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FULL PAPER

Effect of different slaughtering methods on metabolites of broiler chickens using Ultra High-Performance Liquid Chromatography-Time of Flight-Mass Spectrometry (UHPLC-TOF-MS)

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Abstract

Islamic study defined Halal meat as a "thoyyiban" (clean) food source. Halal meat is produced by following slaughtering procedure as determined by the Islamic jurisprudence. Slaughtering methods have gained a worldwide discussion as animal welfare becomes a concern. However, there is lacking of scientific facts to prove which slaughtering methods produce better physiological effects on animals from metabolomics view. Therefore, metabolomics approach by Liquid Chromatography-Time of Flight-Mass Spectrometry (LC-TOF-MS) was used in this study to understand how the metabolites in poultry change when subjected to different slaughtering processes. The broiler chickens were subjected to Halal (Islamic tradition) and non-Halal slaughtering method (neck poking) where pectoral major muscle tissues from the slaughtered meat were selected for UHPLC-TOF-MS analysis. Metabolome data highlighted multiple pathways affected by slaughtering methods including glucose, amino acid, inosine, hypoxanthine and arginine. Higher utilization of energy in non-Halal slaughtering process was observed as indicated by the increase of gluconeogenesis and amino acid breakdown. The result from this study indicated that the method of slaughter affects the metabolites profile of poultry.

1. Introduction

The definition of food security includes the accessibility to nutritious food (Ayala and Meier, 2017). This encompasses the definition of halal and Thoyyiban according to Islam. In developing halal economic sector, the foods or products must meet the halal and Thoyyiban aspects. This requires procedures that must be followed and monitored along the supply chains beginning from farm to fork (Asa, 2017). In Malaysia, food products that are certified with halal signifying the products are permissible and acceptable due to the whole concept of halal determined by Islamic jurisprudence which also includes hygiene, sanitation and safety aspect. However, the production methods (for instance slaughtering method) and distribution of food products (halal foods distribute with non-halal foods during transportation) create numbers of issues. Disputes on various slaughtering methods spring up when there is a potential of slaughtered meat did not meet the halal requirement and Halal slaughtering method is labeled as inhumane.

Previous research conducted by Mohamed and Mohamed (2012); Wong (2014); Hafiz *et al*. (2015); and Salwani *et al*. (2015) discovered different slaughtering methods including halal slaughtering method and non-halal slaughtering method did affect the physical quality of the chicken meat in term of pH, color, water holding capacity, lipid oxidation, haem iron content, texture and mineral content. However, there are no scientific facts of metabolites profiles on chicken subjected to different slaughtering methods. Therefore, this study is aimed to elucidate the effect of halal and non-halal slaughtering method on chicken metabolite profile using metabolomics fingerprinting.

2. Materials and methods

2.1 Ethics statement

This study was carried out in strict accordance with

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the recommendations. The procedure to conduct the study was approved by the Animal Ethics Committee of Universiti Sains Islam Malaysia.

2.2 Research methodology

The study was carried out at Universiti Sains Islam Malaysia (USIM), Nilai. Two groups of male chickens, (*Gallus gallus domesticus)* (halal and non-halal slaughtering method), aged 60 days with approximately the same weight (2 kg) were purchased from a local farm in Kajang. Each group represented by four chickens.

2.3 Halal slaughtering method

The chickens were slaughtered according to the Islamic traditions by severing the jugular veins, carotid arteries, trachea and the esophagus. This method was performed without stunning.

2.4 Non-halal slaughtering method

Chickens in this group were slaughtered by using a sharp-pointed object to poke the neck of the chicken to create a small hole for blood drainage. After slaughter, chickens were immediately processed.

