

## **The Effectivity of Kelubi (*Eleiodoxa Conferta*) Towards Microbial Content and Egg Shell Flour Calcium**

Susan E. Lumban Gaol<sup>1</sup>, Ayutha Wijinindyah<sup>2</sup>, and Ida Ketut Mudhita<sup>2</sup>  
Prodi Peteranakan Fakultas Pertanian, Universitas Antakusuma Pangkalan Bun  
Jln. Iskandar No 63 Pangkalan Bun, Kotawaringin Barat, Kalimantan Tengah  
Corresponding author: Susan E. Lumban Gaol  
Email: susanlgpruden@gmail.com

### **Abstract**

The purpose of this research is to determine the effect of soaking eggshells in kelubi fruit water extract on the microbial content and calcium content of the eggshells. The study was designed using a Completely Randomized Design (CRD). The treatment was soaking the eggshells in kelubi fruit water extract for 0 (without soaking), 3, 4, and 5 hours. The variables observed for the eggshells included calcium levels and testing for *Escherichia coli* and *Salmonella sp.* bacteria using the Most Probable Number (MPN) method. The results showed that the calcium level increased with longer soaking in kelubi fruit water extract. However, soaking in kelubi fruit water extract did not affect the microbial content, as no *Salmonella sp.* or *Escherichia coli* bacteria were found in any of the treatments.

*Keywords : Kelubi fruit water (*Eleiodoxa conferta*), microbial content, eggshell*

### **Introduction**

Broiler chickens are poultry that have the potential to produce meat. The advantages of broiler chickens are that they have a relatively fast growth rate of productivity and are quite efficient in the use of feed, which means that the ratio between the resulting body weight gain is the same as the amount of feed consumed and the product price is relatively affordable. Broiler chickens have a fast growth rate, but the drawback is that it has a fairly high fat content, this will be a good place for the development of spoilage microorganisms which will reduce the quality of the meat so that the impact on the meat carcass will be easily damaged. In addition to relatively fast growth, fat growth will be followed, where high body weight is associated with high body fat accumulation. The high fat content in carcasses will be a concern for consumers. Carcasses that have good quality are carcasses that have low fat content and high protein content. The fat content of broiler chicken is in the range of 5.79-8.44% with a fat mass of  $\pm 10\%$  higher than that of other local chickens, which is 1.18-2.76% (Ismoyowati & Widyastuti, 2003). The content needed by the body is nutrients such as carbohydrates, protein, vitamins, minerals, and no less important, namely fat. Fat is a nutrient that is needed by the body, fat is a source of high calorie-producing energy (Sulistyoningsih, 2014). If you consume too much fat, your body will

experience obesity, high cholesterol, heart disease and fatigue. Therefore, to produce healthy broiler meat carcasses, it is necessary to reduce the fat content so that it is safe for consumption. One of the efforts made is by adding  $\beta$ -glucan.

$\beta$ -glucan here has a role to influence fat absorption by binding fatty acids, cholesterol, and bile salts in the digestive tract. A very easy source of  $\beta$ -glucan is  $\beta$ -glucan from *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* is a type of fungus or yeast that can synthesize  $\beta$ -glucan from its cell wall. The growth process of *Saccharomyces cerevisiae* requires a nutrient medium consisting of carbon, nitrogen, oxygen, vitamins, and minerals. One source of carbohydrate that can be used for *Saccharomyces cerevisiae* is easy to obtain and relatively inexpensive, namely the banana weevil.

Banana weevil is the tuber of the stem that is at the bottom of the banana stem which is under the ground. Besides being cheap, easy to obtain and not competing with humans, the banana weevil also has a high carbohydrate content, namely 66.2%, protein 3.40% and energy 2,450 kcal/kg (Taran, Ballo, & Sinlae, 2015). The use of banana weevil into flour is based on the fact that the weevil is a component of polysaccharides that can be processed into a new source of flour (Saragih, 2013). Banana weevil is rich in dietary fiber (Astawan, 2004). (Rudito, Syauqi, Obeth, & Yuli, 2010) stated that the chemical characteristics of banana weevil were 6.69% water content, 0.11% ash content, and 2.6 mg/kg HCN content. Banana weevil also has a starch composition of 76% and 20% water and the starch resembles tapioca starch and sago flour (Rosdiana, 2009). The crude fiber found in the banana weevil is  $\beta$ -glucan or water-soluble fiber (Tala, 2009). The dietary fiber affects the physical quality of meat such as tenderness, water holding capacity, pH value and cooking loss. Microscopically, the structure of food fiber is in the form of a capillary and has a greater ability to absorb water (Darajat, 2010). The increase in water-holding capacity is not only determined by myofibril proteins, but is also determined by the water-binding component broadly. Swelling of the water-binding component increases with increasing water content, but swelling is by no means infinite.

