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Proximate, Total Phenolic, Antioxidant Activity and Amino Acids Profile of Bali Indigenous Chicken, Spent Laying Hen and Broiler Breast Fillet

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Abstract: Fresh chicken breast fillet of Bali indigenous chicken, spent laying hen and broiler were studied for proximate, total phenolic, antioxidant activity and its amino acids profile. Broiler breast fillet contained higher moisture, fat, and ash contents ($P < 0.05$) compared to Bali indigenous chicken as well as spent hen breast fillets, but protein content, total phenolic content and DPPH radical scavenging activity were lower ($P < 0.05$). The amino acids profile of broiler showed that only methionine contents were higher ($P < 0.05$) compared to the other chicken breast fillet samples. Whilst Bali indigenous chicken had highest ($P < 0.05$) glutamic acid and arginine compared to the other chicken meat, and essential amino acid like valine, phenylalanine, threonine, leucine and lysine contents of Bali indigenous chicken was no differ ($P > 0.05$) with spent laying hen, and had higher ($P < 0.05$) compared to broiler breast fillet.

Key words: Chicken breast fillet, proximate composition, total phenolic content, antioxidant activity, amino acids profile

INTRODUCTION

Chicken meat have been very popular within Indonesian consumers as a relatively cheap nutrients resources and the quality attributes of this meat such as its unique taste and texture could fulfill the needs of consumers for their daily menu. There are many chicken breeds in Indonesia and among those breeds which are popular in Bali island are Balinese indigenous chicken, spent hen layer and commercial broiler.

According to Chuaynukool *et al.* (2007), the meat characteristics of indigenous Thai chicken are similar to the one from spent hen layer although both of them are quite different to broiler meat. Wattanachant *et al.* (2004) and Jaturashitha *et al.* (2008) also noted that indigenous chicken meat had a specific texture and taste compared to broiler meat and in Thailand it was reported that an increase of consumers demand of this meat are due to unique taste and texture. The quality of spent laying hen had also been studied intensively by Mendiratta *et al.* (2012) and reported that this type of meat are tough in texture and fibrous even after cooking. Indigenous chicken was also reported as a slower growth rate than commercial broiler and hence affected the chemical composition of its meat. Leoytarakul and Pimkumhlai (1999) noted that most consumers preferred the indigenous chicken meat due to their believe that this type of meat were free from drugs and hormones in the

production system. The physicochemical characteristics of indigenous chicken meat, spent hen layer and broiler especially in Thailand had been intensively studied as reported by Van Marie-Koster and Webb (2000), Abeni and Bergoglio (2001), Van Heerden *et al.* (2002), Wattanachant *et al.* (2004), Wattanachant *et al.* (2005a,b), Wattanachant *et al.* (2007), Jaturasitha *et al.* (2008) and Wattanachant (2008).

However there are no reports on physicochemical characteristics of indigenous chicken, spent hen and broiler meat produced in Bali island. Therefore the aims of this study were to document the chemical composition, total phenolic content, antioxidant activity and amino acids profile of Bali indigenous chicken meat compared to spent hen layer and broiler meat produced in Bali island.

MATERIALS AND METHODS

Breast fillet preparation: Three strains of chicken were used in this study namely Bali indigenous chicken aged 16 - 20 weeks were bought from local farm, spent laying chicken (ISA-brown) obtained from egg farm in Denpasar after a period of laying of 76 weeks and mixed-sex commercial broiler (CP707) aged 4 - 5 weeks were obtained from commercial poultry farm in central region of Bali. Skinless breast fillet ((*Musculus Pectoralis Superficialis*, left and right breast muscles of each bird)

from those chicken were packed in polyethylene pouches and stored at 4°C before used for laboratory analysis.

Proximate composition: Moisture, protein, fat and ash contents of breast fillet samples were measured following method No. 950.46; No. 928.08; No. 960.39; No 920.153 (AOAC, 2000) respectively.

Total phenolic content: Total phenolics were determined calorimetrically using Folin-Ciocalteu reagent as described by Jang *et al.* (2008) with slight modifications. Each raw breast meat sample (5g) in distilled water (15 mL) was homogenized at 3000 rpm for 2 min. Chloroform (9 mL) was added to the homogenates and the mixture was shaken vigorously 2 to 3 times to separate the lipids. A 1-mL aliquot of diluted sample (1:4, vol/vol) was added to the Folin-Ciocalteu reagent (500 µL), followed by addition of 1 mL of sodium carbonate solution (5%). The reaction mixture was vortexed and the absorbance was measured with a spectrophotometer (SP-870 TURNER Barnstead USA) at 700 nm after incubation for 1 h at room temperature. Samples were measured in nine replicates and absorbance measured in two readings. Quantification was done based on the standard curve generated with gallic acid. The standard calibration curve of gallic acid solution (10, 20, 30 and 40 mg/L) was prepared using the similar procedure.

