IN VITRO ENZYME ASSAY OF COMMERCIAL FEED ENZYMES*

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ABSTRACT

A study was conducted to assess the potency of different commercial feed enzyme preparations available in the market by in vitro. *The cellulase activity of the commercial feed enzyme preparations was ranged from 0.41 IU/g to 145.84 IU/g in Dinitro salicylic acid method. In Glucose oxidase-peroxidase method, the cellulase activity was ranged from 0.26 IU/g to 20.63 IU/g at 42° C for 2 hours incubation and from 0.6 IU/g to 52.21 IU/g at 50° C for 30 minutes incubation. The xylanase, pectinase, protese, amylase and phytase activities of the commercial feed enzyme preparations ranged between 35.09-241.35 IU/g, 76.96-249.30 IU/g, 26.05-254 IU/g, 25.25-904.87 IU/g and 0.9-115.84 IU/g respectively. The* in vitro *evaluation of an enzyme A with sunflower meal (SFM) showed that the increasing the level of enzyme addition from 0 to 15.0 mg/10 g of SFM enhanced the release of reducing sugars, glucose and inorganic phosphorus.*

Key words: Enzyme activity, cellulase, xylanase, pectinase and phytase activity

INTRODUCTION

The major part of poultry feed contains considerable amount of Non-starch polysaccharides (NSP) and phytates. These components reduce the energy utilization, protein digestion and decrease the absorption of other nutrients. The ingestion of soluble NSP like b-glucans increased the digesta viscosity (Choct and Annison, 1992) and depressed the growth rate (White et al., 1983) in broiler chicken. Phytate or phytic acid is a naturally occurring organic complex found in plants and 60-80 % of phosphorus found in the cereal grains, oilseeds as phytic acid (Simons and Versteegh, 1990). Phytate forms stable complex with minerals like Ca, Zn, Cu etc (Erdman, 1979), complex with protein (Cheryan, 1980) in poultry gut and there by reducing their utilization. Hence, supplementation of enzymes will enhance the digestibility of the NSP, utilization of these components to the desired level in the birds.Nowadays various commercial feed enzyme preparations are available in the market. These feed enzymes are full of crude enzyme preparations. Hence, the present study was carried out to assess the actual potency of various feed enzymes.

MATERIALS AND METHODS

Thirteen commercial feed enzyme (Enzyme A to L) preparations marketed by different companies were collected and evaluated interms of cellulase, xylanase, pectinase, protease, amylase and phytase activities by in vitro technique

1. Cellulase, Xylanase, Pectinase Assay

a.Use of Dinitro Salicylic acid method (DNSA) for determination of reducing sugars

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10 g crude enzyme sample was taken in 100 ml of 2 % calcium chloride solution and mixed thoroughly for one hour in a mechanical shaker, centrifuged at 2500 rpm for 10 minutes and the supernatant enzyme extract was diluted at 1:10 ratio with 0.2 M acetate buffer and used for further assay. The substrate carboxy methyl cellulose (CMC) or xylan or pectin was prepared by adding 1g in 100 ml of 0.2 M acetate buffer (pH-5.0) and dissolved by mixing it in a mechanical shaker for minimum period of 12 hours, centrifuged and the supernatant was used for further assay. 0.1 ml of enzyme extract was incubated with 0.9 ml of substrate (CMC or xylan or pectin) at 50° C for 30 minutes. After incubations, the reducing sugars were estimated by Dinitrosalicylic acid method (Miller, 1959). One IU of cellulase or xylanase or pectinase activity is defined as the amount of enzyme that liberates 1 mM of reducing sugars per minute under the assay condition.

b.Specific test for glucose(GOD-POD method)

In the DNSA method along with reducing sugars, glucose, aldehyde groups, disaccharide such as cellobiose, xylobiose and dextrin etc are being released which are not fully utilized by the birds. So, specific test for glucose was done using glucose oxidase peroxidase method (GOD-POD). After incubation of 0.1ml of 10% enzyme with 0.9 ml of 1 % substrate at 42° C for 2 hours (to simulate the condition in poultry gut system) or 50° C for 30 minutes, 20 ml was taken and used for estimation of glucose (Tiets, 1976).

2. Protease and Amylase assay

Protease and amylase activities were estimated by using the method of Kunitz (1947) and Smith and Roe (1949) respectively.

3. **Phytase assay**

The phytase activity was calculated based on the amount of phosphorus liberated per minute under assay condition (Heinonen and Lahti.,1981) .One unit of phytase activity is defined as the amount of enzyme that liberates 1mMol of inorganic phosphorus per minute under assay condition.

4. Assay of in vitro Enzyme -A activity with sunflower meal (SFM)

The Enzyme- A that showed the highest potency in vitro evaluation using specific substrate was selected and evaluated with sunflower meal (SFM) as substrate. Five levels of enzyme 0, 2.5, 5.0, 10.0, 15.0 mg/10 g of SFM was taken in 100 ml beaker with 40 ml of 0.2 M acetate buffer and was incubated at 42° C for 2 hours with periodical shaking. After incubation the content was mixed well and allowed to stand for 10 minutes, then the contents was centrifuged and the supernatant was used for glucose estimation using GOD-POD method (Tiets, 1976) reducing sugars (Miller, 1959) and inorganic phosphorus by Fiske and Subba Rao method (Varley, 1965).

