EVOLVING CUSTOMIZED NON-POLYSACCHARIDASE MIXTURE FOR EFFECTIVE UTILIZATION OF SORGHUM STOVER AND GROUNDNUT HAULM

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ABSTRACT

A study was undertaken with an objective of evolving substrate specific customized non-starch polysaccharidase mixture for effective utilization of sorghum stover and groundnut haulm. Enzyme activity(IU/g) assay revealed that the activity of cellulose, xylanase and pectinase were 1368.33±23.30, 2294.16±65.17 and 930.83±52.22 respectively. All enzymes were found to have associate activity of other enzymes. An invitro trial was conducted to identify the concentration range of individual non-starch polysaccharidase enzymes required for inclusion to sorghum stover and groundnut haulm for maximum hydrolysis. A second invitro trial was conducted to identify the precise concentration of individual non-starch polysaccharidase enzymes required for inclusion to sorghum stover and groundnut haulm for maximum hydrolysis. The third in vitro trial was conducted to optimize the concentration of non-starch polysaccharidase mixture for inclusion to sorghum stover and groundnut haulm for maximum hydrolysis. The efficacy of customized non-starch polysaccharidase mixture was further evaluated at three levels (viz the selected level, 10% higher than selected level and 10% lower than the selected level)to arrive at their optimal level of inclusion separately for sorghum stover and groundnut haulm.

Keywords: *Dry matter digestibility, enzymes, in vitro trials*

INTRODUCTION

Crop residues from cereal and oil seed crop harvesting, forms the staple feed for ruminant livestock in India. However the nutritive values of these crop residues are very poor. Even ruminants, despite their unique and highly efficient digestive system, are not able to extract sufficient energy from low quality highly lignified crop residues to grow or produce milk. To enhance the nutritive value and digestibility of crop residues, physical, chemical and biological treatments have been researched upon. Manipulation of rumen fermentation by using feed additives has been gaining popularity in ruminant nutrition in recent years. One such approach is the use of fibrolytic enzymes in treatment of crop residues to enhance their digestibility.

Many commercial Non-Starch Polysaccharidase (NSPase) enzyme preparations are marketed as feed supplements; however these vary widely in their type and concentration of enzymes they contain and are not substrate specific. Inaddition, the cocktail NSPase enzyme mixture available in the market are non-specific, unnecessary addition or absence of specific enzyme in cocktail enzymes is possible. Unwarranted high

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or low dose of required enzymes have also been observed leading to irrational supplementation. Hence there arises a need to select specific enzymes suited to specific substrates. Hence this study was carried out with the objective of identifying specific NSPase required for effective utilization of sorghum stover and groundnut haulm.

MATERIALS AND METHODS

Enzyme samples viz cellulase, xylanase and pectinase referred to as Non-Starch Polysaccharidase enzymes were procured from six different sources and assayed for their individual enzyme activity and associated activity by dinitrosalicylic acid (DNSA) reducing sugar method Miller (1959). Enzyme activity was calculated based on sugar release (glucose/xylose/ galacturonic acid) using the formula given below and was expressed as IU/g.

IU of cellulase/ xylanase/ pectinase per g =

Concentration of glucose/xylose/ galactouronicacid **X** dilution factor

Incubation time (120 mts.) **X**Molecular weight [glucose (180)/xylose(150)/ galacturonic acid (212.2)]

Based on the activity of the individual enzymes, three sets of in vitro trials were carried out with the intention to evolve substrate specific customized NSPase mixture for sorghum stover and groundnut haulm respectively. The first trial was conducted to locate the range of enzyme activity of individual enzyme required for maximum sugar release from sorghum stover or groundnut haulm. This was followed by another *in vitro* trial to precisely identify the enzymes viz cellulase, xylanase and pectinase needed individually for respective substrates and the third one was to identify the inclusion level of these enzymes in combination to sorghum stover or groundnut haulm. All these trials were conducted in duplicate in three runs.

IN VITRO TRIAL I

This trial was carried out to identify the minimum concentration of enzymes *viz* cellulase, xylanase and pectinase required to bring about maximum sugar release on incubation with sorghum stover or groundnut haulm as substrates at 39 °C for 24 hours. The procedure of Nsereko *et.al.* (2000) was adopted with regard to substrate quantity and incubation protocols. Whereas, to measure sugar release dinitrosalicylic acid (DNSA) reducing sugar method Miller (1959) was followed.

In order to arrive at the range of inclusion of cellulase, xylanase and pectinase to sorghum stover or groundnut haulm, a preliminary screening test was carried out. In the preliminary screening test, cellulase was added at graded concentrations of 400, 800, 1200, 1600, 2000, 2400 and 2800 IU/g to each sorghum stover or groundnut haulm while xylanase was added in graded concentrations of 50, 100, 150, 200, 250, 300 and 350 IU/g to sorghum stover or groundnut haulm. Pectinase was added to sorghum stover or groundnut haulm in graded concentrations of 200, 400, 600, 800 and 1000 IU/g substrate. The minimum concentration of enzymes that caused a maximum sugar release was identified.