The community's need for high-quality chicken meat is increasing, both chemical quality and physical quality. One way that can be done on the physical quality of meat is by optimizing the tenderness, pH value, water holding capacity and reducing the cooking loss of meat (Hamiyanti, Sutomo, Rozi, Adnyono, & Darajat, 2013). The addition of banana hump enriched with  $\beta$ -glucan fiber did not have a negative effect on productivity, abdominal fat, and the relative weight and length of the small intestine (S Imam & Suryadi, 2021; Shokhirul Imam, Suryadi, & Fitriani, 2020). Based on this description, research will be conducted on the effect of banana weevil which is rich in  $\beta$ -glucan fiber on the physical quality of broiler meat including tenderness, water holding capacity, pH value and cooking loss. Based on statistical data from livestock and animal health in Indonesia in 2019, egg production from laying hens amounted to 4.753.382 tons. Eggs are one of the main commodities in the food industry, which produces 250,000 tons of eggshell waste annually (Verna et al., 2018). Waste is material discarded from human activities or natural processes that do not yet have economic value but

negatively impact the environment. The negative impacts are the cost of disposal and cleaning and the potential for environmental pollution (Darmawati, 2015).

The level of eggshell waste is increasing. Eggshell waste needs to be utilized to make eggshell powder, which is rich in calcium and can reduce waste disposal in Indonesia. Eggshells are a plentiful source of calcium but have yet to be widely used (Wijiniandyah et al., 2023). The main composition is  $\text{CaCO}_3$ , the most commonly used calcium salt, because 40% of its components are easily absorbed. One shell of a laying hen's egg contains 108mg/g of calcium (Burn et al., 2013). Eggshells can be processed into powder, and adding the eggshell powder to food ingredients can be an alternative source of calcium (Wijiniandyah et al., 2023). The nutritional content of eggshells is equal to that of eggs, but it currently needs more attention. Eggshells comprise 95.1% inorganic material, 3.3% protein, and 1.6% water. The chemical composition of eggshells consists of 1.71% protein, 0.36% fat, 0.93% water, 16.21% crude fibre, and 71.34% ash (Nursiam, 2011). Based on previous research by Butcher and Miles (2012), chicken eggshells contain 401 7.2 grams or about 39% calcium, in the form of calcium carbonate.

Eggshell waste can be turned into food products, reducing waste from egg-based industries. This is one advantage of eggshells. The disadvantage is the presence of brown spots on the eggshells, called "broad-cast", which contain *Streptococcus* bacteria that can penetrate the shell. Brown spots on eggshells are normal and result from blood vessels breaking during the egg-forming process in chickens (Jansen, 2017). Eggshells can be contaminated with bird droppings, nests, feed, air, and equipment. They contain many bacteria, especially *Escherichia coli* and *Salmonella* sp bacteria, that can disrupt the digestive system, especially gram-negative bacteria that are motile and can enter through the pores of the eggshell, especially if the eggshell is wet (Lily et al., 2018). The problem with applying eggshell powder is the decomposition process of the eggshell, which produces ammonia and hydrogen sulfide. It also produces an unpleasant aroma due to *Escherichia coli* and *Salmonella* sp bacteria (Owuamanam & Cree, 2020).

The Kelubi plant is a sour fruit in certain areas, such as Lampung, South Sumatra, Riau, and Kalimantan in Sumatra (Agung, 2015). Phenolic compounds, flavonoids, saponins, and alkaloids are reported to be present in the *Eleiodoxa conferta* Kelubi fruit (Afriani et al., 2014). The plant belongs to the *Arecaceae* family (salak). Kelubi fruit, a member of the salak family, is believed to contain antioxidants such as vitamin C and phenolic compounds. Antioxidants are compounds or chemical components that, in a certain concentration or amount, can inhibit or slow down the damage caused by the oxidation process (Sayuti et al., 2015). Previous research has used the maceration method to conduct an antibacterial test with crude extract of the sour pay a fruit (*Eleiodoxa conferta*) against *Staphylococcus aureus* and *Salmonella typhi* bacteria. The antibacterial activity test of the crude ethanol extract of the sour paya fruit showed an optimal inhibition zone at a concentration of 80%, with inhibition zones against *Staphylococcus aureus* and *Salmonella typhi* bacteria of 9.63 mm and 17.61 mm, respectively (Safitri et al., 2017).

