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In- vitro Assessment of Non Starch Polysaccharide Hydrolyzing Enzymes in Broiler Diets Based on Guar Meal, Rape Seed Meal and Cotton Seed Meal

M.G. Nikam, V. Ravinder Reddy, M.V.L.N.Raju[1], K. Kondal Reddy, J. Narasimha

Department of Poultry Science, College of Veterinary Science, Rajendra nagar, [1]Directorate of Poultry Research, Rajendra nagar, Hyderabad , India

Abstract: ***The present study was undertaken to assess the In-vitro sugar release with different non starch polysaccharide (NSP) hydrolyzing enzymes, viz. xylanase, ß-D- glucanase, cellulase, mannanase and pectinase in broiler diets based on corn +soya (Diet I) or supplemented with alternate protein (AP) sources like guar meal, rapeseed meal and cotton seed meal, each at two levels i.e. 3 and 6 percent in diet (Diet II and Diet III, respectively). The NSP enzymes were initially supplemented individually to all the diets at different concentrations. Based on the data, the optimum concentration of each enzyme yielding maximum sugar was identified. The enzyme with maximum sugar release was further used in the enzyme combination studies at 3 different concentrations (i.e. 100, 80 and 60 %) in each of the test diet. Similarly a combination of all the enzymes was made at the lowest concentration of each of the enzyme, which was tested at 100, 200 and 400 % on each of the test diet. The maximum sugar release observed in diet-I, II and III was @ 60, 100 and 60 % respectively. i.e. (xylanase, ß-D-glucanase, cellulase, mannanase and pectinase @ 2400, 4800, 1800, 4800, and 2400 IU/kg respectively for diet I, 4000, 8000 ,1900, 10000 and 4000 IU/Kg respectively for diet II and 2400, 4000, 1800, 4800 and 2400 IU/kg respectively for the diet III). Similarly the lowest concentration studies of the enzymes with diet I,II and III revealed a significant sugar release @ 100, 200 and 200 % concentrations i.e. Xylanase ß-D-glucanase, cellulase, mannanase and pectinase @ 200, 120, 100, 100, and 200 IU/kg respectively for diet I, 400, 240, 200, 200 and 400 IU/Kg respectively for the diet II and diet III. It is concluded that the maximum sugar release was reported in the diet I @ 60 % of enzyme concentration.***

Keywords: *In-Vitro*, Non starch polysaccharides, Alternate protein.

1. Introduction

Efficiency in feeding has been the major concern in raising poultry, as feed cost contributes to 65 to 75% of total cost of production. Maize soybean meal based diet is being practiced worldwide for all classes of poultry. The corn-soya diets however contain between 10-75% of non-starch polysaccharides (NSP) (Chot, 2011).

Apart from the NSP content of corn and soybean , the cost of soybean meal is increasing day by day, because of which the poultry farmers are looking for the alternative plant protein sources (APM) like guar meal (GM), (protein 33-45%), rapeseed meal (RSM), (protein 38-43 %) and cotton seed meal (CSM), (protein 40-42%). However, the non-starch polysaccharide (NSP) contents of GM, RSM and CSM are very high (78%, 36.2 and 36.7, respectively).

Most of the NSPs from the cereals and vegetable protein sources can be broken down to the simplest form of the sugars like oligosaccharides and polysaccharides with the help of NSP hydrolyzing enzymes. The amount of simple sugars released on supplementation of the NSP hydrolyzing enzymes can be determined by the phenol sulfuric acid method procedure (Dubois *et.al* 1956).

Soybean meal (SBM) contains about 20% NSP (Malathi and Devegowda, 2001). Similarly, other major ingredients used in broiler and layer diets *i.e.,* maize and rice bran contain 9 and 25% NSP, respectively (Malathi and Devegowda, 201) half of which is cellulose (Saunders, 1986). The NSPs are insoluble (cellulose) and soluble (β-glucose, arabinoxylan, arabinogalactose, xyloglucon etc). The soluble NSPs have the property to immobilize water in their matrix by forming loose gel network which is responsible for increased viscosity, there by depressing the digestibility of fats, proteins and starch. These NSPs impair activity of endogenous enzymes by reducing the contact intensity between nutrients and enzymes, which results in sticky and moist droppings. The diets with NSPs will increase the incidence of vent pasting when birds are fed with the alternate protein sources compared to the normal corn soya diet. It is also observed that the birds receiving diets with the alternate protein source also has the negative relationship between the NSP and energy and nutrient digestibility provided evidence of its anti-nutritive character (Annison and Choct., 1993)

