

Increasing antioxidant activity of quail (*cortunix-cortunix japonica*) eggs with the addition of sweet flag (*acorus calamus*) powder as a feed additive

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Abstract. The aim of this research was to evaluate the addition of sweet flag powder on antioxidant activity and cholesterol activity of quails egg. Sweet flag (*Acorus calamus*) powder was analyzed using the DPPH and cholesterol activity. One hundred sixty (160) quails were divided into 5 treatments in which each treatment had 4 replications with 8 quails per replications. Quails were fed the experimental diets, namely basal diet, 0,1%, 0,2%, 0,3%, and 0,4% of sweet flag powder. Eggs were collected daily in every treatment. The analyze of egg were conducted starting from days 45 of experiments. The data was analyzed using Completely Randomized Design (RAL). The different level was analyzed using Duncan's Multiple Range Test. The addition of sweet flag powder as feed additive has a significant effect on the antioxidant activity of quails egg. The addition of 0,4 % of sweet flag powder improves antioxidant activity of quails egg. The addition of 0.4 % of sweet flag powder decreased cholesterol of quails egg yolk. The conclusion of this research that the addition of sweet flag powder as a feed additive up to 0,4 % had an effect on the antioxidant activity of quail eggs and decreased cholesterol of quails egg yolk.

1. Introduction

In Indonesia, the used of antibiotics as a growth promoter on feed was not allowed by the Government since 1st January 2017. Recently, researchers were looking for the natural bioactive compound as an alternative to changed antibiotic[5]. Antibiotic was used as a growth promoter in relatively small amount as a feed additive but improved feed efficiency and reproduction of livestock, therefore the used of the additive can increase the profit of the farmers. However, the accumulation residue from antibiotic will have a negative effect on the livestock product. The used of antibiotic should be restricted to prevent the problem of increasing resistance in bacteria of human and animal, especially resistance in gram-negative bacteria. Many natural herbs have a beneficial effect on the animal performance through as antioxidant activity [13]. The used of phytobiotic as a natural growth promotor (NGPs) has been identified as an effective alternative to changed antibiotic[8]. Phytobiotic as NGPs was developed as a feed additive, which is antimicrobial, antioxidant, to increase performance, immunity, and also improves a digestive tract of the poultry. Phytobiotics are found into various herbal plants, such as curcumin, ginger, garlic and the other rhizomes of plants[10]. Herbal plants have no chemical compounds where it means has no harmful effect on the health. In hence, the addition of herbal plants as a feed has a good effect on animals, as well as livestock production[21].



Quail is a type of bird that could not fly; the body size is relatively small; short-legged and can be pitted. The benefits of quail can be taken from eggs and meat. Eggs consist of four components namely egg yolks, egg whites, eggshell membranes and eggshells [16]. Male quail has a body weight of about 100-140 g while the female is slightly heavier which is between 120-160 g. Female quails will start laying eggs at the age of 35-42 days [1]. Quail meat contains 21.10% protein, while low-fat content is 7.70% [14].

Egg yolk of quails have high levels of fat and cholesterol, it can cause lowest consumers consumption, so an alternative product of quail egg yolks is low in cholesterol. To reduce cholesterol levels in egg yolks can be done by manipulating feed by adding feed additive consist of the natural bioactive compound.

Phytogenic is the result of plant secondary metabolites that contain nutritionally valuable, non-nutritious, or anti-nutritive compounds [8]. Phytogenic is categorized Generally Recognized as Safe (GRAS) relatively secure for human and animal. Phytogenic used includes herbal plants, spices, or extracts of active components contained in plants. The active component has been known to have anti-microbial activity, antifungal, and antioxidant activity [21]. Generally in a plant contains one or several active molecules [3]. At present, many studies have been carried out using plants or their extracts as feed additives to livestock because compared to synthetic antibiotics, phytogenic has proven to be more natural, free of residues, and more ideal to be used as a trigger for animal growth [7]. Some of the active phytogenic components include essential oils, flavonoids, saponins, and tannins as contained in the sweet flag (*Acorus calamus*).

Sweet flag (*Acorus calamus*) is a perennial wetland monocot plant from the *Acoraceae* family. The plant has been investigated has a number of chemical constituents from the rhizomes, leave and roots. The bioactive compound was identified on the *Acorus calamus* contain aromatic oil that used as a traditional medicine consist of *eugenol*, *asarilaldehyda*, *asarone*, *kalameon*, *kalamidiol*, *isokalamendiol*, *akorenin*, *akonin*, *akroagermakron*, *akolamonin*, *isokolamin*, *sioburin*, *isosiobunin*, *epi-subunit*, *resin*, *amylum* and also *tannin* [9]. The major bioactive compound identified on the sweet flag are saponin and flavonoid [9], α - and β -asarones caryophyllene, isoasarone, methyl isoeugenol, and safrol. Hence, the used of herbal plants expected to provide health effect on livestock and also in the product. The purpose of this experiment was to evaluate the effect of the used sweet flag (*Acorus calamus*) powder as a feed additive on antioxidant activity and cholesterol of quails egg.

