The influence used of calcium and acidifier to performance, internal egg quality, and metabolizable energy of laying hens: *In-vivo* trial

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**Abstract.** The combination between CaCO$_3$ and *Averrhoa bilimbi* L. as a novelty later namely calcidifier conducted to determined possible effect on the growth performance, internal egg quality, and energy of metabolism in laying hens. Eighty-lying hens of Isa Brown strain randomly allocated into five dietary treatments and four replicates with two lying hens each cages. The treatments were formulated as follows: T$_0$ control, basal diet + calcidifier 0.1% (T1), 0.2% calcidifier (T2), basal feed + 0.3% calcidifier (T3), basal feed + calcidifier 0.3% (T4). The statistical analysis were performed according one-way-anova using SAS academic online Ed. It was shown that using those combination concomitantly no significance effect on overall parameters. In summary, the used of calcidifier did not impacted well on lying hens, in line, didn’t help improve on the lying hens.

1. **Introduction**

Since an outbreak N-COV2019, our government made several regulations to inside the live animals and feed product originating from China [1]. Thus, trouble made a several researchers doing alternative to help local farmers by made a novelty including livestock sector [2]. Inline, our nations still imported the main product for feed which is it was impacted to our livestock product [3]. One alternative to support were novelty later called calcidifier. Calcidifier/Ca-Acidifier is an additional type of feed material in the form of an acidifier made from a combination of CaCO$_3$ and starfruit (*Averrhoa bilimbi* L.) which is blended with a blender or juicer, where in the starfruit solution there are several organic acids in the form of citric acid, ascorbic acid, acid oxalate and others, where the acids, when mixed with compounds such as CaCO$_3$, will catalyze into simpler compounds so it is easy to digest because the nature of these acids when reacted with minerals will make complex mineral compounds simpler.

The use of acidifier can be given singly or in combination with other substances such as phytobiotics, so that the use of animal feed acidifiers can be combined with essential minerals such as calcium, where if the acidifier is used together with calcium the organic compounds of the acidifier can break the complex bonds of the mineral so that minerals it decomposes into a simpler form so that it can be absorbed by the livestock body easily, easily the mineral is absorbed by the livestock body and increased nutrient digestibility resulting from the use of an acidifier so that it can be used by livestock for maintenance of the animal's metabolic system. Acidifier can be combined using calcium carbonate,
where calcium carbonate is commonly found in eggshells, shells of marine organisms, snails and charcoal.

Starfruit is a type of plant that is often found in Indonesia, star fruit is famous for its sour taste, where the sour taste contains active substances in the form of organic acid compounds. These organic acids can be used as natural acidifiers in animal feed, especially poultry. The use of acidifier in feed is expected to replace the role of antibiotics in feed, where excessive use of antibiotics is feared to leave residues in the body of livestock, and will be fatal if by-products from chickens are consumed in the long run. The use of acidifier in feed can be useful to reduce or maintain the pH of the chicken digestive tract in optimal conditions, with a pH that tends to be acidic, this will form the ideal conditions for the development of LAB (lactic acid bacteria) such as Lactobacillus Sp and other non-pathogenic bacteria and can kill pathogenic bacteria such as Eschericia coli, Salmonella Sp and others. The population of pathogenic bacteria can be suppressed so that the absorption of food substances in the body of cattle can run optimally. The performance of acidifiers in the small intestine will support the activity and function of digestive enzymes, stimulate feed consumption and reduce the production of ammonia and metabolic pathogenic microbial products which can reduce the rate of livestock growth. Maximum absorption of food substances will have an impact on the productivity value of livestock if in laying hens, the resulting eggs can produce good quality external and internal eggs.

2. Material and method
Eighty-lying hens of Isa Brown strain randomly allocated into five dietary treatments and four replicates with two lying hens each cages. The treatments were formulated as follows: T0 control, basal diet + calcidifier 0.1% (T1), 0.2% calcidifier (T2), basal feed + 0.3% calcidifier (T3), basal feed + calcidifier 0.3% (T4). Restricted feeding around 110 gr-115 gr day formulated in trial. Preparations are made on making calcifiers in several stages. The initial process in making calcifiers is cleaning the starfruit fruit that will be used from the stem. After cleansing from the stem, the starfruit fruit was mashed using a juicer or blender without adding water. The next step was pouring starfruit juice and calcium carbonate in a ratio of 1: 2 onto a tray. 1 for starfruit juice and 2 for calcium carbonate. Stir calcium carbonate and starfruit juice until homogeneous Then the mixture is transferred to aluminum foil in the form of a container. After transferring the mixture of starfruit juice and calcium carbonate is dried using an oven with a temperature of 70-80°C. The last stage is the dry calcifier/ground to get the calcifier in the form of flour which is ready to be mixed into the feed. A composition the diet between maize, rice bran, concentrate, palm kernel meal, and premix are analyses using proximate analysis to determined dry matter, crude protein, fat, and crude fibre (table 1) following Sjofjan et al. (2020) method [4].

Table 1. Ingredient and nutrient composition of the diet.

| Feed nutrient          | (%)     |
|------------------------|---------|
| Maize                  | 49.5    |
| Rice bran              | 15.3    |
| Concentrate            | 29.6    |
| Palm kernel meal       | 5.1     |
| Premix                 | 0.5     |
| **Total**              | **100** |

| Dry matter (%)         | 88.00   |
| ME (kcal/kg)           | 2,773   |
| Ash (%)                | 8.00    |
| Crude protein (%)      | 17.21   |
| Fat (%)                | 5.00    |
| Crude fibre (%)        | 3.25    |
2.1. Data analysis
The statistical analysis were performed according one-way-anova using SAS academic online Ed according to Adli and Sjofjan (2020) method [5].