DPPH radical scavenging activity: The Radical-Scavenging Assay: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was estimated with the aqueous supernatant obtained from raw breast meat according to the method of Blois as described by Jang *et al.* (2008), with slight modifications. 0.1ml quantity of aqueous supernatant diluted to 5ml of water, then take into test tube 1.5 ml and was added 1.5 mL of methanolic DPPH solution (0.2 mM). The mixture was vortexed and left to stand at room temperature for 30 min. A tube containing 1.5 mL of methanol and 1.5 mL of methanolic DPPH solution (0.2 mM) served as the control. The absorbance of the solution was measured at 517 nm (SP-870 TURNER Barnstead USA). Nine sample were prepared and absorbance measured in two replicate readings. The percentage of DPPH radical scavenging activity was obtained from the following equation:

$$RSA = \left(1 - \frac{\text{absorbance value of testing solution}}{\text{absorbance value of control solution}} \right) \times 100$$

Where RSA: Radical scavenging activity

Amino acids profile: The amino acid profile of the muscle samples was determined by the method of

Antoine *et al.* (2001) with slightly modification. The sample obtained were 3 mg protein or 0.015 g chicken breast meat / dry weight and then 1 ml 6 N HCL was added. The mixture was purged with N₂ and then heated in the oven at 110°C for 24 hours. A sample was prepared after the 6 N HCl hydrolysis then dissolved to 5 ml 0.01 N HCl and filtered using Millipore 0.45 µm filter membrane (WHATMAN® diameter 25 mm), followed by adding potassium-borate buffer (pH 10.4). A 5 µL quantity of hydrolyzate protein sample was added with 25 µL of OPA reagent and then injected after 1 minute of derivatization.

Mobile phase A was made up of 0.025M sodium acetate buffer (pH 6.5) 0.5 g Na EDTA, 90 ml methanol and 10 ml THF (80:10:9:1) prepared from analytical grade dissolving to 1 L water Hi Pure. The pH of the acetate buffer (A buffer) was adjusted to 6.5 using NaOH solution, and mobile phase B buffer contain 95% methanol on Hi Pure water. The mobile phases were ultrafiltered through Millipore filter having a pore diameter of 0.45µm (WHATMAN® diameter 25 mm) and degassed by sparging for 5 minutes with pure nitrogen. Gradient elution was generated using solvent delivery module Varian Pro Star Model Number 240 (Chromatography Systems, Walnut Creek, CA 94598 USA Made in USA), was used for controlling the gradient and flow rate (1.0 ml/min) of the mobile phases.

OPA-thiol reagent was made up at least 24 hours before used by dissolving 50 mg o-phthaldialdehyde in 4 ml methanol, and 0.025 ml mercapthoethanol was added. The mixture was thoroughly mixed, then 0.050 ml Brij-30 and 1 ml borax buffer solution were added. The OPA-thiol reagent stored in the dark bottle at temperature 4°C or in a tightly closed container.

The amino acid chicken breast meat were analyzed by cation exchange ICI Instrument High Performance Liquid Chromatography (HPLC) with column Ultra Techsphere ODS 3µ particle size, 4,6 mm x 7,5 cm PARKER 316 (SGE PTY. LTD Victoria Australia) and O-phthaldialdehyde (OPA) precolumn derivatisation. The amino acid standard H (L-Alanine, L-Arginine, L-Aspartic Acid, L-Glutamic Acid, Glycine, L-Histidine, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-Serine, L-Threonine, L-Tyrosine and L-Valine (PIERCE, Rockford Illinois 61105, USA) were used.

Amino acids were analyzed in three replicates, and each replicate of sample was obtained from 3 different whole chicken breast meat (Musculus Pectoralis Superficial is, left and right breast muscles of each bird). The amino acid composition was expressed as g of amino acid per 100g of raw breast meat.

Statistical analysis: The experiments were carried out using Completely Randomized Design with some replications, and the data obtained were analyzed by

using one way Analysis of Variance (ANOVA). The differences among means were determined by Duncan Multiple Range test using SPSS version 13.0 (Chicago, Illinois, USA) at a level of significance of $P < 0.05$. Data were presented as means \pm standard deviation and each analysis were replicated some times for proximate analysis ($n=5$), total phenol content and DPPH scavenging activity ($n=9$), and amino acids content ($n=3$).

