



Research Paper

## The Effect of Phytobiotics and *Lactobacillus acidophilus* of The Diet on Protein Digestibility, Egg Calcium and Protein Mass and Egg Content Weight of Laying Hens

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### Abstract

The study aimed to assess the feeding of a mixture of onion peel flour, garlic peel, bay leaves and *Lactobacillus acidophilus* on crude protein digestibility, calcium mass and egg protein and egg weight in laying hens. The livestock used were 72-week-old *Isa Brown* strain laying hens totaling 144 birds with an average body weight of  $1824 \pm 126.15$  g. The study was arranged in a completely randomized design (CRD) with 6 treatments and 4 replicates, each experimental unit consisted of 6 birds. The treatment given is T0 = basal diet, T1 = basal diet + 1.2 ml *Lactobacillus acidophilus*, T2 = basal diet + phytobiotics 2%, T3 = basal diet + phytobiotics 2% + 1.2 ml *Lactobacillus acidophilus*, T4 = basal diet + phytobiotics 4% + 1.2 ml *Lactobacillus acidophilus*, T5 = basal diet + phytobiotics 6% + 1.2 ml *Lactobacillus acidophilus*. Parameters observed included protein digestibility, egg protein and calcium mass, and egg content weight. Data were analyzed for variance, if the treatment had a significant effect ( $p < 0.05$ ) followed by Duncan's test at the 5% significance level. The results showed that giving a mixture of onion peel flour, garlic flour, bay leaves and *Lactobacillus acidophilus* had a significant effect ( $p < 0.05$ ) on protein digestibility, protein mass and egg calcium and egg weight in laying hens. The conclusion is that the addition of 2% phytobiotic plus 1.2 ml *Lactobacillus acidophilus* to the ration of laying hens can effectively increase protein digestibility, egg calcium mass and egg weight in laying hens.

### Keywords

Egg weight; Laying hen; Phytobiotic; Probiotic; Protein digestibility

## 1. INTRODUCTION

The nutritional value of eggs depends on their internal and external qualities, which are influenced by the quality of the ration. The effect of ration quality on egg products cannot be separated from the ability of chickens to adapt. The weaknesses of laying hens are very sensitive to changes in the environment, so they are easily stressed, not suitable for extensive farming, and have high cannibalism (Hartcher and Jones, 2017). These weaknesses must be supported by providing good quality rations that have balanced nutrition. The use of nutritional rations is expected to be more efficient and effective when supplemented or feed additives. In the previous decades, the use of additives in the form of antibiotics was very intensively given to poultry for the treatment and prevention of disease. The provision of antibiotics in addition to controlling harmful microbes, so that they are resistant to disease, can also increase livestock productivity. Therefore, alternatives to antibiotics are sought with natural additives and do not cause residues in livestock products. Herbal plants with active components of phytobiotics and

probiotics in the form of live microorganisms, such as *Lactobacillus acidophilus*, can be combined as natural additives that can serve as alternatives to antibiotics.

Natural ingredients such as onion skin, garlic and bay leaves each contain active substances. Active substances in shallot skin include phenolics, flavonoids, pectin and phytosterols (Teru et al., 2017), while garlic skin contains flavonoids, allisin, organosulfur, alkaloids, and saponins (Wijayanti et al., 2017). The active substance content of bay leaves includes phytochemicals in the form of essential oils (citric and eugenol), flavonoids, alkaloids, saponins, terpenoids, and tannins (Wilapangga and Sari, 2017). The dominant active substance in the three herbs is flavonoids. Flavonoids and organosulfur act as antimicrobials and antioxidants that are effective in suppressing the growth of pathogenic bacteria (Miekus et al., 2020). Polyphenolic components, including flavonoids, selectively inhibit the growth of pathogenic bacteria (*Escherichia coli* and *Salmonella enterica*) without affecting the viability of probiotics such as *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* (Pacheco-

Ordaz et al., 2018). Therefore, synergistic action between phytobiotics, mostly flavonoids, and probiotics (*Lactobacillus acidophilus*) is likely to occur effectively in this study.