2. Materials and Methods

The NSP enzymes investigated in present study were xylanase, ß-d-glucanase, cellulase, mannanase and pectinase. These pure enzymes were procured from Advanced Bio- Agrotech Limited, Pune, India. The concentrations of xylanase, ß-d-glucanase, cellulase, mannanase and pectinase in the product were 160000, 200000, 1000000, 200000 and 150000 IU/g, respectively. The *in vitro* digestibility studies were undertaken for the corn soya diet and diets with alternate protein meals viz.guarmeal (GM), rapeseed meal (RSM) and cotton seed meal (CSM), which were assessed by two stage *in vitro* digestion assay and the total sugars released from two stage *in vitro* digestion was estimated as per the procedure described by(Dubois *et.al* 1956).

Based on the available literature, various enzyme concentrations were selected to formulate different NSP enzyme combinations for different diets (Table-2 to table 5)

Two stage *In-vitro* digestion assay:

About 0.1g of ground samples containing different NSP hydrolyzing enzymes 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, 1 ml of 1 M $NaHCO_3$ containing 2 mg pancreatin/ml were added and incubated for 2 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 were quantified by phenol-sulphuric acid method as was recorded with enzyme combination. An aliquot of the supernatant (0.5 ml) was diluted to 10 ml with distilled water. To 1 ml of this diluted solution, 1 ml phenol reagent and 5 ml conc. H2SO4 were added, and 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 and was expressed as mg/g substrate/ feed.

The data obtained 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.

3. Results and Discussion

The amount of sugar released with the enzyme supplementation was significantly higher (P<0.001) in all three diets compared to that of unsupplemented control diets. The optimum concentration (OC) of xylanase that released maximum sugar from SBM, APM1 and APM2 was 4000 IU/kg for all three diets. The amount of sugar released at OC of the enzyme in SBM, APM1 and APM2 was 107.11, 108.70 and 110.30 mg/g, respectively and the lowest concentration that yielded statistically comparable sugar release with maximum sugar concentrations in three diets was 4000 IU/kg, for all the three diets (Table 1). The results obtained with xylanase supplementation indicated that the concentration of enzyme required for releasing highest sugar from the three diets increased with increasing the supplemental levels of alternate protein ingredients in the diet. As feed enzymes are most efficacious against soluble fractions of NSP (Chessson, 2001), Xylose and arabinose are the monomers of xylan backbone and are released by breakdown of β-1-4 linkages in the pentosan called arabinoxylan with xylanase supplementation (Massey et al., 2014).

Supplementation of mannanase, pectinase, cellulase and β-D-glucanse also showed similar results as that of xylanase supplementation. The maximum sugar release was obtained at 8000 IU/kg for SBM and APM1, however for APM2 the maximum sugar release was obtained at 10000. The maximum sugar release obtained in respect of pectinase was 4000 IU/kg for all the three diets. In respect of cellulase the maximum sugar release obtained for SBM, APM1 and APM2 diets was at 3000,1900 and 3000 IU/kg respectively. The maximum sugar release obtained from the APM2 diets in respect of β-D-glucanse was at 8000 IU/kg for SBM, APM1, whereas it was at 10000 IU/kg concentration for the APM2. (Table 3, 4, 5 and 6).Thus it clearly indicated that the amount of sugar released at OC of all the enzymes was found to be more in maize soya based diet (T1) compared to alternate protein meals based diets (APM1 and APM2). The sugar release decreased with increasing the levels of alternate protein supplements in the diets. The present findings of NSP enzyme supplementation might be due to higher concentration soluble fraction of mannose, glucose, galactose, galacturonic acid and glucuronic acids in alternate protein supplements compared to that of in SBM. This can substantiate well with increased mannanase, pectinase, cellulase and β-D-glucanse requirement for maximum sugar release, while increasing inclusion levels of alternate protein meals in the diets.

4. Conclusion

The enzyme combination (HC -100) with maximum sugar release in all three diets can be selected as suitable NSP hydrolyzing enzyme cocktail. Thus outcome of present investigation indicates that NSP enzyme combination with maximum sugar release may be taken as a decisive factor for developing a suitable NSP hydrolyzing enzyme cocktail for commercial broiler diets supplemented with alternate protein meals. It can also be concluded that the NSP hydrolyzing enzymes are less effective at lower concentration when compared with the higher concentrations .The NSP,hydrolyzing enzymes possesses a broad spectrum of different modes of action and this way the supplementation of these enzymes affects the nutrient digestion,metabolism,overall performance and health of the birds. The application of the present NSP enzyme cocktail is a tool in designing a least cost and balanced poultry diets. It is further to narrate that the NSP hydrolyzing enzymes selected in the present investigation are substrate specific and will be more effective compared to readily available enzyme combinations in the market.

