doi:10.5455/vetworld.2013.525-529

ORIGINAL RESEARCH

Two-stage *in vitro* digestibility assay, a tool for formulating non-starch polysaccharide degrading enzyme combinations for commonly used feed ingredients of poultry rations

J. Narasimha, D. Nagalakshmi, Y. Ramana Reddy and S. T. Viroji Rao

AICRP on Poultry Breeding, Department of Animal Nutrition, College of Veterinary Science, Rajendranagar, Sri Venkateswara Veterinary University, Hyderabad, Andhra Pradesh - 500030, India
Corresponding author: Narasimha Jatoth, E-mail: simha_vet@yahoo.com
Received: 09-01-2013, **Revised:** 13-02-2013, **Accepted:** 14-02-2013, **Published online:** 24-05-2013

How to cite this article: Narasimha J, Nagalakshmi D, Reddy YR and Rao STV (2013) Two-stage *in vitro* digestibility assay, a tool for formulating non-starch polysaccharide degrading enzyme combinations for commonly used feed ingredients of poultry rations, *Vet World* 6(8): 525-529, doi:10.5455/vetworld.2013.525-529

Abstract

Aim: An attempt was made to assess the effect of pure enzyme combinations with the objective of formulating customized enzyme mixtures based on sugar release when subjected to two-stage *in vitro* digestion assay.

Materials and Methods: A two-stage *in vitro* digestibility assay was carried out for commonly used feed ingredients for poultry viz., maize, soy bean meal, sunflower cake, and de-oiled rice bran supplemented with three concentrations of xylanase (5000; 7500 and 10000 IU/kg), cellulase (50; 100 and 400 IU/kg) and β-D-glucanase (100; 200 and 400 IU/kg) were used to formulate various NSP enzymes combinations. In total 27 NSP enzyme combinations (3x3x3) were formulated and the sugar released due to NSP digestion was quantified by phenol sulphuric acid method.

Results: The total sugar release was significantly ($P<0.05$) higher with supplementation of various enzymes combinations for maize, sunflower cake and de-oiled rice bran where as no significant ($P<0.05$) interaction of various NSP enzymes combinations was observed for soy bean meal. The NSP digestibility was highest in combination (xylanase-5000, cellulase-50 and β-D-glucanase-400 IU/kg), (xylanase-10000, cellulase-50 and β-D-glucanase-200 IU/kg) and (xylanase-7500, cellulase-100 and β-D-glucanase-100 IU/kg) for maize, sunflower cake and de-oiled rice bran respectively. In case of sunflower cake, significant ($P<0.01$) three way interaction was observed among the xylanase, cellulose, and β-D-glucanase enzymes and the two-way interactions between the enzymes were also significant ($P<0.01$).

Conclusion: It is concluded that 'n' number of non-starch Polysaccharide enzymes combinations can be screened for their efficiency to digest non-starch Polysaccharides present in various feed ingredients commonly used in poultry rations by employing two-stage *in vitro* digestibility assay as a tool.

Key words: feed ingredients, *in vitro* digestibility assay, non-starch polysaccharide enzymes

Introduction

Polysaccharides are major components of plant materials used in rations for poultry. They are macromolecular polymers of monosaccharides linked by glycosidic bonds [1]. The Non-starch Polysaccharides are principally non α glucon polysaccharides of the plant cell wall. They are heterogenous group of polysaccharides with varying degree of water solubility, size and, structure [2]. Non-starch polysaccharides (NSP) include celluloses, hemicelluloses, pectines, and oligosaccharides (α - galactosides, etc.). They can also be divided into water-soluble and water-insoluble fractions; fr which have greater relevance to their nutritional values. Birds do not possess endogenous enzymes capable of cleaving and digesting the β*(α)* linked NSP. The water-insoluble NSP can be considered practically undigested by poultry and only soluble NSP has the potential to be digested by birds [3]. However, soluble NSP are known to posses anti-nutritional properties by either encapsulating nutrients and/or depressing overall nutrient digestibility through gastro-intestinal modifica-tions. Hence, supplementation of non-starch polysaccharide degrading enzymes will enhance the digestibility of the NSP and utilization of these components to the desired level in the chicken.