RESULTS AND DISCUSSION

Proximate composition: Proximate analysis data of breast fillet of Bali indigenous chicken, spent laying hen and broiler were presented in Table 1. It is interesting to note that although proximate of those three different breeds were differ significantly ($P < 0.05$) only fat contents of broiler was tended much higher compared to Bali indigenous chicken and spent laying hen breast fillet. Although it was moisture content also significantly higher ($P < 0.05$) compared to other both chicken breast fillet, these is may be affected by ante-mortem factors of the chicken life, such as genetics, physiology, nutrition, management, disease (Fletcher, 2002), and strain of broiler chick, such as low body weights of broiler had more intensely packed protein bands than of heavy broilers. Xlong *et al.* (1993), Abeni and Bergoglio (2001), Van Heerden *et al.* (2002) and Wattanachant *et al.* (2007) noted that chemical compositions of chicken meat were affected by breeds, sex, age, type of muscle as well as carcass processing. However Jaturashita *et al.* (2002) reported that moisture, protein and fat contents of Thai native chicken and broiler meat were not different ($P > 0.05$) although fat content of Thai native chicken meat was lower compared to fat content of broiler meat. Chiofalo *et al.* (2011) reported that of fat content of male broiler breast meat in Italy was 1.66% and protein content 22.19%. In such a case of broiler meat quality in Bali, these is probably due to relatively more affected by time varies of differences in house environmental conditions and management practices which affect bird eating patterns. Our finding was in line with the suggested by Saxena *et al.* (2009) that higher body weights have been related to certain muscle abnormalities and meat quality defects like pale, soft and exudative (PSE), because growth hormone allows relatively more substrate to muscle over adipose tissue accretion in broiler chicken of 4 to 5 weeks of age. Myostatin gene acts as a negative regulator of postnatal skeletal muscle growth and DNA marker in this gene region or its silencing may help in augmenting meat production (Saxena *et al.*, 2009). The protein content of those three different breeds were differ significantly ($P < 0.05$), whereas protein content of broiler was tended lowest compared to both other chicken breast fillet. It is probably due to that the fractional rates of both protein synthesis and protein degradation are significantly greater in younger animals than in older ones. In

addition to varying with animal age, protein synthesis and degradation vary tremendously among different muscles of the same species (Pearson and Young, 1989).

Antioxidative potential of chicken breast fillet

Total phenolic content: In the present study the total phenolic of those three types of chicken breast fillet (Table 2) were in the range of 64.44 to 68.57 mg of gallic acid equivalent/kg of breast fillet and slightly differ significant ($P < 0.05$). However the total phenols content of breast meat from broiler chicken fed Medicinal Herb Extract Mix (MHEM) was in the range of 48.82 (a basal diet as control) to 95.59 (a basal diet with added 0.3% MHEM) mg of gallic acid equivalent/kg of meat at d 0 of storage (Jang *et al.*, 2008). In this context grass intake by Bali indigenous chicken should be considered crucial to scratch while eating and were observed to pick up feed particles more selectively and so to increase antioxidant defenses resulting from the high amounts of carotenoids, tocopherols and polyphenols eaten with pasture. These condition indicate is probably due to present nonessential dietary antioxidants as pyrroloquinoline quinone, and further as described by Decker (2010) that nonessential dietary antioxidants as pyrroloquinoline quinone is a water-soluble phenolic widely distributed in microorganisms, plant, and animal in both free and protein-bound forms. The result showed that total phenolic of breast fillet of Bali indigenous chicken was significantly greater ($P < 0.05$), it is probably due to consume high amounts phenolic antioxidants compounds than of broiler and spent laying hen fed the commercial diet. Furthermore Decker (2010) reported that phenolic compounds influence the quality, acceptability, and stability of foods by acting as flavors, colorants, and antioxidants. In addition the presence of conjugated ring structures and hydroxyl groups allows phenolics to actively scavenge and stabilize free radicals.