The use of a mixture of natural ingredients in the form of onion skin flour, garlic and bay leaves containing flavonoids functions as an antibacterial, so as to increase body resistance and better livestock productivity. The provision of phytobiotics aims to maintain the health of the digestive tract of chickens as host livestock so that the process of digestion and absorption of nutrients is not disturbed by pathogenic bacteria (Obianwuna et al., 2024). The results of research by Zaikina et al. (2022) showed that phytobiotic sourced sweet chestnut wood extract as much as 0.065% can increase protein digestibility in broiler chickens. The addition of probiotics such as *Lactobacillus acidophilus*, on the other hand, is expected to have a positive impact on the efficiency of nutrient use and egg production. The results of research by (Chen et al., 2023) that the dietary supplementation with *Lactobacillus acidophilus* and *Bacillus subtilis* increased the integrity of the intestinal barrier as evidenced by the significant increase in the number of ileal goblet cells, the diversity of gut microbiota, amino acid digestibility and egg production. Probiotics are known to produce antimicrobial compounds that can inhibit the growth of pathogenic bacteria (Biswas and Das Mohapatra, 2023). Thus, feeding a mixture of phytobiotics and probiotics is expected to work in synergy because each produces antimicrobial compounds, resulting in a better bacterial balance that has a positive impact on nutrient digestibility and productivity.

The study was conducted with the aim of examining the effect of feeding a combination of 25% onion peel flour, 25% garlic peel, 50% bay leaves and *Lactobacillus acidophilus* on crude protein digestibility, calcium mass and egg protein in laying hens.

## 2. EXPERIMENTAL SECTION

### 2.1 Animal, Diet and Equipment

The experimental animal were 144 *Isa Brown* strain laying hens aged 72 weeks with a body weight of  $1824 \pm 126.15$  g. The research feed was composed of ground corn, rice bran, soybean meal, meat bone meal, fish meal, salt, grit, methionine and premix with the composition listed in Table 1. A mixture of onion skin meal, garlic, bay leaves containing phytobiotic active components, and *Lactobacillus acidophilus* was used as treatment.

### 2.2 Preparation of herbal combinations as a source of onion, garlic peel and bay leaves

The research began by collecting onion peel, garlic and bay leaves. Onion peels, garlic were obtained from the market where onion sellers and some from household industry waste, while bay leaves were purchased from the market in a wet state. Onion peels, garlic and bay leaves were dried under the sun for 4 days. The three dried ingredients were then pulverized with a blender, so that they became

flour and were ready to be used as a source of phytobiotic additives.

### 2.3 Experimental and treatment

Experimental hens were kept for 7 weeks with 1 week of adaptation period, 5 weeks of treatment and 1 week of total collection. Laying hens were given rations mixed with phytobiotics according to the treatment as much as 2, 4 and 6%. The treatment rations were given twice a day in the morning and evening. *Lactobacillus acidophilus* 1.2 ml was given in the morning mixed with a small amount of feed ( $\pm 25$  g) until it was consumed before the ration was given to meet the needs of the day. Treatments were given from week 2, when the chickens were 73 weeks old until 78 weeks old. Egg samples were collected at week 5 to 6 for physical and chemical quality analysis (protein) based Standar Nasional Indonesia (2006).

The study was arranged in a completely randomized design (CRD) with 6 treatments and 4 replicates (6 replicates each). The determination of treatment levels is based on the results of research by Putra et al. (2022) that the addition of phytobiotic and *Lactobacillus achidophilus* can reduce fat digestibility, relative weight of abdominal fat and increase carcass weight of 3-month-old turkeys. The treatments applied were as follows:

T0 = basal diet without phytobiotic and *Lactobacillus achidophilus*

T1 = basal diet + 1.2 ml *Lactobacillus achidophilus*

T2 = basal diet + phytobiotic 2%

T3 = basal diet + phytobiotic 2% + 1.2 ml *Lactobacillus achidophilus*

T4 = basal diet + phytobiotic 4% + 1.2 ml *Lactobacillus achidophilus*

T5 = basal diet + phytobiotic 6% + 1.2 ml *Lactobacillus achidophilus*

### 2.4 Research Parameters

Parameters measured were protein digestibility, protein mass and egg calcium mass and egg weight. Protein digestibility was measured by the total excreta collection method for 5 days in week 6 using 24 chickens. The total collection method was combined with the use of  $\text{Fe}_2\text{O}_3$  indicator as much as 0.5% of the amount of ration feeding. Excreta samples were collected with a cardboard base covered with black plastic and then coded. Excreta was collected after a red color change. Every 2 hours the excreta were sprayed with HCL to keep the N from evaporating. Wet excreta were weighed and then dried in the sun for 2 - 3 days, and after drying it was weighed again to determine the dry weight. The dried excreta were pulverized with a mortar, then analyzed for protein content. Protein digestibility was calculated using a formula based on Saputra et al. (2016) as follows:

