Mineral contents in flesh of abalone *Haliotis squamata* fed with different live feeds

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**Abstract.** Mineral contents in flesh of abalone *Haliotis squamata* were analyzed by using Atomic Absorption Spectrophotometer method. Samples of abalone *Haliotis squamata* used in this research were treated by three types of live feed, i.e. *Gracillaria* sp., *Ulva* sp., and mix of *Gracillaria* sp. and *Ulva* sp. Flesh of abalone *H. squamata* contained some mineral *i.e* K, Ca, Mn, Zn, Fe, Cu, Hg, and Mg. In the flesh of abalone *H. squamata* fed with those feeds, K had the highest value followed by Mg while the lowest was Cu. In food, *Ulva* sp contain the highest K followed by Ca with the lowest was Cu. In contrast, Ca had the highest value in *Gracillaria* sp followed by Mg while the lowest was Cu.

1. **Introduction**

The utilization of marine resources is turned aside from fishing to aquaculture which is concerning more to economics fish and oysters. However, one of the high economic commodities which can be developed is abalone (*Haliotis* spp.).

Some alternatives in culturing systems of abalone’s culture development are still needed. For many culture systems have been developed whether the traditional or modern, the technology transition is more focused to live feed feeding. The study of live feed feeding will become so important because the foods found to be the important factor and have to be noticed in the abalone culture.

Types of live feed usually used in abalone culture are *Euchema cottonii, Gracillaria arcuata, G. gigas, G. solicornia, G. racillariopsis heteroclada,* and *G. verrucosa* as well as *Ulva* sp. [1]. Those types have been tested and affected significantly to abalone’s growth rate [2, 3].

Beside protein and lipid, mineral also important element the body need that are contained in flesh of abalone. Mineral such as K, Ca, Mn, Zn, Fe, Cu, Hg, and Mg are coenzymes and cofactor of the biochemical process of cellular energy generation [4]. The human body utilizes mineral for the proper composition of bone and blood, and maintenance of normal cell function. Mineral functioned along with vitamins as essential components in enzymes and coenzymes. If an enzyme is lacking the necessary mineral, it cannot function properly no matter how much of the vitamin is available. For example, zinc is necessary for the enzyme that activates vitamin A in the visual process. Without zinc in the enzyme, vitamin A cannot be converted to its active form [5].

This research is aimed to analyze the mineral contents in flesh of abalone fed with different live feeds of *Gracillaria* sp., *Ulva* sp., and mix of *Gracillaria* sp. and *Ulva* sp.
2. Materials and Methods

2.1. Tools and Materials
Materials used in this research were abalone *Haliotis squamata*, alga *Ulva* sp. and alga *Gracillaria* sp. For mineral analysis, the material used were HNO₃, H₂O₂, H₂SO₄, Citric acid, aquades and standard. Mineral analysis was done using Atomic Absorption Spectrophotometer (AAS).

2.2 Sample Preparation
Weight about 5 g of sample (dry weight) into a digestion tube. Add 5 mL of HNO₃ and then 5 mL of H₂SO₄ to the sample. Allow the reaction to proceed. When the reaction slows, place the tubes in a hot-block digestion apparatus and heat at a low temperature (60 °C) for 30 min. Remove the tubes from the hot block, allow to cool, add 10 mL of HNO₃, return tubes to digestion rack and heat slowly to 120 °C. Increase the temperature to 150 °C. Remove the tubes when the samples go black, allow to cool, then add 1 mL of H₂O₂. A vigorous reaction may occur. Return the tubes to the block. Repeat the H₂O₂ additions until the samples are clear. Remove the tubes and make up to 50 mL with deionized water.

2.3 Analysis
Standards should be treated in the same way as samples. It is important that the standards contain the same amount of acid as the samples, especially H₂SO₄, as it will have a viscosity effect that will suppress sensitivity.