RESULTS AND DISCUSSION

In enzyme assay, the cellulase, xylanase, pectinase, protease, amylase and phytase activity of various commercial enzyme preparation are presented in Table 1 and cellulase activity – specific test for glucose (GOD-POD method) in Table 2.

The cellulase activity (IU/g) ranged from minimum of 0.41 IU/g in enzyme L to maximum of 145.84 IU/g in Enzyme A by DNSA method. In GOD-POD method the cellulase activity with respect to release of glucose *in vitro* was also highest 20.43 IU/g in 42 ^{o} C for 2 hours and 52.21 IU/g in 50° C for 30 minutes in enzyme-A, where as other commercial enzyme preparations had a very low activity 0.26 IU/g in enzyme B to 2.20 IU/g in enzyme D at 42° C for 2 hours and 0.60 IU/g in enzyme B to 7.22 IU/g in enzyme D at 50° C for 30 minutes. The xylanase activity (IU/g) was estimated to maximum of 241.35 in Enzyme A and the minimum value of 35.09 in enzyme J. In the pectinase activity (IU/g), Enzyme B, L and E recorded 249.30, 248.28 and 245.09 IU/g respectively where as other enzyme recorded lower activity.

The highest protease activity was observed in enzyme K and D (254 and 242.72 IU/g) where as other enzyme had lower protease activity. The highest amylase (IU/g) activity was noticed in enzyme –E (904.87) followed by enzyme-K (792.4), enzyme–A (777.72) and enzyme G (703.63). The phytase activity (IU/g) in phytase specific enzyme preparation was higher in enzyme B1-115.84. However, 32.95 IU/g phytase activity was recorded in enzyme –A when compared to other enzyme preparations which showed little phytase activity (0.90 to 12.86 IU/g).

The variation in enzyme activity could be mainly due to the crude enzymes of different commercial preparations and the environmental factors including temperature, pH, relative humidity etc may also influence the activities of feed enzymes.

ASSAY OF INVITRO ENZYME ACTIVITY WITH SFM

The mean value of glucose (mg %), reducing sugars (mg %) and inorganic phosphorus (%) as influenced by various levels of enzyme – A in SFM are presented in **Table 3.** The linear increase in the amount of glucose released viz. 4.91, 6.69, 12.07 and 15.03 % in 2.5, 5.0, 10.0, 15.0 mg/10 g of SFM respectively over control. The amount of reducing sugar was 1365.62 mg % in control which increased by 2.45, 4.44, 5.67 and 7.08 mg % by addition of enzyme –A at 2.5, 5.0, 10.0 and 15 mg/10 g of SFM respectively.

The results agree with earlier findings of Slominski and Campbell (1990) who observed an increased hydrolysis of polysaccharides of canola meal when enzyme added at 1 % level.

The amount of inorganic P (%) released in control was 0.052 which increased to 0.056, 0.066, 0.074 and 0.082 % by addition of 2.5, 5.0, 10.0, 15.0 mg/10 g of SFM of enzyme A respectively and increase over the control was 7.14, 21.21, 29.73 and 36.59 % respectively. A similar finding was reported by Simons and Versteegh (1990) who observed that an increase *in vitro* release of phosphate from phytate in maize and soyabean meal incubated with microbial phytase.

From this study it was found that the potency of various commercial feed enzymes were estimated. It is a useful indicator to identify the quality of crude enzyme preparations used in poultry ration. Also, it might be very helpful when formulating the least cost ration contains feed ingredients with high levels of NSP and phytate phosphorus

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Table 1

Cellulase, xylanase, pectinase, protease, amylase and phytase activity (IU/gm) of commercial enzyme preparations

Enzymes	Cellulase	Xylanase	Pectinase	Protease	Amylase	Phytase
A	145.84	241.35	98.10	74.00	777.72	32.95
B	67.26	132.40	249.30	45.25	140.75	1.52
C	19.60	92.04	163.63	26.05	67.30	0.90
D	29.64	101.18	84.92	242.72	137.90	4.56
E	89.44	181.36	245.09	82.29	904.87	1.54
F	34.28	100.00	137.87	50.26	25.25	1.18
G	31.99	132.00	140.12	88.86	703.63	*
H	6.46	40.70	115.25	191.10	78.50	*
I	20.07	129.08	79.22	38.00	60.51	*
J	1.35	35.09	68.88	94.10	67.15	*
K	5.56	85.69	76.96	254.00	792.40	12.86
L	0.41	94.67	248.28	96.00	85.00	*
B1**	-	[illegible]	-	-	-	115.84

* The OD value was equal to the blank
** Phytase specific enzyme

Table 2

Cellulase activity-specific test for glucose (GOD-POD method)

The OD value was equal to the blank

Table 3

Assay of *in vitro* enzyme activity with sunflower meal

Level of enzyme/10 g of SFM	Glucose (mg %) (Specific for glucose test)	Reducing sugar (mg %) (DNSA method)	Inorganic phosphorus (%)
0 mg	138.90 ± 38.20	1365.62 ± 9.07	0.052 ± 0.017
2.5mg	146.07 ± 39.31	1399.85 ± 6.11	0.056 ± 0.019
5 mg	148.86 ± 39.04	1429.01 ± 16.13	0.066 ± 0.02
10 mg	157.96 ± 36.94	1447.74 ± 6.91	0.074 ± 0.025
15 mg	163.46 ± 37.12	1469.61 ± 14.24	0.082 ± 0.028

Mean of five observations