**Table 1.** Composition and nutrient content of research diets

Feedstuff	T0	T1	T2	T3	T4	T5
	----- (%) -----					
Yellow Corn	54.40	54.40	53.33	53.33	52.31	51.32
Rice Bran	8.02	8.02	7.86	7.86	7.71	7.57
Soybean Meal	20.19	20.19	19.79	19.79	19.41	19.05
Meat Bone Meal	1.28	1.28	1.25	1.25	1.23	1.21
Fish Meal	7.29	7.29	7.15	7.15	7.01	6.88
Salt	0.28	0.28	0.27	0.27	0.27	0.26
Grit	3.60	3.60	3.53	3.53	3.46	3.40
Methionine	0.04	0.04	0.04	0.04	0.04	0.04
Premix	4.88	4.88	4.78	4.78	4.69	4.60
Phytobiotic	0.00	0.00	1.96	1.96	3.85	5.66
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient Content*:						
Crude Protein (%)**	17.25	17.25	16.91	16.91	16.73	16.42
Crude Lemak (%)**	3.83	3.83	3.75	3.75	3.68	3.61
Crude Fiber (%)**	5.22	5.22	5.12	5.12	5.02	4.92
Calcium (%)**	4.09	4.09	4.01	4.01	3.93	3.86
Phosphor (%)**	1.01	1.01	0.99	0.99	0.97	0.95
Metabolizable Energy (kcal/kg)***	2813.07	2813.07	2757.91	2757.91	2704.87	2653.84

Source: \*Nutrient content has been customized in 100%.

\*\*Results of proximate and mineral analysis at the Nutrition and Feed Science Laboratory, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang (2024).

\*\*\*Calculation results based on Blair and Bolton (1974) formula (1967).

Protein Digestibility(%)

$$= \frac{\text{protein intake} - \text{amount of excreta protein}}{\text{protein intake}} \times 100\%$$

Egg samples were taken at week 5 to 6 as many as 4 eggs per replicate for protein and calcium analysis. Each egg was weighed then broken and put into a baking sheet with a known weight, then dried in an oven at 55°C. The dried egg samples were pulverized, followed by protein and calcium testing. The protein mass and calcium mass of the eggs were calculated using the formula by adopting the calculation as in meat according to Suthama (2003) as follows:

egg protein mass (g) = egg protein content (%) × egg fill weight (g)

egg calcium mass (g) = egg calcium content (%) × egg fill weight (g)

## 2.5 Statistical Analysis

The observational data underwent statistical testing using analysis of variance at a 5% significance level. If the treatment is found to have a significant impact, Duncan's multiple range test is subsequently applied at the same 5% significance level (Steel and Torrie, 1991).

## 3. RESULTS AND DISCUSSION

The provision of phytobiotics and *Lactobacillus acidophilus* in the diet had a significant effect ( $p < 0.05$ ) on protein digestibility, egg calcium mass and egg weight of laying hens, but no significant effect ( $p > 0.05$ ) on egg protein mass of laying hens. The average results of the parameters are listed in Table 2.

### 3.1 Protein Digestibility

Protein digestibility in the combination of 4% phytobiotic treatment and *Lactobacillus acidophilus* 1.2 ml (T4) and 6% phytobiotic and *Lactobacillus acidophilus* 1.2 ml (T5) showed the highest value, 2% phytobiotic treatment (T2) and 2%

**Table 2.** Average Protein digestibility, calcium mass and egg protein and egg fill weight

Parameter	T0	T1	T2	T3	T4	T5
Protein Digestibility (%)	62.01 <sup>c</sup>	75.96 <sup>b</sup>	79.37 <sup>ab</sup>	79.77 <sup>ab</sup>	84.56 <sup>a</sup>	83.54 <sup>a</sup>
Egg Calcium Mass (g)	4.99 <sup>b</sup>	5.15 <sup>ab</sup>	5.17 <sup>ab</sup>	5.67 <sup>a</sup>	5.65 <sup>a</sup>	5.70 <sup>a</sup>
Egg Protein Mass (g)	9.86	10.15	10.14	10.16	10.35	10.34
Egg Fill Weight (g)	54.21 <sup>b</sup>	54.44 <sup>ab</sup>	54.46 <sup>ab</sup>	54.56 <sup>a</sup>	54.68 <sup>a</sup>	55.56 <sup>a</sup>