3. Result and Discussion

3.1 Mineral contents in flesh of abalone
The result showed that mineral found in flesh of abalone are K, Mg, Ca, Fe, Zn, and Cu at variation values (Table 1).

| Mineral | Mineral contents of flesh of abalone fed with Ulva sp. | Mineral contents of flesh of abalone fed with Gracillaria sp. | Mineral contents of flesh of abalone fed with Mix |
|---------|------------------------------------------------------|----------------------------------------------------------|--------------------------------------------------|
| K       | 2258 mg kg⁻¹                                       | 3506 mg kg⁻¹                                             | 3498 mg kg⁻¹                                     |
| Ca      | 498 mg kg⁻¹                                        | 451 mg kg⁻¹                                              | 827 mg kg⁻¹                                      |
| Zn      | 43.7 mg kg⁻¹                                       | 39.4 mg kg⁻¹                                             | 37.2 mg kg⁻¹                                     |
| Fe      | 67.8 mg kg⁻¹                                       | 36.6 mg kg⁻¹                                             | 153 mg kg⁻¹                                      |
| Cu      | 11.5 mg kg⁻¹                                       | 18.9 mg kg⁻¹                                             | 11.7 mg kg⁻¹                                     |
| Mg      | 1747 mg kg⁻¹                                       | 1688 mg kg⁻¹                                             | 1305 mg kg⁻¹                                     |

The value of mineral in flesh abalone treated with three different feed i.e. *Ulva* sp, *Gracillaria* sp and mix of *Ulva* sp and *Gracillaria* sp varies but showed the same trend. The K values of flesh abalone fed with those feed have the highest value, followed by Mg while the lowest value belonged to Cu.

3.2 Mineral contents in live feeds
Mineral analysis was also conducted to live feeds fed to abalone (*Ulva* sp. and *Gracillaria* sp.) with the result is shown in Table 2.

The value of mineral content of K in *Ulva* sp is the highest, followed by Ca while the lowest is Cu value. On the contrary, the value of Ca is the highest in *Gracillaria* sp, followed by Mg whereas the lowest value belongs to Cu.

Some mineral showed different values between the contents in flesh of abalone and in live feed. Several factors directly or indirectly influenced the levels of mineral in plants and hence the amounts available for humans and animals depend on plants for foods and feeds respectively. The amount of a
particular nutrient in the diet might be insufficient to meet the requirements. However, the metabolism of the animal might be deranged by the interaction of dietary, environmental and genetic factors [6].

| Mineral | Ulva sp. | Gracillaria sp. |
|---------|----------|----------------|
| K       | 24136 mg kg\(^{-1}\) | 783 mg kg\(^{-1}\) |
| Ca      | 9349 mg kg\(^{-1}\) | 10302 mg kg\(^{-1}\) |
| Zn      | 6.1 mg kg\(^{-1}\) | 9.7 mg kg\(^{-1}\) |
| Fe      | 1721 mg kg\(^{-1}\) | 1275 mg kg\(^{-1}\) |
| Cu      | 3.96 mg kg\(^{-1}\) | 7.05 mg kg\(^{-1}\) |
| Mg      | 2350 mg kg\(^{-1}\) | 9676 mg kg\(^{-1}\) |

Mineral K (Potassium) of *Ulva* sp. and *Gracillaria* sp. were found having greater difference of value, which *Ulva* sp. was the highest. The function of K was as cofactor that functions in protein synthesis, activation of enzymes, major solute functioning in water balance and thus affecting osmosis, operation of stomata, and the effect of potassium deficiency was reduced in growth [5]. Sivakumar and Arunkumar (2009) [7] recorded that the mean value of K in the brown and red algae were higher when compared to green seaweeds, however, the mineral analysis result showed difference. It might be caused by the environmental and genetic factors of each alga as the different result showed in mineral contents of flesh of abalone which might be caused by the factors also. Based on the function of K and the negative effect of potassium deficiency mentioned earlier, the result of abalone growth supported this in which the abalone fed with *Ulva* sp. has better growth performance than the abalone fed with *Gracillaria* sp. and mix of both alga as shown in Figure 1. The rapidly growing animals apparently have a higher requirement for potassium, and increasing the protein level increases the requirement [5].