An attempt was made to assess the effect of pure enzyme combinations on commonly used poultry feed ingredients with the objective of formulating customized enzyme mixtures based on sugar release (The addition of selected carbohydrases will break down NSP, releasing nutrients (energy and protein), as well as reducing the viscosity of the contents) when subjected to two-stage *in vitro* digestion assay.

Materials and Methods

The NSP enzymes investigated in present study were cellulase, xylanase, and β-D-glucanase. These pure enzymes were procured from Advanced Bio-Agrotech Limited, Pune, India. The activity of cellulase, xylanase, β-D-glucanase and Protease were 1000000, 1200000, 1600000 and 100000 IU/g, respectively.

The *in vitro* digestibility studies were undertaken

for the commonly used feed ingredients for poultry *viz*., maize, soybean meal, sunflower cake and de-oiled rice bran supplemented with various NSP enzymes oncentrations (xylanase, cellulase and β- D-glucanase) and was assessed by two stage *in vitro* digestion assay of [4] and the total sugars released from two stage *in vitro* digestion was estimated. The total sugar released from *in vitro* digestion was assessed as per the procedure described by, [5]. Based on the available literature various enzyme concentrations were selected to formulate different NSP enzymes combinations for commonly used feed ingredients (Table-1).

Table-1. Various enzyme concentrations selected for feed ingredients.

Enzyme	Concentration (IU/kg substrate)
Xylanase	5000; 7500 and 10000
Cellulase	50; 100 and 400
β-D-glucanase	100; 200 and 400

With three concentrations each of xylanase, cellulase and β-D-glucanase, twenty seven different combinations were formulated. These enzyme combinations were supplemented to each ingredient and subjected to two stage *in vitro* NSP digestibility and total sugars released were measured.

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, 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 was quantified by phenol-sulphuric acid method as described by [5]. 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 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 substrate/ 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 [6].

Results

Maize: The sugars release from maize without enzyme supplementation was 131.2 mg/g. The total sugars release was significantly (*P<0.05*) higher with supplementation of various enzyme combinations (Table-2). Significant (*P<0.05*) interaction was observed for xylanase, cellulase and β-D-glucanase. At 10000 IU/kg xylanase concentration, the NSP digestibility was higher at all combinations containing cellulase and β-D-glucanase (19-27 combinations) compared to control and the sugars release was comparable among all combinations containing 10000 IU/kg xylanase (170.01 – 207.66 mg/g). At 7500 IU/kg xylanase combinations the sugars release was higher in all combinations except in combination containing 400 IU/kg β-D-glucanase. At 5000 IU/kg xylanase supplementation the NSP digestibility was highest in combination 3 (xylanase-5000, cellulase-50 and β-D-glucanase-400 IU/kg) followed by combinations 7 and 1. No effect of enzyme supplementation was observed with combinations 2, 4, 5 and 9 in comparison to control.

Soybean meal: No significant interaction was observed for xylanase, cellulase and β-D-glucanase. Interaction between xylanase and β-D-glucanase, xylanase and cellulase and cellulase and β-D-glucanase were also not significant (Table-2). The *in vitro* sugars release in control was 116.9 mg/g and increased in various enzyme supplementation groups which, ranged between 162.9 – 191.2 mg/g. The *in vitro* NSP digestion increased significantly (*P<0.05*) with xylanase supplementation, irrespective of cellulase and β-D-glucanase concentrations, but dose of xylanase did not show any difference.

