The stability of the BS4 enzymes activity on the process of feed pelleting

T Haryati, P A Sinurat and H Hamid
Indonesian Research Institute for Animal Production, Jl. Veteran 3, Ciawi, Bogor
E-mail: purringcats2001@yahoo.com.au

Abstract. BS4 mannanase enzyme can increase the digestibility of chicken feed containing 20% palm kernel cake. The palm kernel cake was produced by Eupenicillium javanicum molds through coconut cake solid substrate fermentation. In its application as a feed additive, the stability of activity is something that must be considered. This study was conducted to evaluate the stability of the saccharification activity of BS4 enzymes against heating around 90°C in the feed pelleting process. The treatment was used with and without pelleting the process of starter and finisher phases broiler feed with BS4 and commercial enzymes' addition. The parameter measured was the activity of saccharification of palm kernel cake. The results showed that the pelleting process is very influential on BS4 enzymes and can reduce 65.8 to 68.4% of its saccharification activity. While on commercial enzymes, the decrease is about 6%. It can be concluded that the BS4 enzyme is very susceptible to heat in the pelleting process so that technology is needed to protect the stability of its activity.

1. Introduction
In the animal husbandry business, feed is one of the main factors of success because feed is the business’s largest cost component. Several ways are taken to improve the quality of feed, including the use of enzymes as feed additives. Enzymes are increasingly being used, especially after the prohibition of using antibiotics as feed additives or Antibiotic Growth Promoters [1]. The addition of enzymes is expected to increase the digestibility of feed ingredients and/or feed for better feed use efficiency (FCR). These enzymes include protease, xylanase, cellulase, lipase, phytase, and mannanase.

BS4 is a complex enzyme developed by Balitnak, produced by Eupenicillium javanicum BS4 through a solid substrate fermentation process in coconut cake, consisting of β-mannanase, CMCase (cellulase), β-mannosidase, β-glucosidase, and α-galactosidase [2]. Effectively digest cellulose and hemicellulose in palm kernel meal and oil palm sludge [3]. The enzyme aims to increase the nutritional digestibility of low-quality local feed ingredients such as palm oil by-products (palm oil sludge and palm kernel meal). BS4 enzyme effectively improves the digestibility of feed nutrients [4,5] and feed ingredients such as solid palm oil, palm kernel meal, bran, soybean meal, corn [6,7]. Currently, in Indonesia, many enzymes are used commercially by the feed industry and by breeders. Almost all of today's enzymes are imported from America, Europe, Australia, America, and China. This proves that enzymes are needed in the country.

Pelleting is a process that involves impact extrusion of conditioned hot mash through a special mold to obtain shape of length and diameter desired. In the process, steam is introduced under pressure, which is there will be a collision to a high temperature in the feed mixture or mash before entering the pellet mold [8].
The temperature used in pellet machines in factories is around 95°C; the feed industry tends to use even higher and harsher temperatures to control foodborne pathogens such as *Salmonella* and *Campylobacter* [9,10]. Use of temperature higher levels during feed processing to reduce contaminated feed may result in longer exposure retention times and an increase in the vapor pressure used. The temperature in the pelleting process is also known to positively affect the gelatinization of feed starch and pellet quality [8]. This experiment was carried out to evaluate the effect of temperature on the pelleting process on BS4 enzyme activity.

2. Materials and methods

In this experiment using a completely randomized design comparing the pelleting process to the activity of the BS4 enzyme and the commercial enzyme Natuzyme, there were two treatments with and without a pelleting process of starter and finisher phases broiler feed with BS4 and commercial enzymes/Natuzyme. Each treatment was repeated for four times and standard deviation was calculated from the data for each treatment. Saccharification enzyme activity was measured.

2.1. Enzyme productions

Production of the mannanase enzyme was performed through the solid substrate fermentation process of coconut cake using the mold *Eupenicillium javanicum* BS4. Fermentation was carried out in a tray bioreactor at a temperature of 30°C, humidity (RH) 70% with aeration for seven days. The liquid BS4 enzyme was obtained by extracting the fermentation product using sodium acetate buffer and continued with concentration using ammonium sulfate. In contrast, the solid form BS4 enzyme was obtained by immobilizing the liquid enzyme in the pollard flour matrix. The quality of the BS4 enzyme produced was determined by measuring the palm kernel meal's saccharification activity.