DPPH radical scavenging activity (DPPH RSA): The scavenging activity although the DPPH RSA number (Table 2) of local indigenous Bali chicken breast fillet had the higher significant ($P < 0.05$) compared to broiler breast meat fillet, but no significant difference ($P > 0.05$) between of Bali indigenous chicken and spent laying hen breast fillet or it is probably due to lower fat contents). Our finding was in line with of the research by Jang *et al.* (2008) reported that broilers fed contain medicinal herb extract mix (MHEM) as antioxidant compounds showed DPPH RSA from broiler chicken breast meat of 20.82% (a basal diet as control) with of 23.39% (a basal diet with added 0.3% (MHEM) were not significantly or similar of meat at d 0 of storage. Whilst increased the DPPH scavenging effect of broiler meat from broilers fed protein antioxidant mechanisms are

Table 1: Chemical composition of breast fillet of Bali indigenous chicken, spent laying hen and broiler*.

Source of meat	Moisture (%)	Protein (%)	Fat (%)	ash (%)
Broiler	73.85±0.19 ^a	18.94±0.18 ^c	4.70±0.10 ^a	1.78±0.02 ^a
Bali indigenous chicken	72.14±0.19 ^b	22.32±0.23 ^b	1.73±0.05 ^b	1.39±0.02 ^c
Spent laying hen	71.91±0.14 ^c	22.93±0.22 ^a	1.49±0.04 ^c	1.46±0.05 ^b

*Mean ± Standard deviation (n = 5) values with different superscripts in same column differ significantly (P<0.05)

Table 2: The total phenolic content (GAE = mg/kg) and DPPH RSA (%) of breast fillet of broiler, Bali indigenous chicken and spent laying hen*

Meat type	Total phenolic content (GAE = mg/kg)	DPPH RSA (%)
Broiler	64.44 ± 3.58 ^b	19.04±2.36 ^c
Bali indigenous chicken	68.57 ± 3.13 ^a	21.38±1.78 ^b
Spent Laying Hen	65.64 ± 1.31 ^b	20.77±1.79 ^{ab}

*Mean ± Standard deviation (n = 9) values with different superscripts within same column differ significantly (P<0.05)

Table 3: Amino acids profile of Bali indigenous chicken, spent laying hen and broiler breast Fillet*

Amino acids	Type of meat		
	Broiler (%)	Bali indigenous chicken (%)	Spent laying hen (%)
Aspartic acid	1.80±0.12 ^a	1.28±0.23 ^b	2.00±0.25 ^a
Glutamic acid	3.06±0.07 ^c	5.31±0.30 ^a	4.49±0.06 ^b
Serine	0.97±0.06 ^a	0.85±0.19 ^a	0.92±0.09 ^a
Histidine	1.14±0.15 ^a	1.22±0.13 ^a	1.26±0.24 ^a
Glycine	0.73±0.18 ^a	0.46±0.19 ^a	0.76±0.07 ^a
Threonine	1.20±0.10 ^a	2.40±0.05 ^a	2.37±0.19 ^a
Arginine	2.60±0.16 ^c	3.74±0.41 ^a	3.16±0.16 ^b
Alanine	0.86±0.10 ^a	1.43±0.30 ^a	0.92±0.11 ^b
Tyrosine	1.24±0.26 ^a	1.15±0.26 ^a	0.67±0.13 ^b
Methionine	1.34±0.19 ^a	0.27±0.18 ^b	0.51±0.16 ^b
Valine	1.08±0.08 ^b	1.76±0.22 ^a	1.38±0.43 ^{ab}
Phenylalanine	1.29±0.27 ^a	2.19±0.41 ^a	1.98±0.20 ^a
Isoleucine	1.35±0.13 ^a	1.35±0.17 ^a	1.15±0.10 ^a
Leucine	1.52±0.15 ^b	2.92±0.34 ^a	3.17±0.16 ^a
Lysine	2.14±0.10 ^b	2.50±0.54 ^{ab}	2.91±0.22 ^a
Total	22.33±0.70	28.84±1.78	27.66±1.38

*Mean±Standard deviation (n = 3) values with different superscripts within same row differ significantly (P<0.05)

dependent on amino acids composition, because amino acid residues such as histidine, glutamic acid, aspartic acid, phosphorylated serine and threonine are known to bind metals (Elias *et al.*, 2008). In the present study has shown that breast fillet of Bali indigenous chicken contained the significantly higher (P<0.05) of glutamic acid and arginine compared to the other chicken breast fillet samples, whereas these amino acids can donate proton or electron to deficient radical and also interrupt the oxidation reaction. Thus, it is probably due to the other component presence, like as nonessential dietary antioxidant (phenolic compound, conjugated linoleic acid, carnosine, and pyrroloquinoline quinone) in the chicken breast fillet could be partially responsible for their higher DPPH radical scavenging activities. In added, that white fiber muscles chicken meat containing over fivefold more anserine and carnosine (endogenous antioxidant) than red muscles, can be affected by diet and their concentrations vary widely with species and muscle type

