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 Solution). 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 increment 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 ± 2.245a         |
| (1) P1                             | 12.791 ± 0.528c        |
| (2) P2                             | 11.136 ± 0.702b        |
| (3) P3                             | 11.796 ± 0.460bc       |
| 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 success 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 irreversible 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 eggshell 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 decrease 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 ± 1.625c         |
| (1) P1                             | 1.235 ± 1.447bc        |
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 Kozlowski, 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 increment 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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References

1. Abudabos AM. 2011. Effect of enzyme supplementation and wheat middlings as an alternative to corn on laying hens performance. Ital J Anim Sci, 10: e57.
2. Amerah AM, Romero LF, Awati A & Ravindran V. 2016. Metabolism And Nutrition: Effect of exogenous xylanase, amylase, and protease as single or combined activities on nutrient digestibility and growth performance of broilers fed corn/soy diets. Poult Sci 96(4): 807-816.
3. AOAC. 1984. Official Methods of Analysis. 14th Ed. Association of Official Analytical Chemists, Washington, DC. USA.
4. Bedford MR & Partridge GG. 2001. Enzyme in farm animal nutrition. CABI Publishing. UK.
5. Belitz HD, Grosch W & Schieberle P. 2009. Eggs. 11th Chapter. Food chemistry. Springer. Pp: 546-561.
6. Cassir N, Benamar S & Scola BL. 2015. Clostridium butyricum: from beneficial to a new emerging pathogen. Clinical Microbiology and Infection, 22: 37-45.
7. Corring T. 1980. The adaptation of digestive enzymes to the diet: its physiological significance. Reproduction Nutrition Développement, 20(4B): 1217-1235.
8. Craeyveld VV, Swennen K, Dornez E, de Wiele TV, Marzorati M, Verstraete W, Delaedt Y, Onagbesan O, Decuyper E, Buyse J, Ketelaere BD, Broekaert WF, Delcour JA & Courtin CM. 2008. Structurally different wheat-derived arabinonoxylooligosaccharides have different prebiotic and fermentation properties in rats. The Journal of Nutrition, 138: 2348-2355.
9. Darmana W & Sitanggang M. 2003. Meningkatkan produktivitas ayam arab petelur. Agromedia Pustaka, Jakarta.
10. Elaroussi MA, Forte LR, Eber SL & Biellier HV. 1994. Calcium homeostasis in the laying hen. 1. age and dietary calcium effects. Poultry Science, 73(10): 1581-1589.
11. Filho JAV, Geraldo A, Machado LC, de Brito JA, Bertechini AG & Murakami ESF. 2015. Effect of protease supplementation on production performance of laying hens. Acta Scientiarum Animal Sciences, 37(1): 29-33.
12. Gongruttananun N. 2011. Effects of eggshell calcium on productive performance, plasma calcium, bone mineralization, and gonadal characteristics in laying hens. Poultry Science, 90(2): 524-529.
13. Harms RH, Douglas CR & Sloan DR. 1996. Midnight feeding of commercial laying hens can improve eggshell quality. The Journal of Applied Poultry Research, 5: 1-5.
14. Hincke MT, Nys Y, Gautron J, Mann K, Rodriguez-Navarro AB & McKee MD. 2012. The eggshell: structure, composition and mineralization. Frontiers Bioscience, 17: 1266-1280.
15. Hughes DB. 2003. Calcium and protein in bone health. Proceedings of the Nutrition Society, 62(2): 505-509.
16. Iannotti LL, Lutter CK, Bunn DA & Stewart CP. 2014. Eggs: the uncracked potential for improving maternal and young child nutrition among the world’s poor. Nutrition Reviews, 72(6): 1-14.
17. Jazil N, Hintono A & Mulyani S. 2013. Penurunan kualitas telur ayam ras engan intensitas warna coklat kerabang berbeda selama penyimpanan. Jurnal Aplikasi Teknologi Pangan, 2(1): 43-47.
18. Joshua AO. 2016. Effect of protease supplementation on the performance of laying chickens fed marginally deficient protein diets by. Journal for Studies in Management and Planning, 2(11): 20-29.
19. Lampkin N. 1997. Organic poultry production. Welsh Institute of Rural Studies University of Wales Aberystwyth. ISBN 0902124 62 5 Pp. 1-99.
20. Lecker SH, Goldberg AL & Mitch WE. 2006. Protein Degradation by the ubiquitin-proteasome pathway in normal and disease states. Journals of the American Society of Nephrology, 17: 1807-1819.

