RJOAS: Russian Journal of Agricultural and Socio-Economic Sciences
ISSN 2226-1184 (Online) | Issue 1(145), January 2024

UDC 639; DOI 10.18551/rjoas.2024-01.13

POTENTIAL OF ADDITION OF CALCIUM HYDROXIDE FROM CHICKEN EGG SHELLS TO FEED CONVERSION RATIO AND FEED EFFICIENCY ON CULTIVATION OF WHITE SHRIMP (LITOPENAEUS VANNAMEI) IN FRESHWATER CONDITIONS

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ABSTRACT
The purpose of this research was to see the potential for adding calcium hydroxide ($Ca(OH)_2$) from chicken eggshells to the Feed Conversion Ratio (FCR) and Feed Efficiency in white shrimp (*Litopenaeus vannamei*) cultivation in freshwater conditions. This research was conducted for 30 days. The experimental design used was a completely randomized design (CRD) consisting of 4 treatments with 5 replications. The treatments used were P0: commercial feed + 1% CMC, P1: commercial feed + 1% CMC + 1% $Ca(OH)_2$ chicken egg shells, P2: commercial feed + 1% CMC + 2% $Ca(OH)_2$ chicken egg shells, P3: commercial feed + 1% CMC + 4% $Ca(OH)_2$ chicken egg shells. A one-way analysis of variance (ANOVA) was applied to compare the control and treated groups at a significance level of $P<0.05$. Duncan's New Multiple Range Test (DMRT) was used to identify significant differences in the Feed Conversion Ratio (FCR) and Feed Efficiency. The results of this research were significantly different which is the best Feed Conversion Ratio (FCR) in treatment P1 and P2 with an average of 0.551 and 0.717, respectively and the best Feed Efficiency in treatment P1 and P2 with an average of 208.219% and 154.709%, respectively.

KEY WORDS
Shrimp, production, freshwater, feed efficiency.

The increasing cultivation of white shrimp throughout the archipelago in aquaculture areas has recently made some farmers try to cultivate white shrimp in freshwater ponds, and even enter into farmer groups in urban areas to also try to cultivate white shrimp in freshwater by utilizing less productive yards [1]. The location of this culture which is far from the sea causes the cost of cultivation media to increase which requires brackish water as an optimal environment for white shrimp so that the selection of freshwater as a medium for white shrimp cultivation can reduce costs.

The use of freshwater as a medium for white shrimp cultivation on the other hand has a negative impact. The low salinity in the white shrimp environment can limit the intake of calcium minerals in the body. The decrease in salinity when white shrimp is cultivated in low salinity inland waters results in a decrease in the survival and growth of white shrimp. Maintenance of white shrimp at salinity under optimal conditions makes white shrimp have to absorb calcium from other sources [2]. According to [3], shrimp that are in low salinity absorb a lot of water from the environment which causes the shrimp body to change its skin. Shrimp growth is a continuation of the molting process. In the post-molting stage, hardening of the skin occurs through the deposition of calcium in the skin.

Calcium needs can be met from feed and the environment [4]. The role of environmental calcium is very dominant, in the process of hardening shrimp shells; a high enough calcium is needed. The source of calcium in the process of increasing skin calcium levels and the length of the molting cycle, as well as its consequences for the growth of shrimp biomass and the increase in dissolved calcium can lead to an increase in pH [4]. Calcium hydroxide $Ca(OH)_2$ has a positive relationship between skin calcium levels and environmental calcium levels in line with the continuous exchange of calcium between the body and the environment [5].

Feed is a factor that plays a very important and decisive role in the success of a fishery business and the availability of feed is one of the main factors to producing maximum production [6]. Eggs are one type of food that is quite popular among the people of Indonesia. This food is popular because it can be processed into various types of food and can be consumed by both children and adults. In addition, eggs can also be purchased at low prices so that they can be enjoyed by everyone from the lower class to the upper class. Based on data from the Indonesian Central Statistics Agency (BPS) in 2015, egg consumption per capita can reach around 112 chicken eggs per year. If in 2017 there were around 67,851,944 families in Indonesia, then egg consumption in Indonesia could reach around 7.6 billion eggs in a year. This means that there will be quite a lot of waste from eggshells found in Indonesia every year [7].

Unlike eggs, egg shells are usually just thrown away in landfills. The eggshell is a hard layer that protects the egg from environmental conditions. This shell is hard and has a fairly high calcium content. Unfortunately, so far the benefits of egg shells are still rare and not even noticed at all. Even though the calcium contained in it is one type of macronutrient that can be used as a solution to the lack of mineral content in white shrimp culture in freshwater [7].