<sup>a,b,c</sup>Superscripts on row means indicate significant differences (p<0.05).

phytobiotic treatment and *Lactobacillus acidophilus* 1.2 ml (T3) were not significantly different from (T4), while the treatment without the addition of phytobiotic and *Lactobacillus acidophilus* (T0) showed the lowest value (Table 2). The highest phytobiotic addition and the addition of *Lactobacillus acidophilus* increased protein digestibility. The addition of *Lactobacillus acidophilus* supports the activity of lactic acid bacteria (LAB) to grow and develop better and inhibits pathogenic bacteria because it produces short chain fatty acids (SCFA) such as acetate, propionate and butyrate so that the digestive tract becomes better and will ultimately increase protein digestibility. Changes in the environmental atmosphere of the digestive tract in the presence of active substances in the form of flavonoids contained in phytobiotics function as antimicrobials and antioxidants (Sizova et al., 2022).

Increased protein digestibility in the T4 and T5 treatments causes other nutrients, such as protein, not to be carried out with excreta but more digested. The higher the ration consumption, the higher the protein that can be consumed and the impact on increasing protein digestibility. Mathai et al. (2017) stated that high protein digestibility means that the amount of protein that can be absorbed is more than the amount of protein wasted. Protein digestibility besides depending on protein consumption is also related to the rate of digesta. Iqbal et al. (2019) the crude fiber fraction or so-called ration fiber in poultry up to 5% is beneficial for the development of digestive organs, and the improvement of the ecosystem is characterized by transit time, and beneficial microbial colonization is better, but when it is too high it affects the digestive process.

The synergism of flavonoid active substances from phytobiotics as antimicrobials and antioxidants can improve digestive tract health. This research is supported by the report of Yuanita et al. (2019) that the active substance flavonoid from dayak onion extract accompanied by the administration of *Lactobacillus acidophilus* can reduce the number of pathogenic bacteria which causes healthier digestive tract conditions. Improved digestive tract conditions have an impact on the secretion and activity of digestive enzymes maximized, so that digestion and absorption of nutrients are better. Similarly, Fanani et al. (2016) reported that the health of the digestive tract had an impact on increasing

protein digestibility in crossbred local chickens fed inulin from dahlia tubers.

The T0 treatment showed the lowest protein digestibility (Table 2) because it was not given a combination of phytobiotics and *Lactobacillus acidophilus*. Treatment without additional combination of additives means that there is no help from the activity of active substances and *Lactobacillus acidophilus* in relation to the process of digestion and absorption of nutrients, especially protein. Similar research results reported by Saputra et al. (2016) showed that antibacterials in onion skin inhibited the growth of pathogenic bacteria associated with increased protein digestibility. However, the situation in this study was reversed because the T0 treatment was without additional additives of either phytobiotics or *Lactobacillus acidophilus*. Yulianingtyas and Kusmartono (2016) reported that antioxidants help prevent oxidation of digestive tract cells, so that the epithelium becomes healthier and nutrient digestion becomes better. The results in this study were characterized by the lowest protein digestibility due to the absence of both additives.

In contrast to the treatment of T1, T2 and T3 which produced protein digestibility is not different, even T2 and T3 are the same as T4 and T5. statistically. The provision of additives alone can help protein digestibility by increasing the digestibility of other nutrients. However, treatment T2 although only added phytobiotics and T3 with a combination of both additives but phytobiotics at the lowest level. The activity of the additives in these two treatments was not as effective as the synergism as in T4 and T5. Thus, the physiological phenomenon of digestion in the three treatments (T1, T2, and T3) did not occur as stated by Saputra et al. (2016), and Yulianingtyas and Kusmartono (2016).