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Table 1: Concentrations of Non starch polysaccharide hydrolyzing enzymes selected for the *In-vitro* studies of corn soya diet and diets based on alternate protein meals each at 3 and 6 % levels in diet

Xylanase IU/Kg	ß-d-glucanase IU/Kg	Cellulase IU/Kg	Mannanase IU/Kg	Pectinase IU/Kg
200	120	100	100	200
450	480	500	500	800
700	1400	1100	1000	1400
1050	2800	1500	2000	2000
2100	6000	1900	4000	4000
4000	8000	3000	6000	7000
6000	10000		8000	10000
8000			10000	
10000				
20000				
40000				
60000				
80000				

Table 2: Ingredient and nutrient composition of experimental diets

Ingredients	**Diets**		
	SBM	**APM1 3%**	**APM2 6%**
Maize	52.44	50.56	48.68
Soya bean meal 46	40.33	32.76	25.19
Cotton seed meal 36%	0.000	3.00	6.00
Rape seed meal (Ext)	0.000	3.00	6.00
Guar meal	0.000	3.00	6.00
Salt	0.380	0.380	0.380
DCP	1.997	1.946	1.895
Stone grit	1.060	1.058	1.056
DL- Methionine	0.204	0.200	0.196
AB2D3K	0.015	0.015	0.015
B- complex	0.010	0.010	0.010
Choline Chloride, 50%	0.050	0.050	0.050
Toxin binder	0.200	0.200	0.200
Trace mineral Mix	0.100	0.100	0.100
L Lysine HCl	0.072	0.155	0.239
Oil (veg)	3.132	3.565	3.997
Total	100.00	100.00	100.00
Nutrient Composition			
ME (kcal/kg)	2950	2950	2950
Protein (%)	23.00	23.00	23.00
Calcium (%)	0.90	0.90	0.90
Avail Phosphorus (%)	0.45	0.45	0.45
Lysine (%)	1.36	1.36	1.36
Methionine (%)	0.56	0.56	0.56

Table 3:. *In vitro* sugar release (mg/g) from broiler diets supplemented with different levels of Xylanase enzyme

Xylanase, IU/kg	Diet I Corn+ Soya	Diet II Corn + Soya + APM @ 3 %	Diet III Corn + Soya + APM @ 6%
Basal Diet	42.71^{g}	50.64^{g}	48.77^{h}
200	62.64^{f}	74.37^{ef}	27.70^{h}
450	76.37^{cd}	76.44^{ef}	30.10^{h}
700	89.51^{b}	99.77^{b}	71.57^{ef}
1050	90.71^{b}	98.64^{b}	75.84^{e}
2100	92.04^{b}	99.57^{b}	100.17^{bc}
4000	107.11^{a}	108.70^{a}	110.30^{a}
6000	70.31^{de}	10.3.37^{ab}	90.77^{d}
8000	73.04^{cd}	92.17^{ab}	65.51^{fg}
10000	78.24^{c}	86.17^{c}	67.37^{cd}
20000	87.24^{b}	73.30^{d}	95.24^{d}
40000	77.04^{c}	75.97^{c}	67.37^{cd}
60000	64.37^{ef}	78.31^{e}	94.71^{cd}
80000	62.44^{f}	66.50^{f}	59.50^{b}
SEM	2.47	2.52	4.03
P value	0.000	0.000	0.000

Table 4: *In vitro* sugar release (mg/g) from broiler diets supplemented with different levels of ß-D-glucanase enzyme

ß-D-glucanase, IU/kg	Diet I Corn+ Soya	Diet II Corn + Soya + APM @ 3 %	Diet III Corn + Soya + APM @ 6%
0	42.71^{d}	50.64^{d}	48.77^{c}
120	75.97^{c}	34.97^{e}	35.11^{d}
480	93.77^{b}	55.57^{d}	36.31^{d}
1400	97.24^{b}	76.57^{b}	53.64^{b}
2800	96.51^{b}	81.57^{b}	54.17^{b}
6000	98.97^{a}	98.37^{a}	74.04^{b}
8000	109.64^{d}	107.37^{b}	77.11^{a}
10000	45.24^{d}	90.44^{b}	88.37^{a}
SEM	5.04	4.99	3.85
P value	0.000	0.000	0.000

Table 5: *In vitro* sugar release (mg/g) from broiler diets supplemented with different levels of Cellulase enzyme