2. Materials and Methods

An experiment about the addition of sweet flag (*Acorus calamus*) powder as a feed additive on antioxidant and cholesterol of egg was conducted by two steps. The first step of the study was the test of the bioactive content of sweet flag powder. This test was carried out at the Airlangga University Laboratory to determine the flavonoid content. The variables observed were flavonoids and antioxidant activity content of the sweet flag.

The second step experiment was conducted at the Poultry Research Farm, Malang, Indonesia. One hundred sixty (160) quails 45 days old were divided into 5 treatments, in which treatment had 5 replication of 8 quails per replication. Five treatments consist T0 as a control (basal diet), T1 (basal diet + 0,1% of sweet flag powder), (basal diet + 0,2 % of sweet flag powder), (basal diet + 0,3% of sweet flag powder), and (basal diet + 0,4% of sweet flag powder). Quail is placed in 20 units of experimental cages with a size of (60 x 40 x 20) cm with a feeding and drinking area. The battery cage is placed in a cage room, which is equipped with two lighting.

2.1. Cholesterol analysis

Analysis of the chemical quality of quail eggs consists of egg yolk cholesterol content (mg / 100gr) using the *Libermann Burchard Color Reaction* method [11], where the procedure was egg yolk weighed 0.2 gram, then added 1 ml of KOH alcohol stirred until precipitate occurs. Then it was in a heater at 39 °C - 40 °C for 1 hour and added 2 ml of petroleum ether 40 °C -60 °C. After that 0.25 mL H₂O was added and stirred for 1 minute and drying using the oven at room temperature. As much as 4

ml of acetate acid solution was added and stirred, then allowed for 35 minutes and read at 630 nm wavelengths with a gap of 0.5 nm using a spectrophotometer.

2.2. Antioxidant analysis

Preparing the solution of each sample by 100 ppm by dissolving 10 mg extract on 100 ml methanol and prepare a 50 ppm DPPH solution. DPPH stock solution was made by dissolving 5 mg DPPH solids into 100 ml of methanol. Then a comparison solution is prepared, which is a control solution containing 2 ml of methanol and 1 ml of 50 ppm DPPH solution. For the sample test, 2 ml sample solution and 2 ml solution were prepared for DPPH analysis. Then the solution incubated for 30 minutes at a 27 °C until there was a color changed from DPPH activity. All the samples were made Duplo. Analysis of the antioxidant activity of the DPPH method was done by looking at the respective color changes sample after incubation with DPPH. When the DPPH electrons pair with electrons in the sample extract will change the color of the sample starting from dark purple to bright yellow. Then sample absorbance value was measured using spectrophotometer at a wavelength of 517 nm.

Feed content of the basal diet was shown in Table 1. Data was analyzed using Completely Randomized Design (RAL). The differential of the data between treatment was to the analysis of variance followed by Duncan's Multiple Range Test.

Table 1. Feed content of basal diet on laying quails.

Composition	Nutrient (%)
Crude Protein	20,32
Crude Fat	3,92
Crude Fiber	3,50
Ash	10,06
Calcium	2,62
Sodium	0,16
Phosphor	0,67

Proximate analysis PT. JAPFA Comfeed Indonesia

3. Results and Discussion

Antioxidants are compounds that can inhibit reactive oxygen species or reactive nitrogen species (ROS / RNS) and free radicals so that antioxidants can prevent diseases associated with free radicals such as carcinogenesis, cardiovascular and aging [6]. Several epidemiological studies have shown that increased consumption of natural phenolic antioxidants found in plants and their products has great health benefits [4]. This is due to the presence of several vitamins (A, C, E, and folate), fiber, and other chemical substances such as flavonoids and polyphenols that are capable of binding free radicals. Analysis of bioactive compounds and antioxidant activity were taken to determine the chemical content of a substance. The raw material used in this study was sweet flag (*Acorus calamus*) powder where the results of the analysis can be seen in Table below.

Tabel 2. Bioactive compound and antioxidant activity of sweet flag powder.

Plants	Flavonoid (% b/b)	IC ₅₀ (mg/ml)
Sweet flag	33,76	0,22

Flavonoids were found in all parts of the plant including fruit, pollen, and roots. Flavonoids contribute to color in plant organs such as flowers, fruit, leaves, or color in pigments. Flavonoids are useful in attracting insects and other animals to help pollinate and spread seeds [18]. In the flavonoid test of the sweet flag with spectrophotometry method has a positive result about 33.76% b/b. Phenolic compounds consist of flavonoids and alkaloids have the potential as antioxidants which are polar

compounds [19]. These polar compounds will be extracted in the methanol extract fraction because of the polar solvent methanol. The ethyl acetate extract fraction contained moderate polarity compounds and it was also possible to extract a small portion of polar compounds which had antioxidant activity.