2.2. Saccharification activity

The enzyme's saccharification activity was determined by measuring the amount of reducing sugars produced from the decomposition of carbohydrates. In this experiment, a palm kernel meal (PKC) was used as a source of carbohydrate. Saccharification activity was calculated as u/g dm, while the activity saccharification of the enzyme was calculated as u/mg protein. One activity unit is the amount of enzyme in g dm that liberates 1 μmol of glucose per minute under assay condition.

3. Results and discussions

The enzyme activity during production is presented in Table 1. It shows that the average enzyme activity for 14 production batches was 14.1 u/mL with a coefficient of variation of 16.6%. The diversity of enzyme activities is due to the inconsistent growth of each production process. Mold growth is strongly influenced by the substrate, inoculum and the environment. Good growth of mold determines the enzymes produced. BS4 production was best produced for 7 days fermentation [11].

| Description                      | Number of batches | Saccharification activity (U/mL) | Coefficient of variation |
|----------------------------------|-------------------|---------------------------------|--------------------------|
|                                  | 14                | 14.1                            | 16.6                     |

The enzymes used to be mixed in feed are enzymes in powder form or which have been immobilized into the wheat pollard matrix. The saccharification activity measured in this experiment was not only due to the presence of mannanase enzymes but also by the presence of other enzymes such as cellulase and xylanase [11].
Table 2. Change of enzyme activity during treatment

| Treatment              | Added enzyme (U sacch/kg) | Saccharification act after process (U Sacch/kg) | Standard deviation | Reduction (%) |
|------------------------|---------------------------|------------------------------------------------|-------------------|---------------|
| Starter BS4 mash       | 33                        | 182.73                                          | 0.04              |               |
| Starter BS4 pellet     |                           | 62.5                                            | 0.05              | 65.79         |
| Finisher BS4 mash      |                           | 146.99                                          | 0.04              |               |
| Finisher BS4 pellet    |                           | 46.44                                           | 0.04              | 68.40         |
| Starter natuzyme mash  |                           | 116.25                                          | 0.01              |               |
| Starter natuzyme pellet|                           | 109.25                                          | 0.04              | 6.02          |
| Finisher natuzyme mash | According to the manufacturer's recommendation | 130.5                                           | 0.07              |               |
| Finisher natuzyme pellet|                          | 138.75                                          | 0.07              | -6.32         |

Table 2 indicates that the pelleting process’s temperature significantly affects the activity of the BS4 enzyme added to the feed. The decrease that occurred was around 65.79% in starter feed and 68.40% in finisher feed. The enzyme immobilization process in the wheat pollard matrix is insufficient to protect the enzyme against exposure to temperature in the pelleting machine. The immobilization process in the polar matrix is a type of adsorption. The nonspecific interactions in the adsorption process between the enzyme protein and the matrix can cause the enzyme's active site to change or be damaged so that the enzyme activity is lower. However, the adsorption technique can prevent the enzyme from undergoing aggregation, proteolysis, and interaction with the hydrophobic layer, so that enzyme activity remains stable [12].

In the feed with commercial natuzyme enzyme added, the decrease in activity was only 6% in the starter feed type; even in the finisher feed, the enzyme activity was stable; there was no decrease. The commercial enzyme natuzyme, which is a manufactured industrial production, is more resistant to heat exposure in the pelleting process. The preservation process, which is carried out using better techniques, is also protected by a coating process. Various immobilization techniques combined with the coating process have been applied to commercial enzymes to maintain enzyme activity and slow release in the digestion tract according to the purpose of their application.

Pelleting temperature plays a vital role; it must be right because it will cause poor quality of the pellets if it is too low. If it is too high, it can result in maillard reactions that cause color changes and the possibility of destruction of feed additives. Boltz et al (2019) prove that increasing the temperature conditioning from 88ºC within 30 seconds and increasing from 77ºC to 82ºC within 60 seconds can improve the digestibility of amino acid and decrease trypsin inhibitor [13]. Moreover, feed digestibility will decrease due to decreased of extensive protein gelation that lowered protein digestibility.

4. Conclusion

Exposure to high temperatures in the pelleting process greatly affects the saccharification activity of the BS4 enzyme. Enzyme activity decreased between 65.79–68.40%. It is necessary to improve the BS4 enzyme preservation process technique so that it can be stable during storage and in the feed pelleting process.

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