![Figure 1. Growth of abalone (weight increasing) fed with Ulva sp., Gracillaria sp. and mix of Ulva sp. and Gracillaria sp.](image)

The high values of Ca and Mg in *Ulva* sp. and *Gracillaria* sp. were significantly difference with the values in flesh of abalones. It might be caused by the absorption of Ca by abalone to build the shell and muscle. Most aquatic species have no dietary calcium requirement, although it is required for some important functions, including shell or bone building, blood clotting, muscle function and nerve transmission [8, 9]. In this case, abalone appeared to absorb Ca from its dietary food. However, fish,
shrimp and aquatic mollusks were also able to absorb calcium directly from the surrounding water via drinking or direct absorption through the gills, epidermis, or both [9,10,11,12].

Values of Magnesium (Mg) were found similar in flesh of abalones fed with all types of live feeds. While the values showed different and lower compared to Mg values of live feeds itself. Hardwick et al. (1991) [13] reported that absorption, excretion, and retention of Mg in animals were affected by dietary cholecalciferol and phosphorus content. While Zhang et al. (2003) [14] reported that when diets were supplemented sufficient cholecalciferol, Mg concentrations were not significantly influenced by dietary phosphorus. Function of Mg are to activate many enzymes and as component of chlorophylls [5]. Magnesium is absorbed in the intestines and then transported through the blood to cells and tissues. Approximately one-third to one-half of dietary magnesium is absorbed into the body. When a magnesium-deficient diet is fed to young chicks, it leads to poor growth and feathering, decreased muscle tone, ataxia, progressive incoordination and convulsions followed by death [15].

Iron (Fe) at Table 1 and Table 2 showed different values which in flesh of abalone Fe became very poor while the live feeds were rich with iron. It might be caused by the high absorption of other mineral particularly the macros. It also might be caused by the stress condition of abalone during the cultivation. Factors effecting the absorption of iron are: low phosphate diet which increases iron absorption, whereas high phosphate diet decreases iron absorption by forming insoluble iron phosphates [16]. Adrenocortical hormones (glucocorticoids) play a role in regulating the level of plasma iron. During stress, when the hypothalamus, adenohipophysis, and adrenal cortex are activated, regardless of the source, the plasma iron decreases [16]. Iron functions as haemoglobin in the transport of oxygen. In cellular respiration, it functions as essential component of enzymes involved in biological oxidation such as cytochromes c, c1, a1, etc [17]. Biologically important compounds of iron are haemoglobin, myoglobin, cytochromes, catalases and peroxidase [17].

Copper (Cu) and Zinc (Zn) are essential micronutrients found at high levels in the algae and in the tissues of fish an shellfish [18,19]. Zn is available to abalone from both the dissolved phase (e.g. gill uptake) and the diet (e.g. alga ingestion)[20]. Thus it could be suggested that the higher value of Zn of abalone flesh compared to Zn of live feeds were obtained from surrounding water by dissolved phase. As the micronutrient, Cu as well as Zn was needed in small amount, due to some functions of Zn i.e. active in formation of chlorophyll, activates some enzymes, plays a role in formation of auxin, chloroplasts, and starch, and function of Cu as component of many redox and lignin-biosynthetic enzymes [5].

4. Conclusion

In the flesh of abalone Haliotis squamata, the highest mineral contain was Kalium and the lowest was Cuprum for the three treatments of feeding, however the highest concentration was in flesh of abalone fed with mix live feed (Gracillaria sp. and Ulva sp.) while the lowest Cuprum concentration was in flesh of abalone fed with Gracillaria sp.. The minerals contained in flesh of abalone were Kalium, Calsium, Zinc, Ferrum, Cuprum, and, Magnesium.

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