Sunflower cake: The sugars release from sunflower cake with two stage *in vitro* digestion was 133.20 mg/g in control and was significantly (*P<0.01*) increased in enzyme supplemented groups (155.6–175.9 mg/g) irrespective of the combinations (Table 2). Significant (*P<0.01*) three way interaction was observed among the xylanase, cellulase and β-D-glucanase enzyme. The two-way interactions between the enzymes were also significant (*P<0.01*). The highest sugars release was recorded with enzyme combination 20 (xylanase-10000, cellulase-50 and β-D-glucanase-200 IU/kg) and 10 (xylanase-7500, cellulase-50 and β-D-glucanase-100 IU/kg), followed by 24 (xylanase-10000 cellulase-100 and β-D-glucanase- 400 IU/kg), 19 (xylanase-10000,cellulase-50 and β-D-glucanase-100 IU/kg), 17 (xylanase-7500, cellulase-400, β-D-glucanase- 200 IU/kg), 13 (xylanase-7500, cellulase-100 and β-D-glucanase-100 IU/kg) and 10 (xylanase-7500, cellulase-50, β-D-glucanase-100 IU/kg)in that order. The sugars release was higher at xylanase concentration of 7500 and 10000 IU/kg, cellulase concentration of 50 and 100 IU/kg and β-D-glucanase concentration of 200 IU/kg, irrespective of combinations.

De-oiled rice bran: The release of sugars from de-oiled rice bran during *in vitro* NSP digestion was 106.64 mg/g, in control which increased significantly (*P<0.001*) with enzyme supplementation (147.2-187.8) (Table-2). At 5000 IU/kg xylanase

Table-2. *In vitro* NSP digestibility, measured as total sugars released (mg/g) from feed ingredients supplemented with various NSP enzyme combinations

Combination No.	Enzyme combination (IU/kg)			Maize	Soybean meal	Sunflower cake	De-oiled rice bran
	Xylanase	Cellulase	β-D glucanase				
Control	0	0	0	131.2^{f}	116.9	133.2^{m}	106.6^{l}
1	5000	50	100	197.2^{ab}	169.6	162.3^{ijk}	179.2^{ab}
2	5000	50	200	161.4^{bcdef}	172.4	168.3^{cdefg}	174.33^{bcde}
3	5000	50	400	207.4^{a}	164.0	162.2^{ijk}	175.5^{bcde}
4	5000	100	100	142.8^{ef}	171.2	155.6^{l}	174.5^{bcde}
5	5000	100	200	150.9^{cdef}	178.1	169.8^{bcdef}	163.9^{efg}
6	5000	100	400	187.2^{abcd}	177.4	162.0^{ijk}	177.3^{abcd}
7	5000	400	100	197.3^{ab}	190.5	164.5^{ghijk}	159.8^{g}
8	5000	400	200	178.1^{abcde}	178.1	168.1^{defgh}	173.7^{bcdef}
9	5000	400	400	162.1^{bcdef}	171.2	165.3^{fghijk}	173.9^{bcdef}
10	7500	50	100	179.7^{abcde}	171.1	171.1^{abcde}	169.0^{bcdefg}
11	7500	50	200	182.9^{abcd}	163.4	175.1^{a}	166.5^{cdefg}
12	7500	50	400	161.1^{bcdef}	186.8	165.5^{fghijk}	171.7^{bcdefg}
13	7500	100	100	181.0^{abcd}	184.4	171.1^{abcde}	187.8^{a}
14	7500	100	200	164.1^{bcdef}	191.2	173.0^{abc}	173.0^{bcdef}
15	7500	100	400	150.5^{cdef}	179.6	165.6^{fghijk}	179.4^{ab}
16	7500	400	100	171.8^{abcde}	183.4	167.2^{efghi}	167.0^{bcdefg}
17	7500	400	200	178.6^{abcde}	177.5	171.2^{abcde}	164.9^{defg}
18	7500	400	400	149.4^{def}	179.7	163.2^{hijk}	173.0^{bcdef}
19	10000	50	100	207.7^{a}	163.8	172.1^{abcd}	164.8^{defg}
20	10000	50	200	175.6^{abcde}	179.0	175.9^{a}	166.1^{bcdefg}
21	10000	50	400	184.5^{abcd}	174.3	163.3^{hijk}	169.1^{bcdefg}
22	10000	100	100	188.3^{abc}	163.0	166.1^{fghijk}	164.0^{efg}
23	10000	100	200	185.7^{abcd}	166.7	166.6^{efghij}	178.1^{abc}
24	10000	100	400	176.4^{abcde}	177.0	173.8^{ab}	147.2^{h}
25	10000	400	100	172.1^{abcde}	169.0	161.2^{k}	161.6^{fg}
26	10000	400	200	170.0^{abcde}	165.9	163.7^{ghijk}	173.6^{bcdef}
27	10000	400	400	186.5^{abcd}	164.1	164.2^{ghijk}	159.7^{g}
Xylanase	0	---	---	131.2	116.9^{b}	133.2	106.6
	5000	---	---	176.1	174.7^{a}	164.2	172.5
	7500	---	---	168.8	179.67^{a}	169.2	172.5
	10000	---	---	183.0	169.2^{a}	167.4	164.9
Cellulase	---	0	---	131.2	116.9	133.2	106.6
	---	50	---	184.2	171.6	168.4	170.7
	---	100	---	169.7	176.5	167.1	171.7
	---	400	---	174.0	175.5	165.4	167.5
β-D glucanase	---	---	0	131.2	116.9	133.2	106.6
	---	---	100	182.0	174.0	165.7	169.7
	---	---	200	171.9	174.7	170.2	170.5
	---	---	400	173.9	174.9	165.0	169.6
SEM				**2.87**	**2.11**	**2.87**	**1.96**
P value							
Xylanase				0.034	0.037	0.001	0.001
Cellulase				0.025	0.418	0.001	0.051
β-D glucanase				0.136	0.971	0.001	0.875
Xylanase x Cellulase				0.263	0.333	0.001	0.003
Xylanase x β-D glucanase				0.027	0.640	0.001	0.001
Cellulase x β-D glucanase				0.357	0.421	0.001	0.015
Xylanase x Cellulase x β-D glucanase				0.038	0.612	0.001	0.006

