EVALUATION OF NON STARCH POLYSACCHARIDE DEGRADING ENZYMES AND ENZYME COMBINATIONS FOR THEIR ABILITY TO DEGRADE NON STARCH POLYSACCHARIDES OF LAYER DIET BY TWO STAGE *IN VITRO* DIGESTION ASSAY*

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ABSTRACT

The investigation was undertaken to evolve a suitable NSP enzyme combination with potential to improve the digestibility of corn and soybean meal based diets for layers. Two stage in vitro NSP digestibility assay was carried to arrive at a suitable NSP enzyme combination comprising of xylanase, cellulase and â-D-glucanase for corn-soybean meal based layer diets. The degree of NSP digestibility was assayed by measuring the amount of total sugars released. Various concentrations of Non-starch Polysaccharide enzymes viz., xylanase (0-60000 IU/kg), cellulase (0-6400 IU/kg) and â-D-glucanase (0-6400 IU/kg) were screened by in vitro digestibility assay. From that three best concentrations of NSP enzymes were selected for each enzyme to formulate twenty seven (3x3x3) enzyme combinations. The in vitro sugars release for layer diet was significantly higher (P<0.01) for concentrations of 5000, 7500 and 10000 xylanase IU/kg.Where as cellulase concentrations of 400, 800 and 1600 and â-D-glucanase concentrations of 50, 100 and 3200 IU/kg diet resulted in higher (P<0.01) sugar release. Among the twenty seven enzyme combinations of xylanase, cellulase and â-D-glucanase, the combination comprising of xylanase-10000, cellulase-400 and â-D-glucanase-100 IU/kg was selected for layer diet. It can be concluded that In vitro NSP digestibility assay, simulating the gut conditions of poultry could be used as a simple and effective technique to evolve suitable NSP enzyme combination with most effective concentration of enzymes specifically for diets of layers formulated with various feed ingredients and also to arrive at best effective dosage level.

Key words: NSP enzymes, Non-starch Polysaccharides, gut conditions, *in vitro* digestibility assay

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INTRODUCTION

Plant polysaccharides (long-chain carbohydrates) play an important role in feedstuffs. They can function as a major nutritional component in the diet (e.g. starch) but a number of non-starch polysaccharides (NSP's) are detrimental to poultry. Chicken are not capable of digesting these NSP's due to a lack of the relevant enzymes in the digestive tract. Most of the un-conventional cereal resources contain non- starch polysaccharides (NSPs) in the cell wall in higher proportion than maize (Ward, 1995), which are not only indigestible but also interfere with the utilization of the other nutrients. Further, nearly two third of phosphorus in the vegetable feed ingredients is present as unavailable phytate; further, they also have anti-nutritive properties. Early studies indicated that the inclusion of supplemental proteases, á-amylases, â-glucanases, and mixed enzymes may positively influence animal growth (Merstad and McNab, 1975; Petersson and Aman, 1989). Water-soluble, viscous â-glucans (glucans of mixed 01+4 and 01-3 linkages) in barley and other feedstuffs can have deleterious effects on a variety of livestock, especially poultry (Walsh *et al*., 1993). The specific inclusion of â-glucanase supplement in poultry diets containing barley and oats has since been conclusively demonstrated to improve performance (Elwinger and Saterby, 1987). Thus, in the present study, a two stage *in vitro* NSP digestibility assay was carried to arrive at a suitable NSP enzymes combination comprising of xylanase, cellulase and â-D-glucanase on corn soy based layer diets. The degree of NSP digestibility was assayed by measuring the amount of total sugars released (Bedford and Classen, 1993).

MATERIALS AND METHODS

A study was undertaken to screen the effect of various Non-starch Polysaccharide enzymes (xylanase, cellulase and â-D-glucanase) on corn soy based layer diet was assayed by two stage *in vitro* digestion assay of Bedford and Classen (1993) and the total sugars released from two stage *in vitro* digestion was estimated (Dubois *et al,* 1956). Based on the total sugars released, the 3 best concentrations for each enzyme were selected. Accordingly, (3x3x3) enzyme concentrations were formulated with selected enzyme doses and supplemented to standard corn soy based layer diet.