3. Results and discussion
Data on comparation calcidifier (CaCO₃ and Averrhoa bilimbi L.) in feed showed in table 2. Giving calcidifier (CaCO₃ and Averrhoa bilimbi L.) did not improved (p>0.05) on FI, FCR, and egg production. Feed formulation may impacted started from 60-70% [6]. These improvements may from biological appearance [7-8]. The body weight of poultry to be determined would be determined by the consumption of feed with a balanced energy and protein content [9-10]. Last ten years the use of the plant bioactive compound in monogastric are often good among variety, climate, humidity and related factor [10]. Increasing nitrogen digestibility is beneficial to lesser fermentable substrates available for pathogens in the intestine which also contributed to improve microbial balance and gut health as well as to reduce ammonia secretion to the environment [8,11].

Table 2. Effect of calcidifier (CaCO₃ and Averrhoa bilimbi L.) on the performance of laying hens.

| Item              | T0     | T1     | T2     | T3     | T4     | SEM   | P-value |
|-------------------|--------|--------|--------|--------|--------|-------|---------|
| FI, g/bird        | 121.24 | 121.16 | 122.07 | 122.72 | 121.72 | 3.16  | 2.15    |
| F/G               | 2.14   | 2.08   | 2.08   | 2.06   | 2.09   | 1.49  | 0.62    |
| Egg production (%)| 79.03  | 83.51  | 83.51  | 85.47  | 72.57  | 6.27  | 18.00   |

Means within row followed by different superscript differ at p<0.05, BWG (body weight gain), FI (feed intake), F/G ratio, Egg Production (%).

Based on the results of research that has been done, the average effect of the treatment of the egg white index in table 3 shows the treatment of T0 (0.43), T1 (0.43), T2 (0.43), T3 (0.43), and T4 (0.41) in mm. According to Romanoff (2011) the standard egg white index varies between 0.05–0.174 depending on storage time, storage temperature, and feed nutrition [9]. The results of data analysis showed that the addition of calcidifier in the feed had concomitantly significance effect (P<0.01) on the color of the yolk. Addition of calcidifier to feed at the level of 0.4% (T4) gives the highest yield in egg yolk. The increase in the color of the yolk is thought to be due to the role of the calcidifier in the treatment feed which has a different addition level. Another factor is the content of xanthophyll and β-carotene which affects the thickness of the egg yolk found in starfruit. [12] causes the diversity of egg yolk color in addition to being caused by the amount of xanthophyll content and also β-carotene in the feed, it is also caused by differences in strain, individual diversity, and fat in the feed [13].

Table 3. Effect of calcidifier (CaCO₃ and Averrhoa bilimbi L.) in feed on the egg parameters.

| Item             | T0     | T1     | T2     | T3     | T4     | SEM   | P-value |
|------------------|--------|--------|--------|--------|--------|-------|---------|
| Egg yolk index   | 0.43bc | 0.43b  | 0.43c  | 0.43b  | 0.41a  | 0.016 | 0.15    |
| Egg yolk colour  | 8.35a  | 8.53a  | 9.03ab | 9.88b  | 10.0b  | 0.24  | 0.11    |
| Egg yolk volume (ml) | 14.83a | 16.05ab | 15.50ab | 16.43b | 16.08ab | 0.43  | 0.22    |
| Albumin volume (ml) | 30.45a | 33.83b | 34.63b | 35.05b | 34.85b | 0.129 | 0.129   |

Means within row followed by different superscript differ at P<0.05

Based on the results of research that has been done, the average effect of the treatment of the AME in table 4 shows the treatment of T0 (2,730.99), T1 (2,965.65), T2 (2,985.83), T3 (2,969.59), and T4 (3,001.95) in kcal/kg. The results of the calculation of statistical analysis show that the effect of the different effects is very significant (p<0.01). This is due to the application of calcidifiers in dry form in
feed, the benefit of giving in dry conditions is not utilizing one of the technologies to protect feed ingredients from degradation by the digestive organs before the small intestine, giving acidifiers in a way that can actually work optimally in the digestive tract. The high AME value in chickens can be caused by the energy contained in the feed which can be utilized properly by the chickens [14].

Table 4. Effect of calcidifier (CaCO$_3$ and Averrhoa bilimbi L.) in feed on the metabolizable energy.

| Item                  | T0      | T1      | T2      | T3      | T4      | SEM    | P-value |
|-----------------------|---------|---------|---------|---------|---------|--------|---------|
| AME (kcal/kg)         | 2,730.99a | 2,965.65b | 2,985.83b | 2,969.59bcd | 3,001.95bcd | 39.26  | 0.15    |
| AMEn (kcal/kg)        | 2,705.82a | 2,261.18ab | 2,953.1ab  | 2,936.88ab  | 2,968.90b   | 20.24  | 0.10    |
| Protein Digestibility (%) | 62.97a   | 72.92ab  | 74.51ab  | 72.68b    | 74.96ab    | 21.22  | 2.04    |
| NH$_3$ (mM)           | 1.20c   | 0.95b   | 1.04abc  | 0.80c     | 1.95d      | 34.94  | 0.16    |

a-b superscript mean differ significant effect at p < 0.05.

4. Conclusions
In summary, the used of calcidifier did not impacted well on lying hens. Furthermore, need other research to balancing of this novelty product.

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