The optimal feed content can be seen from the Feed Conversion Ratio (FCR) and feed efficiency. Decreasing the value of FCR indicates that the feed consumed has good quality so that it can be utilized efficiently. This is reinforced by the opinion of [8] that the value of feed efficiency is inversely proportional to feed conversion and directly proportional to the increase in body weight of fish, so the higher the feed efficiency value, the lower the feed conversion value. The value of FCR of white shrimp is influenced by the optimal living environment of white shrimp, when white shrimp are given calcium levels that match the needs of shrimp that are reared at low salinity, the appetite of the shrimp becomes high. The high appetite makes the value of the feed conversion ratio of white shrimp low. The addition of nutrients to white shrimp carried out according to the dose will be able to increase the absorption of nutrients from shrimp so that the appetite of white shrimp increases, and FCR is lower [9]. High FCR and low feed efficiency can cause high feed residues in the waters, thus, to determine the level of FCR and feed efficiency from efforts to utilize calcium hydroxide from chicken egg shells in white shrimp culture in freshwater, these are necessary to research the potential for adding calcium hydroxide ($Ca(OH)_2$) from chicken egg shells on FCR in the cultivation of white shrimp (*Litopenaeus vannamei*) in freshwater [10].

METHODS OF RESEARCH

This research is an experimental research method using Completely Randomized Design (CRD) with 4 treatments and 5 replications by randomizing the place of treatment to create diversity and is considered natural [11]. Each aquarium was stocked with 25-day-old white shrimp postlarvae (PL-25) with a stocking density of 15 fish/liter in an aquarium measuring 15x15x20 cm^3. The application of feed with additional calcium is given twice a day. This research was conducted for 30 days. The application of calcium hydroxide ($Ca(OH)_2$) to white shrimp feed with a concentration according to the treatment, namely:

- P0 = Commercial feed + 1% CMC;
- P1 = Commercial feed + 1% CMC + 1% $Ca(OH)_2$ from chicken egg shell;
- P2 = Commercial feed + 1% CMC + 2% from $Ca(OH)_2$ from chicken egg shell;
- P3 = Commercial feed + 1% CMC + 4% from $Ca(OH)_2$ from chicken egg shell.

The shells of chicken eggs are cleaned in running water and the membrane parts are separated. After that, it is dried in the sun for ± 4 days which aims to reduce the water content in the shell, then it is reduced by pounding it to produce a smaller size than the previous size, then the shell is ground using a flour grinder/blender [12]. The results of the milling are then sieved using a 100 mesh sieve to obtain a fine flour result.

Egg shells that have been crushed into flour will be calcined at a temperature of 900°C for 4 hours using a furnace to obtain a white powder (CaO). The calcined CaO powder will be left in the open air for 1 week so that it turns into $Ca(OH)_2$ [13]. Then it will be characterized by XRD and FTIR to ensure that $CaCO_3$ in chicken egg shells has turned into $Ca(OH)_2$ where FTIR has a specific role to identify $CaCO_3$ and $Ca(OH)_2$ compounds while XRD plays a specific role to determine the phase change of $Ca(OH)_2$. Not only that but testing of calcium levels was also carried out on the results of the calcination process.

The mixture of calcium oxide (CaO) (which is the end product of the calcination process) with water (H_2O) produces calcium hydroxide ($Ca(OH_2)$) as a feed additive. The initial step in making feed is to prepare 500 grams of commercial shrimp feed in powder form with the trademark "MS Fengli 0" and then add calcium hydroxide ($Ca(OH)_2$) chicken egg shells according to the dose given, namely 0%, 1%, 2 %, and 4%. The addition of water is carried out as much as 70% of the amount of feed, then stirred evenly. After all the calcium is mixed into the feed, then 1% CMC (carboxy methyl cellulose) is added to the total feed [12].

The feed formulation that will be used in this research is in the form of a crumble. Crumble is a type of ration produced from feed ingredients from a pellet machine and then the pellets are crushed so that they become granules whose size is coarser than powder form and no larger than pellet form. The crumble form of feed was given when the shrimp entered the grower period at the age of 16-45 days. A crumbled feed can give better results because the added $Ca(OH)_2$ can be mixed homogeneously so that it can reduce feed wastage due to more efficient feeding [14].

The making of crumble is done by manually printing the feed using a simple feed printing machine. The printed feed is then cut using a knife which aims to produce the size of green bean seeds, the next step is drying by drying in the sun for ± 3 days so that the feed does not smell rancid and also does not get moldy. After the pellet is dry, the pellet is crushed to form crumble (granules), then the feed is ready to be applied to white shrimp efficiently [14].

