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Coating of Mixed Commercial β -Mannanase and Phytase through Spraying on *Capra Hircus* Pelleted Feed

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Abstract

Pelleting ingredients were processed and subsequently coated homogeneously with mixed solution of β -mannanase and phytase. The objective of this study was to evaluate the efficiency of enzyme coated and colour measurement on goat pelleted feed containing mixed commercial enzymes. The coated enzymes efficiency (%) and colour characteristic of feed were investigated. The high percentage of coating efficiency was obtained. After sprayed pellet demonstrated darker colour of brown compared to initial pellet. High coating efficiency and determination colour values are important attributes for initial step towards enzymes delivery in animal rumen and attributes to the value-added characteristic of the pellet, respectively.

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Keywords: Coating efficiency; colour; β -mannanase; phytase; pelleted feed.

1. Introduction

The physiology of digestion of ruminants is unique among domestic animals because bacterial enzymes play an integral part in absorption, assimilation and metabolism. Actual digestion of feed is aided by the microbial

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population in the rumen *i.e.* microflora. Cellulose and related compounds found in hays and roughages consumed by cattle and sheep is dependent upon enzymes elaborated by micro-organisms living within the digestive tract (Handekar et al., 2010). Feeding can become functional with the addition of value added material (Shaharuddin et al., 2014). Well versed literature on variety of enzyme additives studies on ruminant feeding had been reviewed by Beauchemin et al. (2003). Enzymes are environmentally friendly; they work under moderate conditions of temperature, pH, and pressure, their catalyzed reactions rarely form wasteful side products and the proteins themselves are biodegradable and generally pose no threat to the environment (Saha et al., 2009).

The details of phytase have been described briefly by Greiner and Konietzny (2011), where in Kebreab *et al.* (2012) defined that phytase (EC.3.1.3.26) can hydrolyze phytate to lower inositol phosphates to release inorganic phosphate and it has been widely applied in animal feeds to increase phosphorus availability and reduced phosphorus pollution. Beneficial effect of β -mannanase on variety of animal feed had been wisely discussed by Jackson (2011). β -mannanase (endo-1, 4- β -mannanase; EC.3.2.1.78) is an enzyme that breaks down the compounds known as mannans. These polysaccharides, which are mainly by the D-glucose and D-mannose at a certain molar ratio with β -1, 4 glycosidic bonds, are widely found in nature, wherein the β -mannanase is the key enzyme that catalyzes the random hydrolysis of β -mannosidic linkages in mannan and heteromannan (Singh, et al., 2003).

Through processing of feed manufacturing activities, mainly like an extrusion, pelleting and others, continuous losses of valuable heat sensitive and/ or water-soluble ingredients such as enzymes; normally it is usually related with a high temperature and pressure conditions. Thus, feeds which are exposed to air and water from process of post-manufacturing, *i.e.* storage and handling, often resulting in loss of its valuable ingredients. While, conditions of animal's own system is also included as one of the reasons for loss of valuable ingredients. Therefore, variable of missing risk and continuous loss of valuable ingredients of targeting feed composition for optimal animal performance can be considered costly to feed manufacturing (Street et al., 2009). Hence, the commercially available additives should be able to tolerate the industry trend moving toward harsher feed processing practices. Factors that can cause a denaturation of exogenous enzymes from the conditioning and pelleting process are pressure, heat, retention time and steam quality (Thomas et al., 1998). Thus, high coating efficiency of enzyme is needed to ensure the delivery to targeted area and subsequently provides the advantages to the host.

Visual perception can be described from the appearance of an object and divided into two attributes; colour and geometric (Amookht et al., 2014). One of the quality assurance tests of a product was colour analysis (Chan et al., 2001). In addition, appealing colour could be a trademark for each brand or product's manufacturer that may help in attracting the attention of end user (Panjan et al., 2014). The analysis using colour measurements are easy, fast and comparable, which facilitate better in wide examination of an object and statistical data analysis (Franceschi et al., 2006). Thus, colour measurement analysis will provide data for value-added attributes of a product that may lead to product recognition.

This study was aimed to evaluate the efficiency of enzyme coated and colour measurement on pelleted feed containing mixed commercial β -mannanase and phytase. The fresh pelleted feed loaded combination β -mannanase and phytase (1:1) pellets was prepared by pelleting and spraying. The coating efficiency and colour measurement were examined. Comparisons were performed between pelleted feed of before and after coating process. Pelleted feed without coating was referred as control. Results were statistically analyzed using one way ANOVA and Duncan's multiple range test (DMRT).

2. Materials and methods

2.1. Materials

Commercially available liquid β -mannanase and phytase obtained from MFM Feedmill Sdn. Bhd. (172615-X), Pasir Gudang, Johor Darul Ta'zim, Malaysia. Readily fresh *Capra hircus* pelleted feed also obtained from the same venue. 0.1% Tween 80 (SIGMA ALDRICH Chemical Co., USA) for enzyme extraction was analytical grade. Chemicals were used without further modification. Several equipments were used such as CR-10 Colour Reader (Konica Minolta, Japan) and 7305 Spectrophotometer (Jenway, United Kingdom). Three replications of each analysis were done for each sample.