Determination of IC₅₀ values was carried out to determine the effectiveness of sweet flag powder to prevent 50% free radicals [10]. Based on the results of these calculations, it was obtained information that the sweet flag has 0.22 mg/ml was able to prevent free radicals by 50% in samples. The smaller IC₅₀ value shows the higher antioxidant ability in counteracting free radicals [10]. The antioxidant activity of natural ingredients can be influenced by the levels of flavonoids [20]. Flavonoid compounds consist of phenol groups that are more complex with a higher degree of hydroxylation. The presence of hydroxyl groups in flavonoid compounds causes antioxidant activity. It can be caused by oxygen atoms in the hydroxyl group having free electron pairs that are sufficient to inhibit the reactivity of reactive atoms which make up free radical compounds [2].

The addition of sweet flag powder (*Acorus calamus*) as a feed additive on quail was shown in Table below:

Table 3. Effect of the addition sweet flag (*Acorus calamus*) powder on antioxidant activity and cholesterol on quails egg.

Treatment	Antioxidant Activity (IC ₅₀)	Cholesterol (mg/100 gram sample)
T0	199.17 ^d	604.25 ^c
T1	194.26 ^c	587.99 ^d
T2	191.67 ^b	549.76 ^c
T3	190.55 ^b	524.96 ^b
T4	180.09 ^a	511.76 ^a

^{a-c} Means within a row with the different superscript were significantly different

Based on the data, the addition of sweet flag as a feed additive had a significant effect ($P < 0,01$) on antioxidant activity of quails egg. Antioxidant activity was measured by DPPH. The highest antioxidant activity of quails egg for each treatment shown respectively, T4 (180,09), T3 (190,55), T2 (191,67), T1 (194,26) and T0 (199,17). The results show that the addition of 0,4 % sweet flag had the highest antioxidant activity compared with other treatments. Essential oil of sweet flag had an antioxidant activity to increased quality of feed [12]. The total antioxidant activity of a bioactive compound was influenced by the structure of the test compound and the appropriate method was used for the test. The structure of the bioactive compound analysis was related to the presence of antioxidant groups. High antioxidant activity is obtained in compounds containing ortho-diphenol such as epicatechin, caffeic acid, epigallocatechingallate and others. The presence of phenolic OH groups that can be quickly abstracted by free radicals causes phenolic compounds to have the potential as antioxidants. In addition, antioxidant activity was also influenced by double bonds and carbonyl groups of heterocyclic rings from the core structure which can increase antioxidant activity by stabilizing phenolic radicals through electron conjugation and delocalization (Heim et al, 2002). Flavonoid levels have a positive relationship with antioxidant activity to reach IC₅₀ where the higher the flavonoid content, the higher the IC₅₀. In hence, it can be interpreted that an increased content of polyphenol compounds will increase the concentration value (ppm) of antioxidants to reach IC₅₀, which means that the antioxidant abilities possessed stronger. Antioxidant activity has a correlation with the total components of phenolic compounds [23].

The results of the analysis cholesterol in quails egg with the different treatment had a significant effect ($P < 0,01$). The lowest cholesterol content on quails eggs shown in the treatment 4 with the addition of 0.04 % of sweet flag powder. Cholesterol content was decreased by 2.56 % compared with the basal diet (control). Decreased cholesterol content in quail egg may be due to the high content of flavonoid on the sweet flag powder [5]. Polyphenol compounds such as flavonoids can inhibit oxidation reactions through the mechanism of radical scavenging by donating one electron to unpaired

electrons in free radicals so that the amount of free radicals becomes reduced. Flavonoids were strong inhibitors of lipid peroxidation, as binding of reactive oxygen or nitrogen species, also able to inhibit the activity of lipoxygenase and cyclooxygenase enzymes [6]. Alkaloid compounds have the ability to attach themselves between DNAs so that they interfere with DNA replication [22]. The mechanism of action is to interfere with the formation of a cross-crossing bridge of the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed intact and causes cell death [17]. Flavonoids have become a topic of learning and study because from various studies and existing data show that the function and use of flavonoids as anti-microbial, maintain oxidative stability, antioxidant, antimutagenic, anticarcinogenic, and have the ability to modify gene expression [15].

4. Conclusion

The used of sweet flag powder up to 0.4 % had increased the antioxidant activity and decreased cholesterol of quails egg yolk. The addition of sweet flag powder as feed additive has to increase antioxidant activity and decrease cholesterol on quails egg.

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