The containers needed in this research were 20 aquariums measuring 15x15x20 cm^3. Before use, the aquarium is cleaned of dirt using soap and then rinsed with clean water. Furthermore, a sterilization process is carried out using chlorine as a disinfectant so that the growth medium of white shrimp is protected from bacteria and disease-carrying agents by soaking it for 24 hours, after which the aquarium is dried [15].

Drying is done for 1 day to ensure the aquarium is not damp. The dry aquarium is filled with fresh water which has previously been deposited for 3 days in a water reservoir. Each aquarium was filled with 1 liter of water and given an aerator that was turned on for 24 hours to increase dissolved oxygen. Aquariums were labeled according to each treatment and repetition and randomized according to the research design.

The first thing that needs to be done at this stage is the acclimatization process which is carried out on PL10 white shrimp juvenile. PL10 juveniles were fasted for 24 hours and acclimatized in a holding container for 1-2 days with the intention that the seeds do not experience stress when they are in a new environment. Acclimatization to temperature by floating the bag containing the juvenile in the container and watering slowly. Meanwhile, acclimatization to salinity was carried out on PL12 juvenile with a decrease in salinity of 1-2 ppt per day until the juvenile became PL25. The decrease in salinity can be done by slowly adding fresh water (running) to the storage container [1].

The selection of juveniles is superior and is carried out randomly to be stocked in the aquarium. The stocking of the juvenile was carried out in an aquarium filled with water and carried out in the morning. The criteria for a good white shrimp juvenile are after reaching the size of PL20 or the gill organs are perfect, uniform or flat, the seed body and intestines are visible, swimming against the current.

The research was conducted by rearing white shrimp in fresh water for 30 days. The length and weight of the test shrimp will be measured at the beginning of the research and then put into each aquarium with a stocking density of 15 fish/liter in an aquarium measuring 15x15x20 cm^3. This refers to [16] who argues that rearing shrimp larvae can be maintained at a density of 10-40 fish/liter of water. In addition, the stocking density of 18 shrimp larvae and 24 individuals obtained a high survival rate of up to 88.89% in an aquarium with a size of 75x37x27 cm^3.

Water replacement is carried out every day with as much as 50% replacement water that has been prepared previously, this process is carried out before feeding during the day. Siphoning of leftover feed is carried out once a week before water changes.

Research parameters observed during the rearing period of white shrimp were Feed Conversion Ratio (FCR), absolute length and weight growth, and water quality (ammonia, temperature, pH, and dissolved oxygen). Water media management is carried out carefully so that water quality can be controlled. Observations of temperature, pH, and dissolved oxygen of the media were carried out every day at 05.00 and 15.00 WIB, while ammonia observations were carried out once every 7 days. In contrast to water quality, shrimp length and weight measurements were only carried out at the beginning and end of the research.

The feed given during the white shrimp enlargement process is in the form of crumble. This is due to the relatively small size of the white shrimp's mouth opening. Feed was given in the amount of 8% of wet weight with a frequency of 4 times a day, then adjusted based on the amount of feed consumed. At the age of 1-10 days, the addition of feed per day is 200 grams, 11-20 days is 400 grams, and 21-30 days is 600 grams [16]. The amount of feed given and the remaining feed during the research were recorded to determine the level of feed consumption which would later be used as the basis for calculating the Feed Conversion Ratio (FCR) [17]. Feeding was carried out at 07:00 WIB, 13:00 WIB, 19:00 WIB, and 01:00 WIB.

White shrimp (*Litopenaeus vannamei*) were ready for sampling after 30 days of rearing. In this research, the length and weight of shrimp were measured at the age of 30 days. Sampling was conducted to determine the level of Feed Conversion Ratio (FCR). The sampling process is carried out in the morning by removing water through the water discharge output. Then the shrimp were taken and accommodated into a petri dish according to treatment and replication for the data collection process.

The calculation of the feed conversion ratio or FCR (Feed Conversion Ratio) is the amount (weight of feed that can form a unit in fish. So that what is measured includes the weight of the feed given during the maintenance process and the weight gain of shrimp from the beginning of frying until harvest [18]. [19] state that the calculation of the Feed Conversion Ratio (FCR):

$$FCR = \frac{\mathrm{F}}{(\mathrm{Wt+d})-\mathrm{Wo}} \qquad (1)$$

Where: FCR = Feed Conversion Ratio; F = Amount of Feed (g); Wt = Weight of test animals at the end of the research (g); Wo = Weight of test animals at the beginning of the research (g); d = Total weight of dead shrimp (g).