Each value is the average of triplicate analysis, Means with different superscripts in a column differ significantly (*P<0.05*)

concentration, the various combinations with cellulase and β-D-glucanase were comparable, except lower values in combination 5 (xylanase-5000, cellulase-100 and β-D-glucanse-200 IU/kg) and 7 (xylanase-5000, cellulase-400 and β-D-glucanase-100 IU/kg). At 10000 IU/kg xylanase concentration, the highest sugar release was observed at combination 13 (xylanase-7500, cellulase-100 and β-D-glucanase-100 IU/kg), while other combinations were comparable. Of all the 27 combinations the highest sugar release was observed in combination 13 (xylanase-7500, cellulase-100 and β-D-glucanase-100 IU/kg) and the lowest sugar release was observed in combination 24 (xylanase-10000, cellulase-100, β-D-glucanase-400 IU/kg).

Discussion

Maize: The NSP content of maize as reported by various workers varied from 8.10 to 10.30% [7-11]. While, in barley the NSP content was higher (16.7 and 18.8%) [7,8,12]. In wheat NSP content varied from 11.4 -12.8% [7,8,12] and in rye varied from 13.2-16.10 % [8,13,14]. On the other hand, NSP content of maize was comparable to sorghum (5.6-11.0%) [7,8,9,12], and finger millet [9].

The total sugars released from maize with two stage *in vitro* digestibility assay without enzyme supplementation was 131.20 mg/g (Table-2). The NSP enzymes used *viz*., xylanase (5000,7500,10000IU/kg substrate), cellulase (50, 100, 400 IU/kg substrate), β-D-glucanase (100, 200, 400 IU/kg substrate) increased

(P<0.05) the total sugars release from the maize (142.81 to 207.7 mg/g), except for combination 2 (xylanase 5000, cellulase 50, β-D-glucanase 200), 4 (xylanase 5000, cellulase 100, β-D-glucanase 100), 5 (xylanase 5000, cellulase 100, β-D-glucanase 200) and 9 (xylanase 5000, cellulase 400, β-D-glucanase 400). The sugars release was highest in enzyme combination 3 (xylanase 5000, cellulase 50, β-D-glucanase 400) (207.4 mg/g) and 19 (xylanase 10000, cellulase 50, β-D-glucanase 100) (207.7 mg/g).