2.5 Metabolic extraction

Metabolite extraction was carried out by using the method described by González-Peña *et al*. (2017) with modifications. The tissue of pectoralis major muscle was homogenized to a fine powder by rapid freezing in liquid nitrogen. The freeze-dried chicken powder (250 mg) was extracted with 1 mL cold methanol/water (1:1). The sample was centrifuged for 10 mins at 14000 rpm at room temperature. Supernatants was collected in 1.5 mL centrifuge tubes (Eppendorf) and stored in -80˚C until further analysis.

2.5.1 Liquid Chromatography-Time of Flight–Mass Spectrometry analyses

UHPLC was performed on ACQUITY UPLC I-Class system from Waters (Manchester, UK), consisting of a binary pump, a vacuum degasser, an autosampler and a column oven. Compounds were chromatographically separated using a column ACQUITY UPLC HSS T3 (100 mm x 2.1 mm x 1.8 µm) also from Waters, maintained at 40°C. A linear binary gradient of water (0.1% formic acid) and acetonitrile (mobile phase B) was used as mobile phase A and B respectively. The mobile phase composition was changed during the run as follows: 0 min, 1% B; 0.5 mins, 1% B; 16.00 mins, 35% B; 18.00 mins, 100% B; 20.00 mins, 1% B. The flow rate was set to 0.6 mL/min and the injection volume was 1 µL. The UHPLC system was coupled to a Vion IMS QTOF hybrid mass spectrometer from Waters (Manchester, UK), equipped with a Lock Spray ion source. The ion source was operated in negative electrospray ionization (ESI) mode under the following specific conditions: capillary voltage, 1.50 kV; reference capillary voltage, 3.00 kV; source temperature, 120°C; desolvation gas temperature, 550°C; desolvation gas flow, 800 L/hr, and cone gas flow, 50 L/hr. Nitrogen (>99.5%) was employed as desolvation and cone gas. Data were acquired in high-definition MS^E ($HDMS^E$) mode in the range m/z 50 - 1500 at 0.1 s/scan. Thus, two independent scans with different collision energies (CE) were alternatively acquired during the run: a low-energy (LE) scan at a fixed CE of 4 eV, and a high- energy (HE) scan where the CE was ramped from 10 to 40 eV. Argon (99.999%) was used as collision-induced-dissociation (CID) gas.

3. Results and discussion

3.1 Carbohydrate metabolism

LC-TOF-MS system coupled with an ESI was used to obtain metabolomics data of halal and non-halal chicken meat. The preliminary data was displayed in LC-TOF-MS spectra (BPI Counts Vs Retention time, Figures 1 and 2). Metabolites detected in halal chicken and non-halal chicken and in both halal and non-halal chicken were illustrated in diagram (Figure 3). LC-TOF-MS spectra showed the presence of isomaltose in non-halal chicken meat (Figure 2), but no detection of isomaltose in halal chicken meat (Figure 1). This indicated that gluconeogenesis has been elevated in non-halal chicken meat since isomaltose is a byproduct of glucose metabolism.

It was assumed that non-halal chicken was under stress before the slaughtering process. Under stress condition, the body will respond by increasing the production and release of nutrients (Jasterbski *et al*., 2017). Glucose acts as the major energy source for most tissues notably the brain, thus it is considered as one of the essential nutrients (Svihus, 2014). The glucose can be supply systemically by two metabolic pathways, either by the breakdown of stored glycogen or through gluconeogenesis (Jasterbski *et al*., 2017).

Digestion of starch in poultry is the function of pancreatic amylase which is produced endogenously in the animal (Sanan, 2017). Figure 4 shows starch and sucrose metabolism pathway in chicken. The amylase will break the starch into shorter polymers called dextrin which can be further hydrolyzed into units of maltose, isomaltose and glucose (Donald *et al*., 2002). The maltose and isomaltose can be hydrolyzed from intestinal secretions producing the enzymes maltase and isomaltase that will further hydrolyze the carbohydrate