21. Lee KW, Choi YI, Moon EJ, Oh ST, Lee HH, Kang CW & An BK. 2014. Evaluation of dietary multiple enzyme preparation (natuzyme) in laying hens. Asian-Australasian Journal of Animal Science, 27(12): 1749-1754.

22. Mertens K, Bamelis F, Kemps B, Kamers B, Verhoeest E, Ketelaere D, Bain M, Decuyper E & Baerdemaeker JD. 2006. Monitoring of eggshell breakage and eggshell strength in different production chains of consumption eggs. Poultry Science, 85: 1670-1677.

23. Miranda JM, Anton X, Valbuena CR, Saavedra PR, Rodriguez JA, Lamas A, Franco CM & Cepeda A. 2015. Egg and egg-derived foods: effects on human health and use as functional foods. Nutrients, 7: 706-729.

24. Mombarts R & Mierop KVD. 2012. NSP enzymes play positive role in prebiotic formation. Enzyme Special. All about feed. International Magazine of Animal Nutrition, Sept 2012: 1-4.

25. Motyan JA, Toth F & Tozser J. 2013. Research applications of proteolytic enzymes in molecular biology. Biomolecules, 3: 923-942.

26. Nadeem MA, Ali A, Azim A & Khan AG. 1993. Effects of feeding broiler litter on growth and nutrient utilization by barbary goat. Asian-Australasian Journal of Animal Sciences, 6(1): 73-77.

27. North MO & Bell DD. 1990. Commercial chicken production manual. Van Nostrand Reinhold, New York.

28. Panda AK, Rao SVR, Raju MVLN & Sunder GS. 2009. Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. Asian-Australasian Journal of Animal Sciences, 22(7): 1026-1031.

29. Park JS, Lee EJ, Lee JC, Kim WK & Kim HS. 2007. Anti-inflammatory effects of short chain fatty acids in IFN-gamma-stimulated RAW 264.7 murine macrophage cells: involvement of NF-kappaB and ERK signaling pathways. International Immunopharmacology, 7(1): 70-77.

30. Qi X, Tan D, Wu C, Tang C, Li T, Han X, Wang J, Liu C, Li R & Wang J. 2016. Deterioration of eggshell quality in laying hens experimentally infected with H9N2 avian influenza virus. Veterinary Research, 47(35): 1-10.

31. Ramesh J & Chandrasekaran DC. 2011. Effect of exogenous enzyme supplementation on performance of cockerels. Tamilnadu Journal of Veterinary and Animal Sciences, 7: 29-34.

32. Roberts JR & Ball W. 2003. Egg and egg shell quality guidelines for the Australian egg industry. Proceeding Australian Poultry Science Symposium, 2003: 91-94.

33. Sampurna IP & Nindhia TS. 2008 Analisis data dengan SPSS dalam rancangan percobaan. 1st Ed. Udayana University Press. Denpasar.

34. Scheideler SE, Beck MM, Abudabos A & Wyatt CL. 2005. Multiple-enzyme (Avizyme) supplementation of corn-soybased layer diets. The Journal of Applied Poultry Research, 14: 77-86.

35. Sheppy C. 2000. The current feed enzyme market and likely trends. Editted by Bedford MR. and Partridge GG. CABI Publishing, Wallingford, UK.

36. Sobczak A & Kozlowski K. 2016. Effect of dietary supplementation with butyric acid or sodium butyrate on egg production and physiological parameters in laying hen. European Poultry Science, 80: 1-14.

37. Vieira BS, Barbosa SAPV, Tavares JMN, Beloli IGC, Silva GMM, Neto HRL, Junior JGC & Correa GSS. 2016. Phytase and protease supplementation for laying hens in peak egg production. Semina: Ciências Agrárias, Londrina, 37(6): 4285-4294.

38. Vranjes MV & Wenk C. 1995. The influence of extruded vs untreated barley in the feed, with and without dietary enzyme supplement on broiler performance. Animal Feed Science and Technology, 54: 21-32.

39. Xia WG, Zhang HX, Lin YC & Zheng CT. 2015. Evaluation of dietary calcium requirements for laying Longyan shelducks. Poultry Science, 94(12): 2932-2937.