Similarly [10] reported higher sugars release from maize supplemented with various enzyme combinations with cellulase, xylanase and pectinase. The highest sugars release (315 mg/g) was observed with enzyme combination (cellulase 408, xylanase 2081 and pectinase 369 U/g enzyme) and (cellulase 405, xylanase 2066 and pectinase 369 U/g enzyme).

In vitro studies conducted by [15] revealed higher dose requirement of xylanase (60000 and 240000 IU/kg substrate) along with pectinase and glucanase for barley and rye. The enzyme dose used was higher than that used for maize, used in the present study and as reported by [10] which might be due to higher NSP content in barley and rye compared to maize. Similarly *in-vitro* studies with multiple enzyme addition (high, medium and low) to extruded and non- extruded corn based diets for pigs improved nutritional value in terms of coefficient of apparent digestibility (CAD), starch and NDF [16]. Further [17] also reported that reconstitution of maize with added enzymes improved pepsin pancreatic digestibility (IVPPD) 20% over control.

Soybean meal (SBM): The NSP content of soybean meal as reported by previous workers ranged from 17.1 to 30.3% [7-10,14]. However [11] reported low Non-starch Polysaccharides content in terms of NDF (11-64%), and ADF (7.25%). About 30% of NSP in SBM was galactose with glucose, uronic acids, arabinose and xylose [18]. Higher *in vitro* sugars release was reported with use of xylanase (1080 IU/kg), cellulase (10.8 IU/kg) and pectinase (10800 IU/kg) combination for soy bean meal (4.56 mg/ml) compared to control (3.39 mg/ml) [9].

The *in vitro* sugars release from soybean meal in the present study was 116.9 mg/g and ranged between 163.43-190.5 mg/g, when supplemented with various enzyme combinations (1-27) (Table-2). No significant (*P<0.05*) effect was observed between control and enzyme supplementation for soybean meal (Table-2). Though insignificant (39.35-63.55%) higher sugars release was observed with supplementation of xylanase (5000 to 10000 IU/kg substrate), irrespective of cellulase and β-D-glucanase. The dose rate of 5000 IU/kg was sufficient for soybean meal. The sugars release from soybean meal was in agreement with the sugars release extrapolated from the studies of [18].

The *in vitro* sugars release from soybean meal in studies of [10] was (360 mg/g) and the sugars release increased with supplementation of enzyme combination (cellulase 408, xylanase 2081 and pectinase 369 U/g of enzyme) and (cellulase 405, xylanase 2066 and pectinase 369 U/g of enzyme) followed by (cellulase 468, xylanase 2143 and pectinase 360 u/g of enzyme) and (cellulase 353, xylanase 1975 and pectinase 353 U/g of enzyme).

Sunflower cake: The NSP content of sunflower cake ranged from 21.0 to 55.2% [7-10,19,20]. The NSP content of sunflower cake was higher than rapeseed meal (18.8-32%) as reported by various workers [7,8,21,22].

Higher sugars release for enzyme combination was reported by [10]; (cellulase 408, xylanase 2081 and pectinase 369 U/g of enzyme) and (cellulase 405, xylanase 2066 and pectinase 369 U/g of enzyme) than (cellulase 468, xylanase 2143 and pectinase 360 U/g of enzyme), (cellulase 353, xylanase 1975 and pectinase 353 U/g of enzyme) and control. [9] reported that xylanase and cellulase combination was superior for sunflower meal than xylanase, cellulase and pectinase combination.