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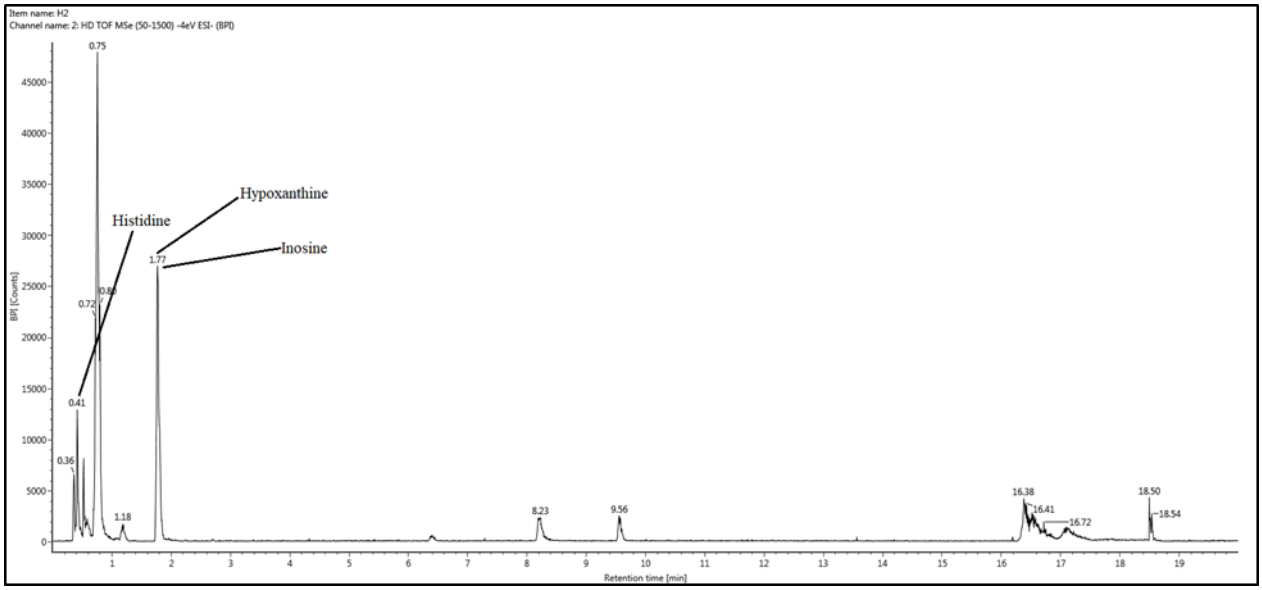

Figure 1. UHPLC-TOF spectra of Halal chicken pectoralis major muscle tissue.