(Chan *et al.*, 1994). Furthermore Min *et al.* (2008) reported that in raw chicken breast and thigh meat at days 0 and 3, respectively, were significantly higher DPPH radical scavenging activities than those of pork and beef loin, and were similar to or greater than those of 500ppm sesame. Castellini *et al.* (2008), reported that oxidative stability of meat during storage depends on initial oxidative status (may be due to lower lipid levels) and from genotype, it is related to the balance between antioxidants and pro-oxidants factor. In the present study it is interesting to note that DPPH RSA parameter was significantly correlation with of alanine, and phenylalanine content of chicken breast fillet from three strains, there share with hydrophobic amino acid. Saiga (2003) reported that the hydrophobic of compounds affected the antioxidant activity, that is, solubility in lipid or it was greater of hydrophobic amino acid content from porcine myofibril proteins by protease treatment and these hydrolyses possessed DPPH radical scavenging activity and chelating activity toward metals ions.

Amino acid profile: Amino acids data of breast chicken meat of Bali indigenous chicken, spent laying hen and broiler were presented in Table 3. It is interesting to note that breast fillet of Bali indigenous chicken contained the highest amount of glutamic acid and arginine, were differ significantly (P<0.05) compared to spent laying hen and broiler breast fillet. While arginine, threonine, phenylalanine and valine were also observed significantly higher (P<0.05) concentration in Bali indigenous chicken compared to broiler breast fillet. No significant differences (P>0.05) were found of essential amino acids, like threonine, methionine, valine, phenylalanine, isoleucine, leucine, and lysine between of Bali indigenous chicken and spent laying hen. Wattanachant *et al.* (2004) also reported that the highest amount of amino acid in Thai indigenous and broiler chicken muscles (pectoralis and biceps femoris muscles) was glutamic acid followed by arginine, leucine, aspartic acid and lysine, however there were no significant differences of amino acids composition between Thai indigenous and broiler muscle samples. The glutamic acid of Bali indigenous chicken breast fillet was significantly higher (P<0.05) than spent laying hen and significantly lowest (P<0.05) of broiler chicken meat. It is probably due to that higher amount vegetables proteins contained on fed of Bali indigenous chicken and of spent laying hen. This difference related with animal age, whereas the adult birds is no longer

growing, and has reached its final, constant weight because all the protein synthesis rate in 35-week chickens is devoted to replacement synthesis, and the fractional growth rate is zero (Pearson and Young, 1989). Furthermore of synthesis of glutamate acid in animals as described, are derivatives of alfa-ketoglutarate may be converted to glutamate by reductive ammunitio and by transamination reaction involving a number of amino acids, although glutamate synthesis varies with cell type and metabolic circumstances, transamination appears to play a major role in the synthesis of most glutamate molecules in eukaryotic cells, although as a component of proteins and as a precursor for other amino acids, it is also used in the central nervous system as an excitatory neurotransmitter (McKee and McKee, 2003). Food science aspect showed that glutamate acid, and inosine mono phosphate, called as "umami" taste, are favorites for consumers and constitute a characteristic taste of chicken meat (Fujimura and Kadowi, 2006). The methionine of broiler chicken breast fillet was significantly higher ($P < 0.05$) than the both to other meat samples. It is probably due to lack or inactive of methionine sulfoxide reductases in transamination reaction to fast growing of broiler chicken. Furthermore Elias *et al.* (2008), reported that the role of methionine as an important free radical scavenger in biological systems can also be argued based on the ubiquity of methionine sulfoxide reductases (enzymes capable of repairing oxidized methionine residues) in virtually all forms of life.

Conclusion: It can be concluded that Bali indigenous chicken and spent laying hen breast fillet contained higher protein and lower fat compared to broiler breast fillet, however broiler breast fillet contained higher fat and ash content. A higher total phenolic content and DPPH RSA value were observed in breast fillet of Bali indigenous chicken.

Bali indigenous chicken contained the highest amount of glutamic acid compared to spent hen layer and broiler breast fillet, while spent hen layer contained higher threonine, arginine, leucine, phenylalanine, and lysine compared to broiler breast fillet. Therefore breast fillet of Bali indigenous chicken and spent laying hen were similar of essential amino acid, are the best from consumers health beneficial.

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