Based on the phytochemical test results in Table 1, it can be seen that Kelubi water (*Eleiodoxa conferta*) contains alkaloids, hydroquinone phenols, saponins, and flavonoids (Ratih et al., 2019). Therefore, a study was conducted to determine the content of Kelubi fruit water on microbial levels and calcium in eggshell powder.

Table 1. Phytochemical Test Results of Kelubi Fruit

Compound	Description
Alkaloid	+
Hydroquinone phenol	+
Saponin	+
Flavonoid	+
Steroid	-

Source: Ratih et.al., 2019

### Materials and Methods

The research was conducted in November 2022 at the Livestock Laboratory of the Faculty of Agriculture, Antakusuma University in Pangkalan Bun, to produce an eggshell powder. Calcium content testing was conducted at the Animal Nutrition Laboratory, Faculty of Animal Husbandry, Lambung Mangkurat University in Banjarmasin, and microbial testing (*Escherichia coli* and *Salmonella sp*) was conducted at the Livestock Production Laboratory, Bogor Agricultural Institute.

The materials used in this study were eggshells from laying hens and kelubi fruit. The equipment used included a digital scale, 80-mesh sieve, blender, tray, cup, spoon, bowl, and calcium and microbial testing equipment.

The Kelubi fruit (*Eleiodoxa conferta*) was obtained from Kumai Village, West Kotawaringin, Central Kalimantan. The kelubi fruit was peeled, and the flesh was separated from the seeds (Figure 1), sliced into small pieces, then ground with a blender and filtered to extract the juice. The juice was then squeezed using a thin cloth and centrifuged to obtain pure kelubi juice (Figure 2).

Figure



Figure 1. Klubi Fruit



Figure 2. Kelubi Fruit



Figure 3. Eggshell and Kelubi Fruit

The eggshells were obtained from a pastry shop in Pangkalan Bun. The eggshells were washed with running water and cleaned from the membrane. The cleaned eggshells were air-dried at room temperature for one day and then crushed to obtain smaller pieces (Figure 3). The eggshells were then soaked in kelubi fruit juice and air-dried at room temperature for 24 hours. After the soaking process, the eggshells were ground using a blender and sifted through an 8-mesh sieve to obtain an eggshell powder.

The study was designed using a Completely Randomized Design (CRD) with 4 treatments, and each treatment was replicated 3 times. The treatments in this study are as follows:

P1: Eggshell 250 g without soaking

P2: Eggshell 250 g + 100 g Kelubi water + soaked for 3 hours

P3: Eggshell 250 g + 120 g Kelubi water + soaked for 4 hours

P4: Eggshell 250 g + 140 g Kelubi water + soaked for 5 hours

Calcium testing was done by weighing 5 g of the sample in a porcelain crucible, then heated using a hot plate and cooled in a desiccator for 30 minutes. The dried sample was put into a furnace at 550° C for 3 hours and then left to cool in a desiccator. The sample that had turned into ash was dissolved in distilled water with HNO<sub>3</sub> 1:1 as much as 10 ml, then heated until the volume became 5 ml. The mixture was then filtered to obtain a filtrate in a 25 ml measuring flask. The filtrate was added with distilled water to the measuring line, then analyzed with AAS (Sowmya et al., 2015).

Microbial content testing for *Salmonella sp* and *Escherichia Coli* used the Most Probable Number (MPN) method (Blodgett, 2006).

The data analysis of the study results used ANOVA, with the design used is a Completely Randomized Design (CRD), with 4 treatments and 3 replications. ANOVA calculations were performed using SPSS program version 26, and if there were differences, it would be followed by a Duncan test at a 5% significance level (Steel and Torrie, 1991).

## Results and Discussion

### Calcium Level

Table 2. The measurement of calcium content in eggshells using kelubi water with 5 hours of soaking showed the highest value

No	Treatment	Ca Content %
1	P1 No soaking	34,806±0,05
2	P2 Soaking 3 Jam	35,012±0,10
3	P3 Soaking 4 Jam	35,526±0,02
4	P4 Soaking 5 Jam	36,717±0,07

Sumber: Data Hasil Analisis (2022).