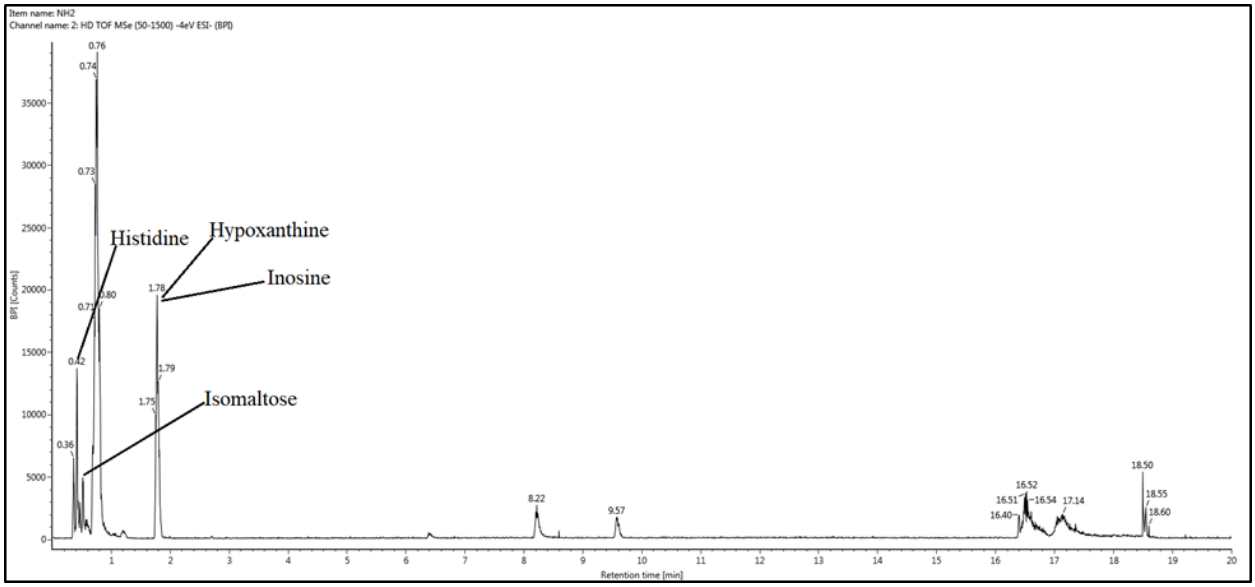

Figure 2. UHPLC-TOF spectra of non-Halal chicken pectoralis major muscle tissue

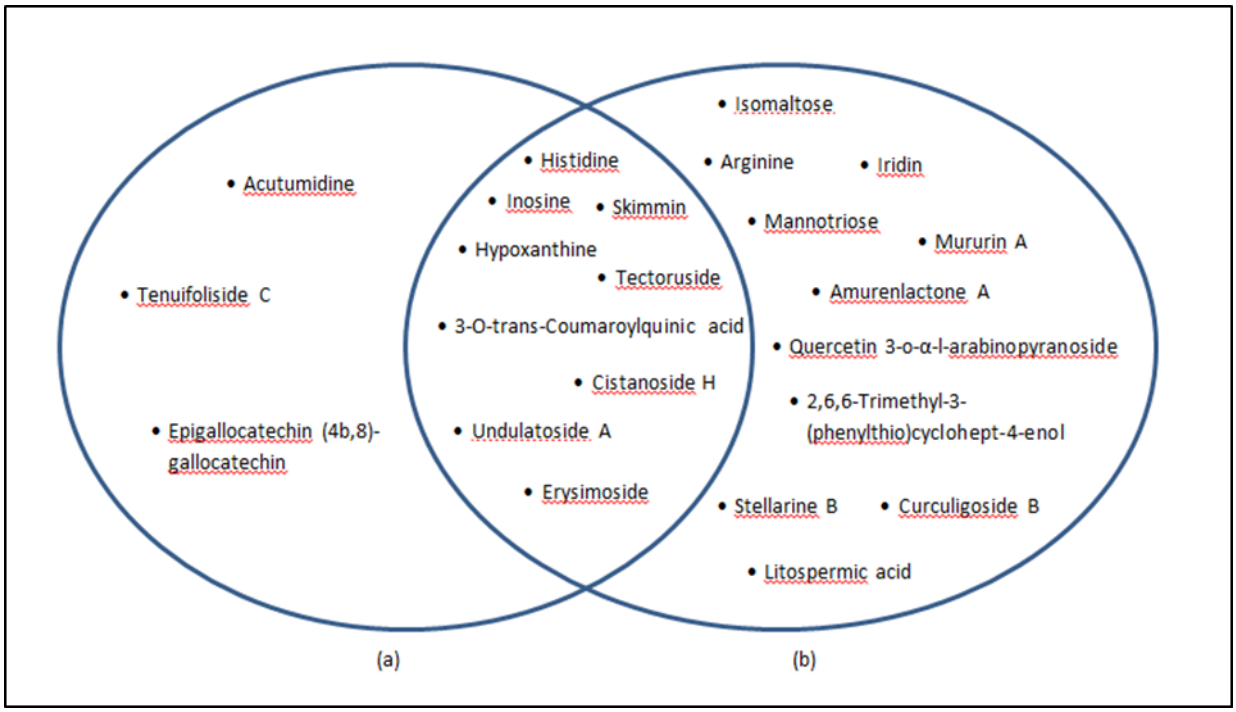

Figure 3. (a) Metabolites that are detected in halal chicken meat. (b) Metabolites that are detected in non-halal chicken meat. (c) Metabolites that are detected in both halal and non-halal chicken meat.