Two Stage *in vitro* Digestion Assay

About 0.1g of ground samples containing different enzyme combinations in triplicate were incubated with 3 ml of 0.1 N HCl containing 2000 IU pepsin/ml at 40°C for 45 minutes to simulate the peptic / gastric phase. To the same tubes after 45 minutes, one ml of 1 M $NaHCO_3$ containing two mg pancreatin/ ml were added and incubated for two hours at 40°C to simulate the pancreatic/intestinal phase. At the end, contents were centrifuged and the supernatant was stored in ice for total sugar estimation.

Total Sugar Estimation

After pancreatic phase, the total sugars released due to NSP digestion was quantified by phenol-sulphuric acid method as described by Dubois *et al.* (1956). An aliquot of the supernatant (0.5 ml) was diluted to 10 ml with distilled water. To one ml of this diluted solution, one ml phenol reagent and five ml concentrated

H_2SO_4 was added, and was allowed to stand for 20 minutes at room temperature and the absorbance was read in double beam UV spectrophotometer at 490 nm. The concentration of sugars in the sample was calculated using glucose standard graph. The total sugar released was expressed as mg/g substrateor feed.

Statistical Analysis

The data on total sugars released was subjected to statistical analysis using SPSS 16th version and comparison of means was tested using Duncan's multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSION

To evolve a best NSP enzymes complex comprising of xylanase, cellulase and â-D-glucanase for the corn-soybean meal based layer diet, first various concentration of the single enzyme were screened by *in vitro* NSP digestibility assay (Table.1) From that, three best concentrations for each enzyme were selected to formulate twenty seven (3x3x3) enzyme combinations. These twenty seven combinations supplemented to corn-soybean meal diet were again subjected to *in vitro* NSP digestibility studies to arrive at the best NSP enzymes combination

Eight doses of xylanase (1000 to 60000 IU/kg diet), cellulase (50 to 6400 IU/kg diet) and â-D-glucanase (50 to 6400 IU/kg diet) were tested for corn- soybean meal based standard layer diet containing 2600 kcal ME/ kg diet. Supplementation of xylanase, cellulase or â-D-glucanase at all tested doses increased ($P<0.05$) the NSP digestibility of layer diet compared to unsupplemented diet. The sugars release for layer diet was highest for concentration of 7500 IU of xylanase, 800 IU cellulase and 3200 IU â-D-glucanase. The ranking of various doses of three enzymes are given in Table 2.

The three concentrations selected were 5000; 7500 and 10000 IU/kg xylanase, 400; 800 and 1600 IU/kg for cellulase and 50; 100 and 3200 IU/kg for â-D-glucanase. With the above concentration of enzymes twenty seven combinations (3x3x3) were formulated for layer standard diet. The amount of sugars released by *in vitro* digestion with various enzyme combinations is presented in Table 3. The sugars release were significantly higher ($P<0.001$) for enzyme combination 20 (xylanase-10000, cellulase-400 and â-D-glucanase-100) followed by 5 (xylanase-5000, cellulase-800 and â-D-glucanase-100 IU/kg), 11 (xylanase-7500, cellulase-400 and D-glucanase-100 IU/kg), 14 (xylanase-7500, cellulase-800 and â-D-glucanase-100 IU/kg), 15 (xylanase-7500, cellulase-800 and â-D-glucanase- 3200 IU/kg), 17 (xylanase-7500, cellulase-1600 and â-D-glucanase-100 IU/kg), 23 (xylanase-10000, cellulase-800 and â-D-glucanase-100 IU/kg) and 26 (xylanase-10000, cellulase-1600 and â-D glcanase100 IU/kg)in that order. The amount of sugar release for layer diet without supplementation of enzyme was 138.36 mg/g and enzyme supplementation increased ($P<0.05$) the sugar release (205.53 to 381.43mg/g). At 5000, 7500 and 10000 IU xylanase/kg, the NSP digestibility was comparable among the various formulations with no effect of cellulase and â-D-glucanase. Malathi and Devegowda (2001) reported higher sugars release for enzyme combination containing xylanase, cellulase and â-D-

glucanase for corn soybean meal based broiler diet compared to enzyme combination of xylanase, cellulase and pectinase, which was attributed to higher cellulase (5.79%) and pentosan (5.4%), with comparatively less pectin (2.98%) in the diet. From the above study it can be concluded that *In vitro* NSP digestibility assay, simulating the gut conditions of poultry could be used as a simple and effective technique to evolve suitable NSP enzyme combination with most effective concentration of enzymes specifically for diets of layers formulated with various feed ingredients and also to arrive at best effective dosage level.