The measurement of calcium content in eggshells using kelubi water with 5 hours of soaking showed the highest value (Table 2). The effect of kelubi fruit water on eggshell soaking resulted in the highest calcium content of 36.717% in

5-hour soaking at room temperature. The results showed that the longer the eggshell soaking time, the higher the calcium content in the eggshell powder. In a previous study (Brun et al., 2013), the average calcium content in eggshells was 32.8% with the wet sterilization (autoclave) process. Rahayu and Shofia (2017) stated that the average calcium content in eggshells after boiling sterilization was 33.5%. This research showed that the effectiveness of kelubi fruit greatly affects the calcium content. This is likely due to the highly acidic properties of kelubi water containing secondary metabolites, flavonoids, tannins, and saponins. Each solvent has the same principle of opening up the eggshell pores, thus creating a space easily reached by the solvent. The dielectric constant value influences the binding of eggshell minerals by the solvent. The higher the dielectric constant value of a solvent, the more polar it is. The polarity level of a solvent will affect the effectiveness of the solvent in attracting or dissolving some components and compounds of the material (Purnamasari, 2013).

### Microbial levels

Table 3. The results of the *Salmonella sp* microbial tests using the MPN (Most Probable Number) method

Treatment	Results	Method
P1 No soaking	Negative/25g	Fda/Bam Chapter 5
P2 Soaking 3 jam	Negative/25g	Fda/Bam Chapter 5
P3 Soaking 4 jam	Negative/25g	Fda/Bam Chapter 5
P4 Soaking 5 jam	Negative/25g	Fda/Bam Chapter 5

Source: Analysis Results Data (2022).

Table 4. The results of the *Salmonella sp* and *Escherichia coli* microbial tests using the MPN (Most Probable Number) method

Treatment	Unit	Result	Method
P1 No soaking	MPN/25g	<3	Fda/Bam Chapter 4
P2 Soaking 3 jam	MPN/25g	<3	Fda/Bam Chapter 4
P3 Soaking 4 jam	MPN/25g	<3	Fda/Bam Chapter 4
P4 Soaking 5 jam	MPN/25g	<3	Fda/Bam Chapter 4

Source: Analysis Result Data (2022).

The results of the *Salmonella sp* and *Escherichia coli* microbial tests using the MPN (Most Probable Number) method are presented in Tables 3 and 4. Test levels of *Salmonella sp* bacteria using the Most probable number (MPN) method in immersing eggshells using kelubi fruit water did not show microbial growth between treatments. Adding kelubi fruit water as an antimicrobial showed no difference in each treatment. This study stated that *Salmonella sp.* bacteria were not detected by adding water from the fruit of the sea cucumber with a long immersion time on the egg shells. The tannins in kelubi water are used as antibacterials because they can precipitate proteins and damage cell membranes, inhibiting fungal growth. (Sudira et.al., 2011) Tannin compounds are organic compounds that can inhibit microbial growth by forming bonds with functional proteins of microbial cells and damaging microbial cell walls. Tannin's antibacterial mechanism can inhibit the synthesis of chitin needed for forming cell walls in fungi and inhibits fungal growth by damaging cell membranes. Tannins are lipophilic compounds that can damage cell walls because they easily bind to cell walls.

*Escherichia coli* bacteria concentration test Most probable number (MPN) in eggshell immersion using kelubi fruit water did not show microbial growth. The results of this study stated that by adding kelubi fruit water to eggshells, *Escherichia coli* bacteria of less than three were not detected, which means negative for bacterial growth. From these results, eggshell flour soaked in kelubi fruit water can kill bacteria and is safe for consumption as food fortification. The food ingredient limit for bacteria detection <3 This means negative, the maximum threshold for microbial contamination in food ingredients (BPOM 2009). Flavonoids and ethanol found in sea cucumber water work as antibacterials. The nucleic acid synthesis mechanism inhibits the cytoplasmic membrane's function and inhibits the energy metabolism of bacteria. Flavonoids act as antibacterials to form complex compounds with extracellular proteins and dissolved proteins so that they can damage the bacterial cell membrane and be followed by releasing intracellular compounds (Nuria et al., 2018). In a study by Surtina et al., 2020, it was stated that adding a 40% concentration of kelubi fruit acid gave moderate inhibition. 60%, 80%, and 100% Concentrations provide strong resistance. The greater the concentration used, the larger the diameter of the bacterial inhibition zone. The ethanol extract of kelubi fruit pulp has antibacterial activity against *S. aureus* and *E. coli* bacteria characterized by forming strong inhibition zones. They know the greatest antibacterial activity of ethanol extract and each fraction and the correlation between antibacterial activity and flavonoid content.

### Conclusion

The research concluded that calcium levels were higher with increasing water immersion of kelubi fruit. However, immersion in kelubi fruit water did not affect microbial levels. *Salmonella sp* and *Escherichia coli* were not found in all kinds of treatments.

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