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Increasing Eggs Protein Level and Eggshell Integrality Performed by Addition of Xylanase, Amylase, Protease (Avizyme® 1502) In Layers Feed

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Abstract

The high number of broken eggshells on laying hens in Indonesia is very detrimental to farmers, so that needs to be overcome. A total of 480 Lohman chickens with 40 weeks of age were divided into 4 treatment groups with the addition of different doses of Avizyme® 1502 respectively P0 as a control that got 0 g/Kg of feed, P1 got 1 g/Kg of feed, P2 got 2 g/Kg of feed, P3 got 3 g/Kg of feed. Avizyme® 1502 contains enzymes xylanase, amylase, and protease. This treatment was given daily for 30 days. The sampling of eggshell checking was recorded when start of administering enzymes to the end of the administration and at the end of the study, 6 eggs from each treatment were taken randomly to measure the protein level. The results showed that the addition of 1 g/Kg of Avizyme® 1502 on feed provided the highest protein level in eggs. During the research period, eggs produced by the groups of chicken under treatments of Avizyme 1502® showed a decrease in cracked eggshell.

Keywords: Avizyme® 1502, Xylanase, Amylase, Protease, Protein, Eggshell.

Introduction

Eggs have been consumed by human since ancient times (Belitz *et al.,* 2009). Eggs are the perfect food, because they contain complete nutrients for the growth of organism (Iannotti *et al.,* 2014). Eggs are known as a sources of protein that play an important role in basic nutrition (Miranda *et al.,* 2015). The quality of egg protein level should be maintained and improved (Roberts and Ball, 2003). Many efforts have been done to increase egg quality by adding some enzymes as a feed supplements (Bedford and Partridge 2001; Abudabos, 2011; Lee et al. 2014; Vieira *et al.,* 2016).

The superiority of eggs as a nutrient-rich poultry product also has an obstacle because eggs can be easily damaged. The damage can be a physical damage, chemical damage, and damage caused by microbial attacks through the pores of eggshell (Mertens *et al.,* 2006; Qi *et al.,* 2016). It will reduces the quality of eggs and make losses for farmers and the people who consume them.

In the laying hens business, there are many obstacles (Lampkin, 1997). Besides the diseases, many eggs produced with eggshell cracks (Harms *et al.,* 1996). The rise of broken eggshells that occurs in laying hens in Indonesia is still one of the biggest causes of losses (Jazil *et al.,* 2013). Therefore, it was necessary to conduct a

research by adding enzymes as supplements to improve the efficiency of the use of feed which will also increase the protein in eggs and make the shells stronger and thicker so that they are not easily cracked.

Materials and Methods

These research used a total of 480 forty-weeks old Lohman chickens, with 85% productivity of egg production. All chickens separated into 4 groups, 1 group as a control (P0) and 3 others were the treatment groups (P1-P3). The treatments using a different doses of Avizyme® 1502 were given to the Lohman laying hens for 30 days (Tab 1). The addition of enzymes used in this study were Avizyme® 1502 that contains 600 IU/g of xylanase, 800 IU/g of amylase, and 8,000 IU/g of protease. All samples were given the same feed CP 124® produced by PT Charoen Pokphand Indonesia and adlib water.

Table 1 Treatments of Avizyme® 1502 on a group of Lohman laying hens.

Treatments group	Dosage of Avizyme® 1502 (g/Kg feed)
P0	0
P1	1
P2	2
P3	3

The eggshell cracked data were obtained by observation during the treatment period to all groups of treatments hens. After 30 days of treatment, a total of 24 eggs were randomly taken to test the protein level that contained in the egg. The level of protein were deternined by AOAC methods (1984).

Data analysis

The obtained data of egg protein levels were analyzed using Analyze of Variance. And the number of cracks in egg samples were tested using the Univariate test to determine the effect of treatment. This analysis calculation was done using SPSS software (Statistical Product and Service Solotion). If there were significant differences in treatment, it would be continued by Duncan's Test (Sampurna and Nindhia, 2008).

Results and Discussion

Egg Protein Level

Duncan's test results showed that the average protein content produced in eggs with the addition of Avizyme® 1502 with the dosage of 1 g/Kg of feed provide the highest protein level. The addition of Avizyme® 1502 with the dosage of 3 g/Kg of feed produced similar level of protein between dosage 1 and 2 g/Kg. The addition of Avizyme® 1502 provided a contribution to increament of egg protein level (Tab 2). The xylanase, amylase and protease enzymes work for fiber breaker, protein, starch and pitat acid (North and Bell 1990). These things probably caused by xylanase and amylase enzymes capable of breaking non starch polysaccharides that are insoluble in feed and convert them into simple sugars. The nutrients that are initially entangled in the hemicellulose cell wall and which cannot be absorbed entirely by the body will be released to be digested and can be utilized by the body optimally for egg formation which increased the egg production and quality (Sheppy, 2000).

Table 2 Average of egg protein levels produced by addition of different dosage of Avizyme® 1502 in the feed.

Dosage of Avizyme® 1502 (g/Kg feed)	Egg protein level ± SD
(0) P0	9.574 ± 2.245[a]
(1) P1	12.791 ± 0.528[c]
(2) P2	11.136 ± 0.702[b]
(3) P3	11.796 ± 0.460[bc]
Significance Score	0.002

Description: Values with unequal letter subscripts show significant differences (P<0.05).