Feed efficiency is the result obtained from the wet weight of shrimp meat in each unit weight of pellet/dry feed given or is a percentage of the weight of shrimp produced compared to the weight of the feed given [18]. [19] states that to calculate feed efficiency the following formula is used:

$$FE = \frac{(\mathrm{Wt+d})-\mathrm{Wo}}{\mathrm{F}}\ x\ 100\% \qquad (2)$$

Where: FE = Feed efficiency; F = Amount of Feed (g); Wt = Weight of test animals at the end of the research (g); Wo = Weight of test animals at the beginning of the research (g); d = Total weight of dead shrimp (g).

The data were tested for normality and homogeneity with the Kolmogorov Smirnow test and Levene test, respectively. If the data is normal and homogeneous, then the data is analyzed using one-way Analysis of Variance (ANOVA) (One Way Anova) with a significant degree of 5%. If the ANOVA has a significant effect, then it is continued with Duncan's Multiple Range Test (DMRT) to compare between treatments. If the data is not normal and not homogeneous, it is tested using the Kruskal-Wallis test and if it has an effect then it is continued with the Mann-Whitney test [20].

RESULTS AND DISCUSSION

The results of the calculation of the Feed Conversion Ratio (FCR) in white shrimp can be seen in Table 1.

Table 1 – Average Feed Conversion Ratio (FCR) for one month.

Treatments	FCR ± SD
P0	2,776[b] ± 1,374
P1	0,551[a] ± 0,229
P2	0,717[a] ± 0,215
P3	1,378[a] ± 0,376

Table 2 – Average Feed Efficiency for One Month

Treatments	FE (%) ± SD
P0	44,448[a] ± 22,330
P1	208,219[b] ± 81,973
P2	154,709[b] ± 65,072
P3	77,633[a] ± 24,099

Feed Conversion Ratio (FCR) in white shrimp with the addition of calcium hydroxide $Ca(OH)_2$ 0% in feed was significantly different ($p<0.05$) with the addition of $Ca(OH)_2$ 1%, $Ca(OH)_2$ 2% and $Ca(OH)_2$ 4%. Feed Conversion Ratio (FCR) in white shrimp with the addition of 1% calcium hydroxide $Ca(OH)_2$ in feed was significantly different ($p<0.05$) from the addition of 0% $Ca(OH)_2$. Feed Conversion Ratio (FCR) in white shrimp with the addition of calcium hydroxide $Ca(OH)_2$ 2% in feed was significantly different ($p<0.05$) with the addition of 0% $Ca(OH)_2$. Feed Conversion Ratio (FCR) in white shrimp with the addition of calcium hydroxide $Ca(OH)_2$ 4% in feed was significantly different ($p<0.05$) with the addition of Ca(OH)2 0%.

Feed efficiency on white shrimp with the addition of calcium hydroxide $Ca(OH)_2$ 0% in feed was significantly different ($p<0.05$) with the addition of $Ca(OH)_2$ 1% and $Ca(OH)_2$ 2%. Feed efficiency on white shrimp with the addition of 1% calcium hydroxide $Ca(OH)_2$ in feed was significantly different ($p<0.05$) with the addition of 0% $Ca(OH)_2$ and 4% $Ca(OH)_2$. Feed efficiency on white shrimp with the addition of calcium hydroxide $Ca(OH)_2$ 2% in feed was significantly different ($p<0.05$) with the addition of $Ca(OH)_2$ 0% and $Ca(OH)_2$ 4%. Feed efficiency on white shrimp with the addition of calcium hydroxide $Ca(OH)_2$ 4% in feed was significantly different ($p<0.05$) with the addition of $Ca(OH)_2$ 1% and $Ca(OH)_2$ 2%.

The results of statistical calculations using One Way ANOVA showed that the highest Feed Conversion Ratio (FCR) of white shrimp was the addition of 0% $Ca(OH)_2$ in feed that was kept for 30 days (2.776), while the lowest Feed Conversion Ratio (FCR) was found in the addition of $Ca(OH)_2$ by 1% in the feed reared for 30 days (5.77%). %). The results of P1 and P2, it is not much different from the results of research by [21] suggesting that the value of the feed conversion ratio of white shrimp is between 0.59-0.91. Feeding in sufficient quantities at the right time can increase the growth rate of cultivated biota [22]. The highest feed efficiency of white shrimp was the addition of 1% Ca(OH)2 in the feed that was kept for 30 days (208.219%), while the lowest feed efficiency was found in the addition of Ca(OH)2 of 0% in feed reared for 30 days (44.448