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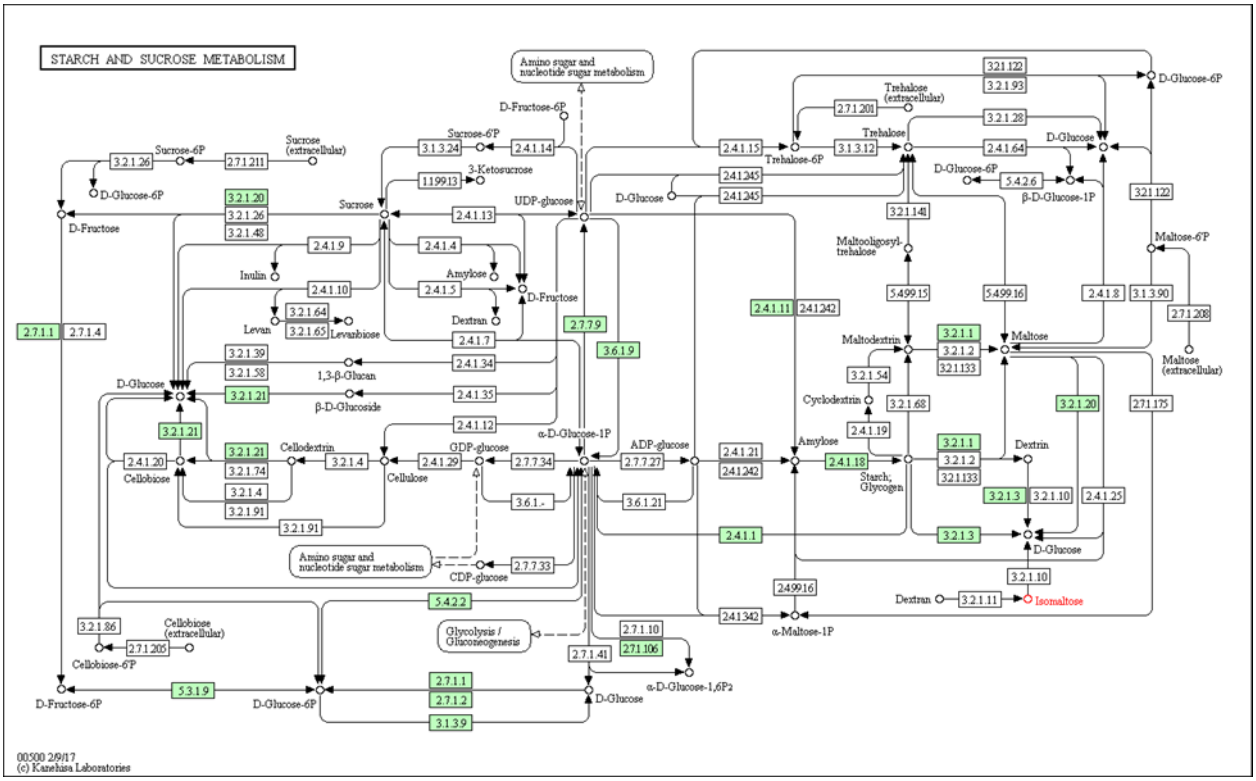

Figure 4. Starch and sucrose metabolism pathway in chicken (*Gallus gallus domesticus*)

into glucose units. The glucose then, can be actively absorbed in the intestine of poultry. Carbohydrate that is being absorbed can be metabolized: 1) for lipid formation, 2) for structural components formation such as chondroitin sulfate in cartilage, 3) for production of chemical energy that can be utilized by the poultry for productive purposes, and 4) during acute stressful situation where synthesizing of reserve glucose source called glycogen will occur which then will restore the depleted energy (Donald *et al*., 2002). Therefore, this study suggested that the increase of glucose export in non-halal chicken is due to higher energy utilization during the struggling of death and stress faced by the chicken.

