Determination of β-Mannanase in Feeds by Immunoassay

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Abstract

The β-Mannanase (EC 3.2.1.78) enzyme catalyzes random hydrolysis of beta-1,4- glycosidic bonds within the main chain of mannans and various non-starch polysaccharides. The compound is added to animal feed to help swine or poultry improve their growth performance and immunity. A competitive enzyme-linked immunosorbent assay (ELISA) for detection of β-mannanase was developed. Rabbit polyclonal antibodies produced after immunization were used for assay development. Total assay time was 80 minutes. The detection limit for β-mannanase in PBS buffer was 0.1 ppm, and the calibration curve ranges from 0.1 ppm to 10 ppm with a semi-log curve fit. β-Mannanase coated plate was stable more than 3 months at 37 °C. β-mannanase spiked in pig or chicken feed were extracted with PBS buffer by vortex and centrifugation. Further dilution was made with PBS buffer for analysis. The recoveries of spiked β-mannanase (10, 50 and 100 ppm) ranged from 68.4 to 141%. The results indicate that the ELISA kit developed in this study (96-well plate format) can be used as a rapid, predictive tool for the determination of β-mannanase in feed.

Introduction

Soybean meal is a major source of protein in livestock and poultry feeds in many countries. One of the undesirable components in soybean meal is the high content of β-mannan, which significantly decreases egg production, egg weight, and feed intake in laying hens. In addition, β-mannan has also been demonstrated to stimulate the innate immune system in animals, causing an increase in macrophage and monocyte proliferation and cytokine production, which often leads to increased severity of disease symptoms and a decrease in the efficiency of nutrient utilization. β-mannan is a type of hemicellulosic polysaccharide found in the plant cell wall and is composed of repeating units of mannose, with galactose and glucose often attached to the β-mannan backbone. β-Mannanase (endo-1,4- β-mannanase; EC 3.2.1.78) catalyzes hydrolysis of beta-1, 4-mannopyranosyl within the main chain of mannans and various non-starch polysaccharides. It has been known that the addition of β-mannanase to soybean meal can improve the performance of poultry and swine, by improving feed:gain ratio and decreasing water:feed ratio and dry fecal output of broilers. Production can be achieved by microbial fermentation with *Bacillus lentus*, *Trichoderma reesei* or *Paenibacillus cookii*. The most common method for detecting β-mannanase levels is by enzymatic method, in which β-mannan is hydrolyzed to reducing sugars, which are then measured. However, this enzymatic method is good for only purified or partially purified enzyme. It is difficult to apply this method for feed samples. Therefore, it is necessary to develop a simple and easy assay for detection of β-mannanase in complex matrices such animal feeds. In this study, a competitive enzyme-linked immunosorbent assay (ELISA) was developed and evaluated to determine β-mannanase content in swine and poultry feeds.

Methodology

Polyclonal antibody production:
In the development of this assay, β-mannanase was coupled to a modified protein carrier, and it served as an immunogen. The antibody was produced through the immunization of New Zealand white rabbits. The serum was collected monthly to evaluate the antibody titer.

Sample preparation:
Feed samples (5 g) were measured in a 50 mL plastic conical centrifuge tube and 20 mL of PBS buffer was added. The tube was vortexed for 2 minutes for extraction. Upper layer (1 mL) was transferred into a micro-centrifuge tube and centrifuged for 5 minutes at 12,000 rpm. Dilution of the supernatant was made with PBS buffer to give 1:10 dilution (0.1 mL + 0.9 mL).

Assay protocol:
Calibrators or samples (50 µL) were added to test wells of antigen coated plate followed by antibody solution (50 µL). They were incubated for 15 minutes at room temperature. The plate was washed with PBST 4 times. GAR-HRP (100 µL, Goat Anti Rabbit- Horse Radish Peroxidase) conjugate was added and incubated for 30 minutes. The plate was washed 4 times again. Substrate (100 µL) was added to the wells and incubated for 30 minutes. The reaction was stopped with 100 µL of stop solution and the color intensities of the wells were read at 450 – 650 nm with a microtiter plate reader.

Results

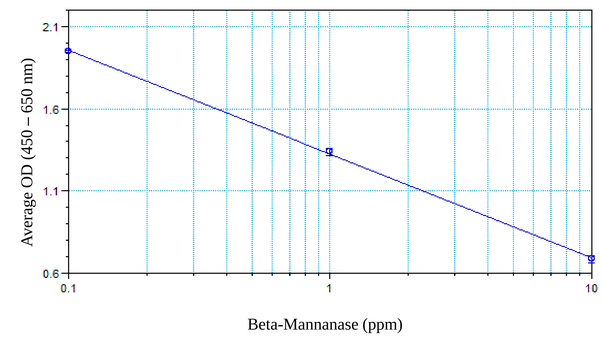
Table I. Recovery of β-Mannanase spiked in chicken and pig feeds

Type of Feed	Spiked Level of β-Mannanase in Feed (ppm)	β-Mannanase Quantitation by ELISA (ppm)	% Recovery
Chicken feed (#1)	10	8.23	82.3
	50	49.3	98.6
	100	90.0	90.0
Chicken feed (#2)	10	14.1	141
	50	61.8	124
	100	115	115
Pig feed (#1)	10	8.17	81.7
	50	34.2	68.4
	100	69.0	69.0
Pig feed (#2)	10	10.4	104
	50	51.3	103
	100	87.2	87.2

Table II. Typical ELISA values - Calibrators

Well Contents	OD	Average OD ± SD	%RSD	%Bo
Negative Control	2.142 2.206	2.174 ± 0.045	2.1	100
0.1 ppm Calibrator	1.943 1.952	1.947 ± 0.006	0.3	90
1.0 ppm Calibrator	1.354 1.324	1.339 ± 0.021	1.6	62
10 ppm Calibrator	0.701 0.669	0.685 ± 0.022	3.2	32

Results



CALIBRATION CURVE ($R^2 = 1$)

Discussion

A competitive immunoassay was developed for determination of β-mannanase in animal feeds (poultry and swine). The detection limit for β-mannanase in PBS buffer was 0.1 ppm, with a calibration curve ranging from 0.1 ppm to 10 ppm (semi-log curve fit). A 40 fold dilution factor used in the feed preparation method provides analyte detection in feed ranging from 4.0 - 400 ppm.

The kit demonstrated excellent stability during heat stressed stability testing (3 months at 37 °C). This data predicts kit stability for > 2yrs. at 4 °C based on a conservative Q10 temperature prediction model. Assay performance was demonstrated by spiking β-mannanase into multiple chicken and pig feed formulations at 3 different levels (10, 50 and 100 ppm) within the assay range of performance. The recoveries ranged from 68.4 to 141% (Table I). The results indicate that the ELISA kit developed in this study can be used as a rapid, predictive tool for the determination of β-mannanase in animal feed samples.

References

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