The sugars release from sunflower cake without any enzyme supplementation in the present study was 133.2 mg/g and was lower than the value reported by [10] (Table-2). NSP enzyme supplementation to sunflower cake resulted in increased (*P<0.01*) total sugars release compared to non supplemented group. The NSP enzymes supplemented to sunflower cake at various doses and combination, the *in vitro* sugars release was significantly (*P<0.01*) higher than control. Among various enzyme combinations comprising of xylanase, cellulase and β-D-glucanase tested, the xylanase combination consisting of (7500 or 10000 IU/kg), cellulase (50 IU/kg) and β-D-glucanase (200 IU/kg) recorded higher total sugars release (175.94 mg/g). The 27 combinations tested have shown considerable increase in total sugars released and ranged between 161.18 and 175.94 mg/g. The sugars release was higher at xylanase combination of 7500 and 10000 IU/kg. While for cellulase 50 and 100 IU/kg had comparatively higher sugars release than 400 IU and β-D-glucanase 200 IU was having highest sugars release.

Similarly [23] reported significant increase in the release of glucose, other reducing sugars and in organic phosphorous due to addition of enzyme mixtures to sunflower meal. [10] also reported improved sugars release in enzyme supplemented group. The influence of cellulase was clearly seen in the present study and was in accordance with the findings of the [9,10].

De-oiled rice bran (DORB): The *in vitro* sugars release from de-oiled rice bran (DORB) without enzyme supplementation was 106.6 mg/g and was much lower than the sugars release of the other ingredients (maize, soybean meal and sunflower cake) tested (116.9 to 133.2 mg/g). This might be due to higher NSP content in the DORB than other ingredients. The NSP content of DORB as reported by [9,11,24] was 53.54, 59.97 and 65.3%, respectively.

The *in vitro* digestibility of DORB with various enzyme combinations significantly improved sugars release (147.19 to 187.80 mg/g) than no enzyme supplementation (106.64 mg/g). The enzyme combination of xylanase (7500 IU/kg), cellulase (100 IU/kg), β-D-glucanase (100 IU/kg) yielded highest sugars release (187.80 mg/g) than other combinations tested and varied between 147.19 to 187.80 mg/g (Table-2).

The sugars release from DORB was highest (*P<0.01*) for 5000 and 7500 IU/kg concentration of xylanase compared to 10000 IU/kg and a trend of higher (*P<0.01*) sugars release was observed for 50 IU and 100 IU/kg compared to 400 IU/kg cellulase. No effect of β-D-glucanase was observed in tested range (100-400 IU/kg). Similarly, [9] reported that xylanase (1080 IU/kg), cellulase (10.8 IU/kg) and pectinase (10800 IU/kg) combination was superior to the other enzyme combinations having β-D-glucanase. *In-vitro* study conducted by [25] showed increased total sugar release of 36.97% to 53.64% from cocoa pod husk with addition of commercial extra cellular exogenous fibro lytic enzymes.

Conclusion

It is concluded that 'n' number of NSP enzyme combinations can be screened for their efficiency to digest NSPs present in various feed ingredients commonly used in poultry rations by employing two-stage *in vitro* digestibility assay as a tool.

Authors' contribution

JN and DN designed the experiment, implemented the design, analyzed data and prepared the manuscript; YRR and STVR revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The Authors are thankful to authorities of Sri Venkateswara Veterinary University for providing fund and infrastructure for this study.

Competing interests

The authors declare that they have no competing interest.