Table 2 :
Ranking of enzymes concentration based on *in vitro* NSP digestibility for layer standard diet

Rank	Activity of enzyme (IU/kg diet)		
	Xylanase	Cellulase	â-D-glucanase
1.	7500	800	3200
2.	10000	1600	1600
3.	5000	400	100
4.	20000	200	50
5.	2500	100	200
6.	40000	50	400
7.	60000	3200	6400
8.	1000	6400	800

Table 1:
***In vitro* NSP digestibility, measured as total sugars released (mg/g) from standard Layer diet (2600 kcal ME/kg) supplemented with various NSP degrading enzyme.**

Enzyme	Enzyme concentration (IU/kg)	Sugars released (mg/g)
Xylanase	0	125.6^{e}
	1000	148.4^{d}
	2500	216.0^{abc}
	5000	219.3^{abc}
	7500	240.3^{a}
	10000	228.2^{ab}
	20000	218.4^{abc}
	40000	212.9^{bc}
	60000	196.3^{c}
	SEM	**7.37**
	P value	**0.001**
Cellulase	0	148.3^{e}
	50	211.7^{b}
	100	211.2^{bc}
	200	211.6^{bc}
	400	219.8^{ab}
	800	239.0^{a}
	1600	228.6^{ab}
	3200	188.9^{cd}
	6400	177.8^{d}
	SEM	**5.55**
	P value	**0.001**
â- D glucanase	0	133.5^{e}
	50	218.1^{abc}
	100	237.9^{ab}
	200	203.9^{bcd}
	400	183.9^{cd}
	800	175.7d
	1600	237.8^{ab}
	3200	249.8^{a}
	6400	183.6^{cd}
	SEM	**7.76**
	P value	**0.001**

Each value is the average of triplicate analysis

Means with different superscripts in a column differ significantly (P<0.05)

Table 3:

***In vitro* NSP digestibility, measured as total sugars released (mg/g) from standard layer diet supplemented with various NSP enzymes combinations**

Combination	Enzyme combination (IU/kg)			Sugars released (mg/g)
	Xylanase	Cellulase	â-D glucanase	
Control	0	0	0	138.4^{h}
1	5000	400	50	250.7^{defg}
2	5000	400	100	275.7^{bcdefg}
3	5000	400	3200	271.2^{cdefg}
4	5000	800	50	205.5^{g}
5	5000	800	100	354.9^{ab}
6	5000	800	3200	233.2^{fg}
7	5000	1600	50	263.3^{defg}
8	5000	1600	100	262.6^{defg}
9	5000	1600	3200	289.6^{bcdef}
10	7500	400	50	231.3^{fg}
11	7500	400	100	346.5^{abc}
12	7500	400	3200	272.2^{cdefg}
13	7500	800	50	281.0^{bcdefg}
14	7500	800	100	329.1^{abcd}
15	7500	800	3200	344.8^{abc}
16	7500	1600	50	248.9^{defg}
17	7500	1600	100	327.0^{abcd}
18	7500	1600	3200	286.7^{bcdefg}
19	10000	400	50	215.1^{fg}
20	10000	400	100	381.4^{a}
21	10000	400	3200	278.7^{bcdefg}
22	10000	800	50	232.6^{fg}
23	10000	800	100	326.0^{abcd}
24	10000	800	3200	237.7^{efg}
25	10000	1600	50	222.3^{fg}
26	10000	1600	100	318.1^{abcde}
27	10000	1600	3200	207.6^{fg}
		SEM		**6.57**
		***P* value**		**0.001**

Each value is the average of triplicate analysis

Means with different superscripts in a column differ significantly (P<0.05)

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