Comparative study of herbal and non-herbal egg protein profiles using sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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Abstract. Chicken egg yolk and albumen contain some bioactive proteins. The present study was conducted to compare the protein profile of yolk and albumen of herbal and non-herbal chicken egg. The herbals that supplemented into chicken feed consisted of turmeric, sambiloto leaves, soursop leaves, ginger and lemongrass. The protein profiles were measured in term of the numbers of bioactive peptide fraction and their molecular weight by SDS PAGE using descriptive qualitative analysis. The antioxidant binding protein were also evaluated. The result show that SDS-PAGE can separate the egg yolk protein fraction and albumen protein with molecular weight between 14-97 kDa. Egg yolk protein fraction of herbal eggs separated better than non-herbal eggs, whereas giving herbal to laying hens does not affect the number of fractions in albumen protein. Antioxidants from herbals were indicated binding the apoprotein and apolipoprotein of egg yolk, also avidin and ovalbumin of albumen. It can be concluded that herbals supplementation affects the bioactive protein fraction in chicken eggs.

1. Introduction
The livestock industry around the world currently banned the use of antibiotics as growth promoter. Antibiotics banned because its residue were danger to consumers of animal product such as meat, eggs, and milk. Oxytetracycline residues were found in egg shells and albumen on the second day of feeding containing antibiotics growth promoter. Antibiotic residues were also found in egg yolks on the 4th day of feeding and still found 0.01 ppm residues in albumen and egg yolk after 7 days of stopping feeding [1]. This triggered the layer farm industry to search for antibiotic replacement solutions by applying herbs as a natural additive ingredient for layer hens feed.

Herbs are medicinal plants that contain bioactive compounds and can be used in the livestock industry as a feed additive [2]. Giving herbs as feed additives can provide more value as organic eggs. Herbs contain various bioactive compounds including antioxidants [3] that can synergize with protein or bioactive peptides from egg [4]. This study is a case study in "Sekuntum Farm" East Lampung, which feeds laying hens using herbal supplementation. The purpose of the study was to determine the differences in profiles of bioactive protein compounds from the eggs produced by Sekuntum farm, and to find out the antioxidant interactions of herbs with bioactive peptide compounds on egg yolks and albumen.
2. Materials and methods

2.1. Materials
This study used herbal egg and non-herbal egg from "Sekuntum Farm" East Lampung, Lampung Province. Chicken eggs were taken in the last three days of 28-days production cycle. Chemicals used in this research included 1 M Tris-Cl pH 6.8; 10% w/v SDS, 0.5M EDTA, β-mercaptoethanol, glycine, 30% w/v acrylamide, 10% w/v APS and TEMED. The tool used is a set of Bio-Rad protean mini electrophoresis devices.

2.2. Methods
Fifty four egg samples material from Sekuntum Farm were analyzed at laboratory of FALITMA, Faculty of Biology, Universitas Gadjah Mada using SDS PAGE electrophoresis methods [5].

2.2.1. Preparation egg sample. Herbal eggs and non-herbal eggs were broken, then the yolk and albumen were separated using egg separator. One ml of egg white and egg yolks were added to 9 ml of PBS solution and homogenized. Then 100 μl of egg whites mix with PBS were added with 100 μl sample buffer. Preparation of the egg yolk sample was performed in the same procedure as albumen. Pour the mixture into a centrifuge tube, vortexed, and incubated at 60ºC for 5 minutes. Store the sample below -20°C until ready for use. Sample buffer composition is shown in Table 1.

| Material                          | Total |
|----------------------------------|-------|
| HCL tris solution 1 M pH 6.8     | 1.25 ml |
| SDS 10% v/v solution             | 4 ml  |
| Glycerol solution of 70% v/v     | 2 ml  |
| 0.5M EDTA solution               | 0.5 ml |
| B-mercaptoethanol solution       | 0.2 ml |
| Aquabidest                       | 2.05 ml |
| Bromophenol Blue 1% w/v          | 4 mg  |

2.2.2. Polyacrylamide gel preparation. The composition of stacking gel and resolving gel shown in Table 2. The gel sandwich plates were assembled according to the Mini-PROTEAN cell instructions. The gel slabs were poured between two glass plates fitted with two 1.5-mm spacers. The gel sandwich was placed in the gel casting apparatus.