References

1. Rodica, Capriţa., Adrian, Capriţa. and Calin, Julean. (2010) Biochemical Aspects of Non-Starch Polysaccharides. *Animal Science and Biotechnologies* 43 (1) 368-375.
2. Carre, B. (1993) *European Symposium Poultry Nutrition, WPSA,* 5-9 September, Jelena Poland, pp. 148-1 63.
3. Bedford, M. R. and Classen, H. L. (1993) An *in vitro* assay for prediction of broiler intestinal viscosity and growth when fed rye-based diets in the presence of exogenous enzymes. *Poultry Science.* 72 : 137-143.
4. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers P. A. and Smith F. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*. 28 : 350-356.
5. Duncan, D. D. (1955) Multiple range and multiple ' F' test. *Biometrics*. 11:1-42.
6. Carre, B. and Brillouet, J. M. (1986) Yield and cell wall residues isolated from various feed stuffs used for non-ruminant farm animals. *Journal of Science Food Agriculture*. 37 : 341-351.
7. Classen, H. L. (1996) Enzymes in action. *Feed Mix.* 4 (2): 22-28.
8. Malathi, V. and Devegowda, G. (2001) *In vitro* evaluation of non starch polysaccharide digestibility of feed ingredients by enzymes. *Poultry Science.* 80: 302-305.
9. Chandra, Reddy (2003) Influence of enzyme supplementation on son-starch polysaccharide content in feed ingredients. M.V.Sc. Thesis ANGR Agricultural University, Hyderabad.
10. Bharathidasan, A., Chandrasekaran, D., Natarajan, A., Ravi, R., Viswanathan, K. and Ezhilvalavan, S. (2008) Non-starch polysaccharides and phytate phosphorus content of commonly available poultry feed ingredients. *Tamilnadu Journal of Veterinary Animal Sciences* 5: (1) 20-24.
11. Nyman, M. M., Siljestrom, B., Pedersen, K. E., Batchknudsen, N. E., Asp, C. G., Johansson, E. O. (1984). Dietary fibre content composition in six cereals at different extraction rates. *Cereal Chemistry.* 61 (1) : 14-19.
12. Englyst, H. (1989) Classification measurement of plant polysaccharides. *Animal Feed Science and Technology* 23 : 27-42.
13. Smits, C. H. M. and Annison, G. (1996) Non starch plant polysaccharides in broiler nutrition towards a physiologically valid approach to their determination. *World Poultry Science Journal.* 5: 203-221.
14. Castonan, J. I. R., Flores, M. P. and Petterson D. (1997) Mode of degradation of non-starch polysaccharides by feed enzyme preparations. *Animal Feed Science and Technolog.* 68: 361-365.
15. Li, Yong., Fang, Zhengfeng., Dai, Jinjun., Partridge, Gary., Ru, Yingjun and Peng, Jian (2010) Corn extrusion and enzyme addition improves digestibility of corn/soy based diet by pigs: In vitro and in vivo studies. *Animal Feed Science and Technology* 158(34):146-154.16.
16. Manvar, S. J. and Mandal, A. B. (2008) Availability of certain nutrients in cereals reconstituted with or without enzymes for chickens. *Journal of the Science of Food and Agriculture* 88: (4) 699–706.
17. Meng, X. and Slominski, B. A. (2005) Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. *Poultry Science.* 84: 1242–1251.
18. Dusterhoft, E. M. and Voragen A. G. J. (1991) Non starch polysaccharides from sunflower (*Helianthus annus)* meal and palm kernel (*Elaeis guineenis)* meal preparation of cell wall material and extraction of polysaccharide fractions. *Journal of Science Food and Agriculture* 55: 411-422.
19. Bharathidasan, A., Chandrasekaran, D., Natarajan, A., Viswanathan, K. and Ezhilvalavan, S. (2009) *In vitro* enzyme assay of commercial feed enzymes. *Tamilnadu Journal of Veterinary Animal Sciences* 5: (1) 20-24.
20. Slominski, B. A. and Campbell, L. D. (1990) Non-starch polysaccharides of canola meal: Quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. *Journal of Science Food and Agriculture* 53: 175-184.
21. Siddiqui, I. R. and Wood, P. (1997) Carbohydrates of rapeseed: A review. *Journal of Science Food and Agriculture* 28 : 530-538.
22. Ramesh, J. and Chandrasekaran, D. (2012) *In vitro* evaluation of feed grade enzymes. *Indian Veterinary Journal.* 89 (4) : 76-78.
23. Annison, G., Moughan, P. J. and Thomas, D. V. (1995) Nutritive activity of soluble rice bran arabinoxylans in broiler diets. *British Poultry Science* 36: 479-488.
24. Francis, Alemawor., Dzogbefia, V. P., Emmanuel, O. K. Oddoye., Oldham, J. H. (2009) Enzyme cocktail for enhancing poultry utilization of cocoa pod husk. *Scientific Research and Essay* 4 (6) pp. 555-559.

****