### 3.2 Egg Calcium Mass

Egg calcium mass in the phytobiotic mixture treatments of 2, 4 and 6% and *Lactobacillus acidophilus* 1.2 ml (T3, T4, and T5) produced the highest egg calcium mass. Phytobiotics at all levels (2, 4, and 6%) when mixed with *Lactobacillus acidophilus* produced the same high egg Ca mass (Table 2), meaning that the two additives can work synergistically. The synergism of the active components of phytobiotics and *Lactobacillus acidophilus* can be attributed to their effect

on Ca metabolism characterized by increased Ca retention. Ca metabolism starting from the intestinal mucosa until it is transported into the target tissue always binds to a protein called calcium binding protein/Ca-BP (Li et al., 2017). Calcium binding protein (Ca-BP) is very likely to be formed higher because protein digestibility also increases which in turn has an impact on increasing egg Ca. The results of this study are in line with previous reports that there was an increase in albumen viscosity and Haugh unit of eggs due to thymol combined with synbiotics (Abdel-Wareth, 2016), and effective microorganism mixed with turmeric (Kinati et al., 2021). The increase in albumen viscosity and Haugh units is an indication of increased Ca and protein deposition in eggs, resulting in high egg Ca mass.

The T0 treatment produced the lowest egg calcium mass (Table 2) because it was not supplemented with phytobiotics or *Lactobacillus acidophilus*. Treatment without additional additives means there is no help from the activity of active substances and *Lactobacillus acidophilus*. An important factor affecting Ca absorption or retention is protein digestibility. Higher calcium retention means better calcium absorption. The absorption and transportation of Ca biochemically binds to a protein called (Ca-BP) calcium binding protein (Li et al., 2017).

In contrast, T1 and T2 treatments resulted in statistically similar calcium mass to T0, as well as to T2, T3 and T4. Feeding phytobiotic (T1) or *Lactobacillus acidophilus* (T2) separately resulted in lower egg Ca mass, although not different from T3, T4 and T5 (Table 2). This phenomenon implies that the single activity of either the active components of phytobiotics or *Lactobacillus acidophilus* is not as effective as the combination of the two, but still functions in assisting the digestive process. These results suggest that the administration of phytobiotics or *Lactobacillus acidophilus* alone can mediate the performance of the digestive tract so that the Ca-BP formed and absorbed contribute positively to egg Ca mass. Thymol supplementation, which contains active components, can prevent villous damage that determines the effectiveness of digestion (Hashemipour et al., 2016) and has an impact on improving egg quality based on egg mass and shell weight (Abdel-Wareth, 2016). Similarly, the effect of a single administration of *Lactobacillus acidophilus* can increase the specific gravity and viscosity of albumen (Abdel-Wareth, 2016; Carvalho et al., 2023).

### 3.3 Egg Protein Mass

Egg protein mass in the T4 and T5 treatments originated from the health of the digestive tract due to the synergistic work of phytobiotics and *Lactobacillus acidophilus* and tended to increase protein digestibility (Table 2). Increased protein digestibility at least has an impact on the protein that can be deposited into the egg in the form of protein mass. The results of this study have a similar phenomenon with the report of Rahman et al. (2021) that the quality of egg contents (albumen and yolk) is better by giving a mix-

ture of herbal flour as much as 15 g/kg and 20 g/kg of feed. The higher weight of egg contents, especially albumen, is an indicator that protein deposition is better. Protein mobilization into the egg was not as high as fat but due to the activity of phytobiotic active substances assisted by *Lactobacillus* at T4 and T5 tended to increase egg protein mass (Table 2).

The T0 treatment produced the lowest egg protein mass (Table 2) because it was not treated with a combination of phytobiotics and *Lactobacillus acidophilus*. The treatment without additional additives means that there is no help from the active substances of phytobiotic and *Lactobacillus acidophilus*. The phenomenon that occurred in this treatment is evidenced by the low calcium absorption and protein digestibility (Table 2) affecting protein deposition into the egg, resulting in low egg protein mass (Table 2). Mobilization of protein into the egg is not as high as fat since there is no contribution of phytobiotic active substance activity assisted by *Lactobacillus*, resulting in low egg protein mass (Table 2). Research by Salas et al. (2017) using isotopes showed that broiler layers directly use ration energy for egg fat deposition or consistently send nutrients into the egg. Based on this metabolic phenomenon, it can be stated that the availability of energy to support the protein deposition process is less available because it is used for yolk fat. However, the mass of egg protein in the T0 treatment in addition to the mechanism mentioned above is also related to low protein digestibility due to the absence of assistance from the work activities of both additives, phytobiotics and *Lactobacillus acidophilus*.