3.2 Amino acid metabolism

LC-TOF-MS spectra showed a lower concentration of histidine in non-halal chicken meat (Figure 2) as compared to halal chicken meat (Figure 1). Histidine is categorized as basic amino acids including lysine and arginine. In poultry, these amino acids are considered essential as the body cannot produce these compounds endogenously (Jun *et al*., 2018). These amino acids exert their roles in several metabolite pathways. Metabolite roles of arginine are showed through the byproduct production including nitric oxide (NO), L-ornithine (L-Orn), polyamines, proline, glutamate, creatine and agmantine (Morris, 2004). Histidine is transformed to histamine by enzyme histidine decarboxylase and also histidine degraded to glutamate (Stifel and Herman, 1971).

Amino acid also plays its role as an energy source in poultry apart from functioning as substrates for proteins biosynthesis and physiological messengers in the body (Yamane *et al*., 2019). Amino acid can be grouped into ketogenic amino acid and glucogenic amino acids depending on byproducts produced after hydrolyzation. Amino acid that hydrolyzed to acetyl-CoA or acetoacetyl -CoA fall into the ketogenic amino acid group due to its ability to give rise to ketone bodies or fatty acid. Amino acids that degraded to pyruvate, α-ketoglutarate, succinyl -CoA, fumarate or oxaloacetate are termed as glucogenic amino acids (Yamane *et al*., 2019). Both hydrolyzation processes contribute to energy production in poultry.

Due to increased metabolic demands during non-halal slaughtering, the chicken is likely to increase the breakdown of amino acids for energy as shown by the metabolome data. Therefore, it is hypothesized that the lower concentration histidine which is 13500 BPI in non-halal chicken meats may be due to increased recruitment and utilization of this amino acid in order to provide energy substrates for the cell at the brink of death.

3.3 Inosine metabolism

It was observed that different slaughtering methods had affected the inosine metabolism of chicken. Figure 2 shows that a higher concentration of inosine was detected in non-halal chicken meat compared to halal chicken meat (Figure 1). The lower concentration of hypoxanthine which is 20000 BPI was observed in non-halal chicken meat as recorded in the metabolome data (Figure 2). Inosine represents a metabolite of ATP

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degradation which can be converted to hypoxanthine released into the blood circulation (McConell *et al*., 2005; Bishop, 2010). A higher concentration of hypoxanthine (27000 BPI) was recorded in halal chicken suggested that muscle ATP could have been catabolized to supply energy within the cell. The increase of energy depletion was observed due to the lower value of inosine in halal breast muscle and the increasing concentration of hypoxantine as recorded by the metabolome data.

However, it is interesting to note how inosine and its byproducts affect the flavor profile quality of poultry. Amino acids and inosine monophosphate (IMP) are among the major compounds contributing to meat flavor (Zhu and Lu, 1996). IMP is considered as a key index of meat flavor as it plays a major role in meat flavor (Suzuki *et al*., 1994; Fuiymura and Chen, 1998). Hall (1964) stated that IMP and its corresponding nucleoside and base, inosine and hypoxanthine are normally found in meat and these substances are derived from the degradation of adenosine triphosphate (ATP). Therefore, the degradation of ATP is a desirable metabolic activity, as it can contribute to flavor quality of meat.

4. Conclusion

Metabolome data illustrated the metabolomics activity of gluconeogenesis, amino acid breakdown, inosine hydrolyzation as well as hypoxanthine production in broiler chickens when subjected to different slaughtering methods. Non-halal slaughter resulted in higher utilization of energy as indicated by the elevated amino acid and glucose breakdown. Halal slaughtering method favors less energy usage, hence keeping the welfare of the animal as well as the meat quality.

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