A good FCR value when the results obtained have a low value. This is following the statement of [23] which explains that the lower the FCR value, the more efficiently the feed is converted into meat. [24] also stated that the smaller the FCR value, the better, this shows that the smaller the costs incurred for the purchase of feed, the higher the profit obtained. [25] explained that the size of the feed conversion ratio is influenced by several factors, namely the quality and quantity of feed, species, size, and water quality. Treatment with doses of 1% and 2% had good FCR results because the addition of calcium at these doses was the optimal dose. [26] stated that the addition of calcium at a dose of 1.33% in feed can increase the growth and feed efficiency of white shrimp. Meanwhile, [27] stated that the addition of a dose of 2% calcium in the feed was proven to be effective in increasing the frequency of molting. P0 and P3 have FCRs that are not as optimal as P1 and P2 because, in the P0 treatment, the maintenance of vannamei shrimp with sub-optimal salinity requires additional minerals, one of which is calcium for the molting process [28]. In the P0 treatment, the consumed feed did not produce optimal meat due to disturbances in the growth process. Growth in white shrimp occurs when the shrimp has gone through the molting process. [29]

explained that molting in crustaceans is a biological process that plays an important role in the growth and reproduction of shrimp. This molting process requires more calcium intake. [30] explained that in the molting phase the size of the shrimp meat will increase while the outer shell does not increase, so for adjustment, the shrimp will release the old shell and reshape the new shell with the help of calcium. So if there is no calcium preparation at below optimum salinity conditions, shrimp can only utilize the available energy to maintain their life [28]. As for the P3 treatment, the addition of calcium at a dose of 4% had an FCR that was not as optimal as P1 and P2 because the increase in calcium levels in the feed could inhibit protein retention. Calcium content of 4% did not significantly affect the protein synthesis activity of PL white because calcium can have an inhibitory effect on the absorption of phosphorus and other important minerals [31, 32].

Feed Efficiency (EP) for treatments P1, P2, and P3 is good, this result is not much different from [19] with a semi-intensive method which has feed efficiency results between 72.42% -83.57%. Feed efficiency at P0 was not as optimal as other treatments because at P0 there was no addition of calcium under conditions of sub-optimal salinity. P1 and P2 were thought to have a feed efficiency of more than 100% due to cannibalism and eating them and supported by an optimum dose of calcium. This is supported by research by [33] where the feed efficiency value of all treatments is more than 100%. The increase in body weight for each treatment was more than the amount of feed given. This can happen because of cannibalism and eating among the shrimp that are kept so that the body weight of the shrimp does not only come from the amount of feed given but also from the body weight of the shrimp that is cannibalized and eaten. Shrimp itself has a protein content of 61.78%, so if consumed it can support growth [21]. The total weight of the number of shrimp that died due to cannibalism cannot be calculated, because all that is left of the cannibalized shrimp is only small pieces such as the head which has become part of the shrimp that cannibalizes and eats it. After all, the body weight of the shrimp is not only from feed, the value of feed efficiency can be more than 100% and in the calculation, only the amount of feed given is taken into account. The doses of calcium at P1 and P2 are both optimum, but there is a difference in the efficiency of feed P1 and P2, this is also caused by the cannibalism process and eating fellow shrimp. [33] explained that this difference was caused by cannibalism and eating fellow shrimp, so that the increase in body weight of the shrimp and the protein stored in the shrimp's body did not only come from the feed given but also from the shrimp eaten, the total weight of which was not known. However, on the other hand, the addition of calcium at a dose of 1.33% in the feed can increase the growth and efficiency of white shrimp feed, while the addition of a dose of 2% calcium in the feed is effective in increasing the frequency of feeding [34, 26]. As for P3, calcium levels of 4% did not significantly affect the protein synthesis activity of PL white shrimp because calcium can have an inhibitory effect on the absorption of phosphorus and other important minerals [35, 32]. In the P0 and P3 treatments, cannibalism and eating also occurred but the feed efficiency did not exceed 100%, this was due to environmental conditions that were not optimal so the availability of secondary feed had not been able to increase feed efficiency up to 100%. At P0, shrimp that are below optimal salinity and do not receive additional minerals can only utilize the available energy to sustain their life [28].

CONCLUSION

The addition of calcium hydroxide $Ca(OH)_2$ from chicken egg shells to the feed can affect the Feed Conversion Ratio (FCR) and feed efficiency in the cultivation of white shrimp (*Litopenaeus vannamei*) in fresh water.

ACKNOWLEDGMENTS

Authors would like to acknowledge assistance and encouragement from colleagues, special work made by technical staff, and financial support from host organizations.

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