| Material                        | Stacking Gel | Resolving Gel |
|---------------------------------|--------------|---------------|
| 30% acrylamide                  | 0.67 ml      | 4 ml          |
| **Stacking gel buffer solution**| 1.25 ml      | -             |
| Solution for buffer resolving gel| -            | 2.4 ml        |
| APS solution                    | 25 μl        | 50 μl         |
| TEMED                           | 5 μl         | 5 μl          |
| Aquabidest                      | 3.4 ml       | 4 ml          |

The comb was inserted to form the wells for the samples. Make a mark with a lab marker on the outside of each glass sandwich about 0.5 cm below the comb. Carefully mix each solution and immediately transfer to the casting apparatus using a Pasteur pipet. The air was prevented to being blown into the mixture by evacuating the air from the pipet before placing it into the mixture. The mixture was pipetted directly between the glass plates for both gels. The mixture should be pipetted up to the mark made on the glass sandwich so that there will be room for the stacking gel and comb on top. The 1.5 mm combs were carefully inserted into the top of the gel sandwich, starting from one end.
and lowering down into the solution so that no air bubbles are trapped underneath the comb. Allow the stacking gel to stand and polymerize.

2.2.3 Operational electrophoresis. The polymerized gels were removed from the gel casting apparatus and placed on the Mini-PROTEAN cell electrophoresis tool. As much as 20 μl standard samples and proteins (SeeBlue® Protein Standard kit 3-198 kDa) were inserted into the gel using a micropipette. Electrode buffer was added to the lower buffer chamber, and fill the upper chamber with electrode buffer. Electrophoresis tools was operated at a mains voltage of 100 V and 30-110 mA electric current, and discontinued after the marker protein reaches the lower limit of the resolving gel.

Gel being visualized using coomasie brilliant blue by soaking the gel with 1% w/v coomasie solution brilliant blue in methanol for one night. Residue of coomasie brilliant blue were destaining with destaining solution (10 μl acid acetate, 30 ml methanol, and 70 ml aquabidest ). After that, gel were captured with the camera and stored in JPEG file. Interpretation of molecular weight based on the Rf fraction. To determine the relative mobility (Rf) of a protein, divide its migration distance from the top of the separating gel to the center of the protein band by the migration distance of the tracking dye from the top of the separating gel.

2.2.4 Statistic analysis. Data for the protein profiles were calculated by its molecular weight from SDS PAGE using descriptive qualitative analysis.

3. Results and discussion

Migration distance of each fraction and marker were calculated to determine the Relative mobility factor (Rf) of each fraction by linear regression analysis. Standard curve between Rf and molecular weight of standard protein fraction was Y = 2.08-1.60X (Figure 2).

Figure 1. Description of non-herbal egg protein fractions and herbal eggs on SDS PAGE. KTK: non-herbal egg yolk protein fraction; KTH: herbal egg yolk protein fraction; PTK: non-herbal egg white protein fraction; PTH: herbal egg white protein fraction

Y is the standard molecular weight fraction of protein logarithm and X is Rf standard protein fraction. Egg yolk and albumen can be separated by electrophoresis into specific fraction based on each molecular weight. Figure 1 shows that electrophoresis SDS-page can separate non-herbal egg yolk protein into 8 fractions and separate herbal egg yolk protein into 9 fractions. Interaction of herbal egg yolk protein with antioxidants shown in band number 3, 6, and 7 (Figure 1).
Table 3. shows Rf and molecular weight of each herbal and non-herbal egg protein fraction. By knowing the molecular weight of each fraction, it can be estimated the type of protein fraction (Table 3).

**Table 3.** Number of fractions, Rf, molecular weight, and estimates of bioactive protein compounds from heralbs and non-herbal eggs.