2.2. Pelleting and enzyme coating

Basal ingredient which consists of a palm kernel and wheat-based diet was formulated and through a pelleting process on pellet mill (Buhler, Singapore) for pressing to formed pellets. Pellets were then through a cooling system and immediate sprayed homogenously with mixed β -mannanase and phytase using COMCO liquid system at 1.2 ton dosing for 5000 ton *Capra hircus* pellets. The pellets were collected after cooling and after the coating was took place comparison.

2.3. Enzyme extraction and determination of coating efficiency

Slight modification had been made on Ramachandran et al. (2005) methods for extraction of crude enzyme, wherein a known quantity fermented pellet at 5 g was mixed in distilled water containing 0.1% Tween 80 solution and homogenized by a rotary shaker at 180 rpm for 1 hour at room temperature. After that, the sediments were removed by filtration using Whatman filter paper no. 1. Then, the solution containing the enzymes was used for absorbance measurement at λ_{595} using UV/Vis Spectrophotometer (Jenway Model 7305, United Kingdom). Standard curve was illustrated using pure mixture of both enzymes (1:1). The efficiency of coating was determined and calculated according to Eq. (1):

$$\text{Coating efficiency (\%)} = \frac{\left[\left(\frac{D}{n} \right) \div 0.055 \text{abs / ml} \right]}{0.24 \text{ ml / g}} \times 100 \quad (1)$$

where,

- D = Different of absorbances between before and after enzyme coating
- n = number of pellet used in extraction
- 1 ml = 0.055abs (as illustrated in standard curve of mixed enzymes absorbances)
- 0.24 ml g⁻¹ = maximum concentration of enzyme coated in 1 g

2.4. Surface colour measurement

The surface colour measurements of pelleted feed samples of before and after spraying process were measured using Colour Reader (CR-10, Konica Minolta, Japan). Parameters measured were the level of brightness of material (*L*), redness and greenness (*a*), and yellowness and blueness (*b*), of the samples. Measurements of colour were recorded.

2.5. Statistical analysis

Results were analyzed using the software package SPSS (Statistical Package for Social Science) version 20.0 in Windows platform. Mean and Standard Deviation of each traits were estimated using one-way ANOVA and Duncan's multiple range tests.

3. Results and discussions

3.1 Coating efficiency

Table 1 demonstrated the absorbance values of both pellet samples (before and after sprayed). The coating efficiency of mixed enzymes was 75.00% upon differentiation of before and after sprayed on pelleted feed. It indicated high coating efficiency of mixed enzyme on pelleted feed. This was due to easy adsorption and absorption on the surface of the pellet. It was also supported by the high adsorption and absorption potentials of dried pellet before the coating which affected from the heat exposure during pelleting process. In addition, the major ingredients of pellet basal were palm kernel and wheat-based diet which have high water absorption potential and

hygroscopicity (Mulinari et al., 2010).

Table 1. Absorbance values on pelleted feed (5g) before and after sprayed.

Pelleted feed	Absorbance of enzyme (abs)
Initial pellet	0.94 ± 0.31 ^a
After spray pellet	0.99 ± 0.04 ^a
Different	0.05

^aMeans with same superscripts within a column were not significantly different [$P < 0.05$, $n = 4$].

$$\begin{aligned} \text{The coating efficiency (\%)} &= \frac{(0.05/5)/0.055 \times 100}{0.24} \\ &= 75.00\% \end{aligned}$$

3.2 Colour measurement

Colour values evaluated on both pellet were stated in Table 2. The colour measurements of L, a and b values are following Hunter system (Chan et al., 2001; Khairuddin et al., 2014). There were significant differences in all colour measurement on both feed samples. The colour values for initial pellet and after enzyme coating pellet were ($a^+5.00 \pm 0.35$, $b^+15.97 \pm 0.25$, $L39.43 \pm 0.29$) and ($a^+4.10 \pm 0.30$, $b^+14.70 \pm 0.30$, $L37.57 \pm 0.51$), respectively. After sprayed pellet demonstrated darker colour of brown compared to initial pellet. This findings may contributed by the initial colour of mixed enzyme which was dark brown in colour. High efficiency of enzyme coating also may affect in end colour of sprayed pellet.

Table 2. Colour of pelleted feed before and after enzyme coating.

Pelleted feed	a+	b+	L+
Initial pellet	5.00 ± 0.35 ^b	15.97 ± 0.25 ^b	39.43 ± 0.29 ^b
After spray pellet	4.10 ± 0.30 ^a	14.70 ± 0.30 ^a	37.57 ± 0.51 ^a

^{ab}Means with different superscripts within a column were significantly different [$P < 0.05$, $n = 3$].

4. Conclusions

The mixed of β -mannanase and phytase enzymes was successfully coated at high efficiency on sprayed pelleted feed. Colour of coating pelleted feed was significantly change compared to initial pelleted feed. The alterations allow more efficient action of digestive enzymes on the feedstuffs. Colour attributes may contribute to value-added product characteristics. The homogenous of coated should be considered upon applying exogenous enzymes products to a ration of ruminants to improve feed energy utilization.

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