Protease enzymes are the main component of the digestive enzyme that acts on starch in food (Motyan *et al.,* 2013), it has a function to break down feed proteins into simpler parts (oligopeptides) so that they are more easily absorbed and rearranged into egg proteins. Protein is very important because protein is the main ingredient in egg and meat (Hughes, 2003). The succession in the management of feed protein is a benchmark of success in efficiency (Filho *et al.,* 2015; Joshua, 2016). In the majority of laying hens, before the digestive enzymes complete a process of protein remodeling, other feeds have begun to enter the digestive tract. So that the protein feed that can be completely overhauled to be absorbed by the body becomes not maximal (Vranjes and Wenk, 1995; Sheppy, 2000).

Protease catalyzes proteolysis, an irevesibel process that breaks down proteins into amino acids and other components (Lecker *et al.,* 2006). Proteolysis cuts the peptide bonds between amino acids in proteins. The amount of feed protein is also wasted with feces (Nadeem *et al.,* 1996). The addition of xylanas, amylase and protease enzymes on feed will increase the amount of feed protein that can be overhauled, absorbed, and rearranged into egg (Hughes, 2003; Amerah *et al.,* 2016).

Eggshell cracked

Duncan's test results showed that the average egshell cracked cases in chicken were given the feed with the addition of Avizyme® 1502 with the dosage of 3 g/Kg of feed provide the lowest cases of eggshell cracked. The addition of Avizyme® 1502 provided a contribution to dcrease the egg shell cracked cases (Tab 3). The higher doses of Avizyme® 1502. The P0 group of eggs produced 2,845 eggs and 43 cracked eggs (1.494%) for 30 days. P1 chickens produced 3,013 eggs and 38 eggs (1.235%) for 30 days. Group of P2 chickens produced 2,892 eggs and 26 eggs (0.857%) for 30 days. P3 chicken group produced 2,894 eggs and cracked eggs as many as 24 eggs (0.811%) for 30 days.

Table 3. Average of cracked of eggshell produced by addition of different dosage of Avizyme® 1502 in the feed.

Doses of Avizyme® 1502 (g/Kg feed)	Eggshell cracked ± SD
(0) P0	1.494 ± 1.625[c]
(1) P1	1.235 ± 1.447[bc]

(2) P2	0.857 ± 1.178[ab]
(3) P3	0.811 ± 1.171[a]
Significance score	0.001

Description: Values with unequal letter subscripts show significant differences (P<0.05)

Based on the results of the analysis, between P0 and P1 was not significantly different (P>0.05), but P0 with P2 and P3 was significantly different (P<0.05). This is probably caused by the addition of xylanase enzymes, amylase and protease in feed will be able to optimize the digestibility of calcium contained in food (Scheideler *et al.,* 2005). Ramesh and Chandrasekaran (2011) reported that enzyme supplementation in poultry feed resulted in the increment of the protein metabolizability, NSPs digestibilities, apparent metabolizable energy (AME) and retention of calcium, phosphorus, phytate phosphorus and nitrogen in chicken. Xylanase, amylase and protease as catalysts to accelerate the reaction of breaking the complex organic compounds into simpler ones (Amerah *et al.,* 2016). The catalyst will participate in the reaction and experience physical changes during the reaction, but will return to its original state when the reaction has been completed. Eggshells are mostly (40-60%) composed of calcium (Hincke *et al.,* 2012). During laying eggs, hens need calcium 20 times more than normal (Xia *et al.,* 2015). If calcium in the oviduct is not enough when the eggshell formed, the calcium is mostly absorbed from free Ca contained in blood plasma and food (Gongruttananun, 2011). If the preparation of calcium in the hen's body is not fulfilled, then the formation of eggshells can be disrupted, resulting in soft eggshells and cracks. Absorption of calcium in the blood plasma will not fulfilled the need for calcium when the chickens lay their eggs, so the absorption of calcium from food is needed (Elaroussi *et al.,* 1994; Darmana and Sitanggang, 2003).

The addition of xylanase, amylase and protease enzymes on chicken feed also greatly affected villus length and villus/crypt ratio increased 24% and 42% respectively. The results show that nutrase xylanase and amylase are able to work well in influencing the microbial profile through a combination of providing fragments that are easily fermented together with changes in the composition of digest as a whole as a substrate for the intestinal microbiota and increasing butyric acid (Alireza *et al.,* 2015). Increased butyric acid will be a vital energy source for the growth and development of epithelial cells in the small intestinal villi tissue. Intestinal's villies that grow and develop well will increase the ability of the intestine to absorb nutrients (Panda *et al.,* 2009). Butyric acid has the effect of suppressing the growth of pathogenic bacteria in the gastrointestinal tract, especially E. Coli, Salmonella spp and Clostridium perfringens (Cassir *et al.,* 2015). Butyric acid can increase the digestibility of protein, carbohydrates and fat by stimulating the secretion and production of digestive enzymes (Corring, 1980). Butyric acid has an anti-inflammatory effect, repairing damaged cells and stimulating abnormal cell release (apoptosis) in the small intestine tissue (Park *et al.,* 2007). Butyric acid can also increase the absorption of calcium and other minerals so it has a positive impact in: Increasing nutrient absorption power, improving eggshell quality, increasing egg production, increasing feed efficiency up to 5%, increasing egg's weight up to 3%, in older chickens can decrease the number of eggs broke and cracked up to 70%, maintaining egg quality in old chickens (>66 weeks), improving the quality of hatching eggshells (Sobczak and Kozlwski, 2016). The use of nutrase xylanase and amylase can reduce or replace the addition of butyrate, the price of feed will decrease significantly along with the positive impacts that produced (Craeyveld *et al.,* 2008; Mombaerts and Mierop, 2012).

Conclusion

The hen feed with addition of xylanase, amylase and protease enzymes (Avizyme® 1502) resulted the increament of protein level of eggs and reduced eggshell cracks. The dosage of 1 g/Kg of Avizyme® 1502 provided the highest protein level of egg.

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