| Egg Type       | Fraction Number | Rf (cm) | Molecular Weight (kDa) | Estimated Bioactive Protein Fraction |
|----------------|-----------------|---------|------------------------|-------------------------------------|
| Non-herbal egg yolk | 1               | 0.06    | 96.38                  | Livetin                              |
|                 | 2               | 0.08    | 89.53                  | Livetin (α-albumin)                  |
|                 | 3               | 0.14    | 71.77                  | LDL-1 apoprotein                     |
|                 | 4               | 0.20    | 57.54                  | LDL-2 apoprotein                     |
|                 | 5               | 0.26    | 46.13                  | LDL-3 apoprotein                     |
|                 | 6               | 0.28    | 42.85                  | β-α2-glycoprotein-1                  |
|                 | 7               | 0.30    | 39.81                  | β-α2-glycoprotein-2                  |
|                 | 8               | 0.52    | 31.91                  | Apolipoprotein                       |
| Herbal egg yolk | 1               | 0.06    | 96.38                  | Livetin                              |
|                 | 2               | 0.08    | 89.53                  | Livetin (α-albumin)                  |
|                 | 3               | 0.10    | 83.17                  | LDL-1 + antioxidant apoprotein       |
|                 | 4               | 0.16    | 66.06                  | LDL-2 + antioxidant apoprotein       |
|                 | 5               | 0.20    | 57.54                  | LDL-2 + antioxidant apoprotein       |
|                 | 6               | 0.24    | 49.65                  | Apoprotein LDL-3 + antioxidant       |
|                 | 7               | 0.30    | 39.81                  | β-α2-Glikoprotein-2                  |
|                 | 8               | 0.36    | 36.98                  | Apolipoprotein + antioxidant        |
|                 | 9               | 0.52    | 31.91                  | Apolipoprotein                       |
| Non-herbal egg white | 1               | 0.06    | 96.38                  | EW135 non reducing                   |
|                 | 2               | 0.16    | 66.68                  | Avidin                               |
|                 | 3               | 0.20    | 57.54                  | Ovalbumin                           |
|                 | 4               | 0.28    | 42.85                  | Ovoglobulin                         |
|                 | 5               | 0.48    | 22.08                  | Ovoglycoprotein                     |
| White herbal eggs | 1               | 0.04    | 103.75                 | EW135 non reducing                   |
|                 | 2               | 0.14    | 71.77                  | Avidin + antioxidant                |
|                 | 3               | 0.22    | 53.45                  | Ovalbumin                           |
|                 | 4               | 0.28    | 42.85                  | Ovoglobulin                         |
|                 | 5               | 0.48    | 20.51                  | Ovoglycoprotein                     |

The same results were also shown in calculating the molecular weight of egg white. Ovalbumin is the most reactive bioactive protein fraction in egg white. Egg whites contain ovalbumin protein fractions (45 kDa), ovotransferrin (77.7 kDa), ovomucoid (28 kDa), ovomucosin (8,300-23,000 kDa), avidin (15.6 kDa), ovoglobulin (36-45 kDa), lysozyme (14.4 kDa), ovomakroglobulin (760-900 kDa), ovoflavoprotein (32-36 kDa), cystatin (12.7 kDa), ovoglycopoeterin (24.4 kDa), and ovomucin (116 kDa) [6]. Egg white contains a type of protein called EW135 because it has a molecular weight of 135 kDa, but in a non-reduced [7]. EW135 has a molecular weight about 100 kDa. Ovalbumin can bind antioxidants such as L-ascorbic acid, α-tocopherol, procyanidin B3, β-carotene and astaxanthin through hydrophobic interactions. Referring to this, herbal antioxidants are bound to ovalbumin. Ovalbumin has the capacity to bind polyphenol antioxidants such as khrisin, quercetin, and routine [8]. Chicken egg whites fed with herbal feed appeared an ovotransferrin protein fraction [9]. This is because herbal bioactive compounds affect the biosynthesis of egg proteins. Herbal feed mostly contains components of herbal bioactive compounds which can influence the appearance of bioactive protein compounds in egg white. Furthermore, herbs generally work in ribosome cells by influencing protein biosynthesis.

These results indicated that herbs can influence the formation of bioactive protein fraction. Herbs have the ability to bind antioxidants that can accumulated in eggs yolk and albumen, so that the eggs contain antioxidants. It can cause the formation of more bioactive protein fraction with antioxidant
characteristics on herbal eggs. Nevertheless, the findings in this study need further elaboration through other methods of analysis, including quantitative analysis of antioxidants and qualitative analysis that can show the types of antioxidants that are bound to bioactive peptides in eggs.

4. Conclusion
Based on the results, it can be concluded that SDS PAGE analysis could separate egg protein into specific fraction based on its molecular weight. Herbs supplementation affect the bioactive protein fraction in chicken eggs because it can bound antioxidant in yolk and albumen.

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