 2541 |
| 18 | L-Valine | 11.210 | 30278.57 | 0.56 | 18292 |
| 19 | L-Isoleucine | 12.054 | 19281.44 | 0.35 | 15006 |
| 20 | L-Leucine | 12.102 | 38895.07 | 0.71 | 31074 |
| 21 | L-Phenylalanine | 12.164 | 27577.80 | 0.51 | 21907 |
| Sum | | | 5451787.68 | | |

**Figure 1.** Chromatogram of amino acids profile of male OCC head skin.




| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | AMQ | 2.605 | 5141174.02 | 73.88 | 590109 |
| 2 | NH3 | 3.880 | 67898.56 | 0.98 | 15443 |
| 3 | L-Histidine | 4.801 | 12401.89 | 0.18 | 3521 |
| 4 | L-Serine | 6.240 | 68875.37 | 0.99 | 22781 |
| 5 | L-Arginine | 6.380 | 94066.61 | 1.35 | 31148 |
| 6 | Glycine | 6.649 | 575866.92 | 8.28 | 180244 |
| 7 | L-Aspartic Acid | 7.272 | 59433.09 | 0.85 | 22081 |
| 8 | L-Glutamic Acid | 7.853 | 100222.86 | 1.44 | 40939 |
| 9 | L-Treonine | 8.282 | 35271.29 | 0.51 | 14359 |
| 10 | L-Alanine | 8.862 | 153540.07 | 2.21 | 62662 |
| 11 | L-Proline | 9.656 | 181065.05 | 2.60 | 81548 |
| 12 | AABA | 10.428 | 51493.38 | 0.74 | 27989 |
| 13 | Derivated Peak | 10.522 | 206826.01 | 2.97 | 126116 |
| 14 | L-Cystine | 10.632 | | | |
| 15 | L-Lysine | 10.744 | 63757.94 | 0.92 | 41890 |
| 16 | L-Tyrosine | 11.050 | 10785.39 | 0.15 | 7093 |
| 17 | L-Methionine | 11.140 | | | |
| 18 | L-Valine | 11.280 | 38497.72 | 0.55 | 23320 |
| 19 | L-Isoleucine | 12.116 | 21755.99 | 0.31 | 17056 |
| 20 | L-Leucine | 12.163 | 46336.84 | 0.67 | 36956 |
| 21 | L-Phenylalanine | 12.224 | 29453.12 | 0.42 | 23407 |
| Sum | | | 6958722.12 | | |

**Figure 2.** Chromatogram of amino acids profile of female OCC head skin.

Chromatography was a general term for various methods of separation based on samples between the moving phase which can be gas or liquid, and the stationary phase which can be liquid or solid. This chromatography was discovered by Tswett in 1903 [9]. HPLC (High Performance Liquid Chromatography) was the method of choice for the analysis of amino acids because it was a versatile method, had a high capacity, and could be trusted. The most amino acids did not have absorption in either the ultraviolet or visible regions, they could not be detected using the UV-Vis spectrophotometer detector which was the most widely used detector in HPLC. Several attempts had been made to develop derivatization procedures that mad amino acids easier to detect. A derivative reagent must have the following conditions: a) the resulting product must be able to absorb either ultraviolet or visible light or be able to form fluorescent compounds so that it could detected by spectrofluorometric, b) the derivatization process must be fast and produce the largest possible product and c) derivatized products must be stable during the derivatization and detection processes [14]. HPLC was most often used to determine levels of certain compounds such as amino acids, nucleic acids, proteins in physiological fluids, determine levels of active drug compounds, process by-products of synthetic processes, monitor samples originating from the environment. , purify a compound in a mixture [15]. Chromatography was a technique in which solutes or solutes were separated by differences in elution rates, because these solutes pass through a chromatographic column. The separation of the solutes was governed by the distribution of the solute between the mobile and stationary phases. The mobile phase must have pure properties, not react with the packaging, suit the detector, be able to dissolve the sample, have a low viscosity, allow recovery of the sample and the price is quite affordable [9].The peak absorption of mass spectrophotometry between samples in males and females shows different.. Based on the results of the amino acid characterization study, the OCC scalp amino acid characterization was recommended using HPLC because it was simple and compatible. Amino acid analysis of OCC edible head skin using HPLC had the following advantages: high masssamples, non-volatile molecules, low fragmentation, and direct analysis of liquids.

## 4. Conclusions

There were several difference between male and female amino acids profile of the OCC edible head skin, including: *L-glutamic acid, L-alanin, L-arginin, glysine, L-lisin, L-aspartid acid,* and *L-proline* of female OCC was the higher than male OCC. Laboratory analysis using HPLC was suitable for using to characterize amino acids in the OCC edible head skin.

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