Cellulase, IU/kg	Diet I Corn+ Soya	Diet II Corn + Soya + APM @ 3 %	Diet III Corn + Soya + APM @ 6%
0	42.71^{d}	50.64^{d}	48.77^{e}
100	90.97^{b}	35.84^{e}	72.84^{d}
500	67.17^{c}	92.11^{c}	83.51^{c}
1100	85.44^{b}	107.24^{b}	89.77^{a}
1500	88.71^{a}	110.91^{b}	112.17^{b}
1900	122.11^{a}	131.57^{b}	99.17^{a}
3000	122.24^{a}	115.31^{b}	114.84^{a}
SEM	5.93	7.39	4.84
P value	0.000	0.000	0.000

Table 6: *In vitro* sugar release (mg/g) from broiler diets supplemented with different levels of ß-Mannanase enzyme

ß-Mannanase, IU/kg	Diet I Corn+ Soya	Diet II Corn + Soya + APM @ 3 %	Diet III Corn + Soya + APM @ 6%
0	42.71^{d}	50.64^{g}	48.77^{de}
100	119.24^{b}	55.91^{g}	64.71^{c}
500	142.77^{a}	67.51^{f}	68.57^{bc}
1000	107.84^{c}	89.04^{e}	56.11^{d}
2000	122.44^{b}	111.64^{d}	66.91^{bc}
4000	123.64^{b}	114.91^{cd}	71.44^{bc}
6000	142.71^{a}	120.84^{b}	73.37^{b}
8000	149.04^{a}	138.04^{b}	89.24^{a}
10000	115.64^{b}	162.77^{a}	44.57^{e}
SEM	5.89	7.10	2.61
P value	0.000	0.000	0.000

Table 7: *In vitro* sugar release (mg/g) from broiler diets supplemented with different levels of Pectinase enzyme

Pectinase, IU/kg	Diet I Corn+ Soya	Diet II Corn + Soya + APM @ 3 %	Diet III Corn + Soya + APM @ 6%
0	42.71[c]	50.64[f]	48.77[e]
200	121.04[ab]	125.57[de]	109.37[c]
800	140.17[ab]	119.04[e]	115.37[c]
1400	93.11[b]	126.97[d]	114.97[c]
2000	138.84[ab]	149.24[b]	131.51[b]
4000	150.84[a]	161.04[a]	142.97[a]
7000	118.97[ab]	156.11[ab]	130.24[b]
10000	108.31[ab]	134.91[c]	99.04[d]
SEM	8.28	6.81	5.67
P value	0.007	0.000	0.000

Table 8: Concentrations of NSP hydrolyzing Enzymes used for combination studies

Lower combinations (LC) – for Diets with corn+soya and APM @ 3 % and 6 %					
Percentage of Enzyme	*Xylanase (IU/kg)*	*β-D-glucanase (IU/kg)*	*Cellulase (IU/kg)*	*Mannanase (IU/kg)*	*Pectinase (IU/kg)*
100%	200	120	100	100	200
200%	400	240	200	200	400
400%	800	480	400	400	800
Higher Combinations (HC)					
SBM					
100%	4000	8000	3000	8000	4000
80%	3200	6400	2400	6400	3200
60%	2400	4800	1800	4800	2400
APM1					
100%	4000	8000	1900	10000	4000
80%	3200	6400	1520	8000	3200
60%	2400	4800	1140	6000	2400
APM2					
100%	4000	10000	3000	8000	4000
80%	3200	6000	2400	6400	3200
60%	2400	8000	1800	4800	2400

Table 9: *In vitro* sugar release (mg/g) from broiler diets supplemented with NSPHE (Xylanase, β-d glucanase, cellulase, β-mannanase and pectinase) at lower as well as higher concentrations

Concentrations		*Diet I Standard Corn + soybean meal diet*	*Diet II Standard diet + APM each @ 3 %*	*Diet III Standard diet + APM each @ 6 %*
1	**Basal**	42.71[f]	50.64[f]	48.77[f]
2	**100% LC**	106.91[cd]	73.51[e]	90.84[d]
3	**200% LC**	93.11[de]	115.77[ab]	102.31[c]
4	**400% LC**	89.51[e]	109.57[bcd]	70.17[e]
5	**100 % HC**	111.11[c]	116.11[ab]	109.84[abc]
6	**80 % HC**	130.44[ab]	113.11[abc]	117.24[ab]
7	**60 % HC**	135.77[a]	105.91[cd]	120.57[a]
	SEM	5.11	4.01	4.24
	P value	0.001	0.001	0.001

Values bearing different superscripts within a column are significantly (P<0.01) different