In contrast to treatments T1, T2 and T3 which produced the same egg protein mass with values classified as medium level and not different. Egg protein mass is closely related to protein digestibility because the high protein deposited can be influenced by the amount of protein digested. Increased protein digestibility values can cause the amount of protein digested by chickens to increase, so that the protein deposited for the formation of egg mass will increase. Saadaoui et al. (2021) stated that the amount of protein digested and absorbed will result in a higher amount of protein that can be utilized by livestock for growth and egg formation. Protein intake in the body plays an important role in the process of egg protein deposition, besides that calcium in the ration which plays a role in the process of deposition of digested egg protein will be absorbed by the body and enter the blood and will be transported to body tissues that need it, especially in egg formation. The activity of additives, phytobiotics and *Lactobacillus acidophilus*. in the three treatments was not as effective as the synergistic work as in the T4 and T5 treatments.

### 3.4 Egg Fill Weight

A mixture of 6% phytobiotic and 1.2 ml *Lactobacillus acidophilus* (T5) resulted in higher egg fill weights supported by increased protein digestibility (Table 2). Increased pro-

tein digestibility is not entirely for egg content but at least has an impact on the protein that can be deposited into the egg. Protein is not only used for egg production but also for tissue repair. The use of protein has a very complex dimension in addition to meeting basic needs as well as for growth and tissue repair and egg production (Fan and Wu, 2022). The results of this study have similar phenomena with the report of Rahman et al. (2021) that the quality of egg contents (albumen and yolk) is better by giving a mixture of herbal flour as much as 15 g/kg and 20 g/kg of ration compared to the control. The higher mixture of phytobiotics and *Lactobacillus*, especially at T5, in this study means that the synergism of the work of the two additives is increasingly effective. Previous research using isotopes as tracers showed that broiler layers either directly utilize energy for egg fat deposition, or consistently send nutrients into the egg (Salas et al., 2017). In addition, energy intake is required as a mediator of reactions in the process of protein synthesis. The process occurs more intensively with the contribution of the work of both additives, active substances of phytobiotics and probiotics, starting from the digestive tract. Higher egg fill weight, characterized by higher yolk weight and protein deposition in the form of protein mass.

The T1 treatment produced low egg fill weights, although not statistically different, (Table 2) because it was not supplemented with a combination of additives, but only *Lactobacillus acidophilus*. Nutrient intake, especially protein and calcium, plays an important role in egg formation. Lukic et al. (2011) stated that low calcium absorption is less supportive of the process of forming shells and egg contents which can affect egg weight. When associated with the T0 treatment, without added additives, egg fill weights tended to be better than T1. The weight of egg contents between treatments T0 and T1 is different but not significantly, it can be assumed that the response of chickens given a ration without probiotics is little different from those that get additional additives. The results of this study contradict those reported by Alaqil et al. (2020) that the administration of *Lactobacillus acidophilus* increased egg mass in Hy-Line Brown laying hens. The difference in results occurred mainly with high levels of *Lactobacillus acidophilus* ( $3 \times 10^9$  CFU), but lower levels ( $1 \times 10^9$  CFU) showed egg mass as a reflection of egg content similar to this study. Thus, additive level determines the effectiveness of nutrient transport, especially protein and calcium, into the ovaries.

There was little difference with the three treatments, T2, T3 and T4, which produced the same low egg fill weights. This phenomenon means that the active components of phytobiotics, either alone or mixed with *Lactobacillus acidophilus*, were not as effective as the T5 treatment, but still functioned in helping to improve the condition of the digestive tract. The population of lactic acid bacteria (LAB) in the three treatments was similar and higher than *Coliforms*, which is the effect of the activity of phytobiotic active substances

either alone (T2) or with *Lactobacillus acidophilus* (T3 and T4), which is related to the improvement of digestive tract health. Similar results were reported by Mangisah et al. (2020) that digestive tract health is better and protein digestibility increases in boilers fed rations mixed with herbal ingredients not containing flavonoids but inulin combined with *Lactobacillus casei*.

#### 4. CONCLUSION

The addition of phytobiotics plus 1.2 ml *Lactobacillus acidophilus* (T3) to the feed can effectively increase protein digestibility and egg calcium mass in laying hens.

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