IN VITRO TRIAL II

Since the previous experiment was conducted by incorporating individual NSP enzymes at graded concentration in wider range, this experiment was conducted by testing the respective NSP enzymes at narrow graded concentration level within the range identified earlier, to fix the precise level of inclusion of these enzymes to sorghum stover or groundnut haulm. In this experiment five graded concentrations (identified concentration, two levels below and two levels above the identified concentration) of addition of cellulase (increment of 100 IU/g substrate), xylanase (increment of 20 IU/g substrate) and pectinase (increment of

100 IU/g substrate) to sorghum stover/groundnut haulm were tested. The minimum concentration of enzymes added to sorghum stover/groundnut haulm that brought about maximum sugar release was considered as the selected level of enzymes.

IN VITRO TRIAL III

Based on the results of the previous *in vitro* trial, the third in vitro trial was designed. The selected concentration of enzymes (cellulase, xylanase and pectinase) for sorghum stover/ groundnut haulm was combined to form an enzyme mixture (NSPase mixture). The NSPase mixtures were evaluated at three levels to arrive at their optimal level of inclusion separately for sorghum stover and groundnut haulm. The three levels were 1. cellulase, xylanase and pectinase at selected concentration of 1200 IU/g, 100 IU/g and 700 IU/g for sorghum stover and 1600 IU/g, 100 IU/g and 600 IU/g for ground nut haulm. 2. cellulase, xylanase and pectinase at 10 per cent below selected concentration and 3. cellulase, xylanase and pectinase at 10 per cent above selected concentration. This in vitro trial was also carried out in duplicate in three runs. For this trial also, the procedure adopted by Nsereko *et.al.* (2000) was adopted with regard to substrate quantity and incubation protocol. Sugar release was assayed by dinitrosalicylic acid (DNSA) reducing sugar method Miller (1959). The levels of NSPase mixtures that evoked maximum sugar release for sorghum stover and groundnut haulm respectively was designated as customized NSPase enzyme mixture for respective crop residue and was taken for further validation studies.

RESULTS AND DISCUSSION

IN VITRO TRIAL I

The total sugar (µg/g) released (glucose/ xylose/galacturonic acid) on incubation at 39°C for 24 hours from sorghum stover/groundnut haulm with enzymes cellulase, xylanase and pectinase at graded concentrations in wide range so as to determine the approximate enzyme activity (IU/g) required for maximum hydrolysis is presented in Table 1. For sorghum stover, increasing the concentration of cellulase led to significant ($P<0.05$) increase in sugar release up to a level of 1200 IU/g. Thereafter additional increase in enzyme concentration did not result in any further increase in sugar release. In the case of groundnut haulm, sugar release increased significantly ($P<0.05$) and up to a concentration of 1600 IU/g and thereafter no further significant increase in sugar release was observed. As regard to xylanase, for both sorghum stover and groundnut haulm sugar release increased significantly ($P<0.05$) up to 100 IU after which the sugar release was consistently same. A similar trend was evident for pectinase. Sugar release increased significantly ($P<0.05$) up to 600 IU/g, then plateaus off. Since the interval between the graded concentrations of enzymes tested was wide, the values identified for the respective enzymes against the crop residue needs to be further tested at narrow interval on the located range.

IN VITRO TRIAL II

The total sugar released from sorghum stover and groundnut haulm on incubation with enzymes (cellulase, xylanase, pectinase) at graded concentration were measured and the results are presented in Tables 2, 3 and 4. With regard to cellulase significantly ($P<0.05$) highest sugar release from sorghum stover was evident when it was added at 1200 IU/g. Beyond 1200 1U of cellulase sugar release was consistent. As 1200 IU/g was the minimum concentration of cellulase that brought about maximum hydrolysis, it was chosen as the selected concentration for sorghum stover. In case of groundnut haulm cellulase added at 1600 IU/g resulted in significantly highest ($P<0.05$) sugar release and further increase in enzyme concentration resulted in similar sugar release. Therefore 1600 IU/g of cellulase was

selected, as it was the minimum concentration that brought about maximum hydrolysis.

Addition of xylanase at the concentration of 120 IU/g brought about significantly highest (P<0.05) sugar release from sorghum stover. Further increasing the concentration of xylanase, did not lead to any further increase in sugar release. For groundnut haulm significantly highest (P<0.05) sugar release was achieved on addition of xylanase at 100 IU/g No further increase in sugar release was observed on increasing the concentration of enzyme Thus for xylanase 120 IU/g with regard to sorghum stover and 100 IU/g with groundnut haulm were the selected concentration.

Pectinase when added to sorghum stover at 700 IU/g led to maximum hydrolysis and caused a significantly highest (P<0.03) sugar release. No further increase in sugar release was observed on increasing the enzyme concentration. Pectinase at 600 IU/g brought about significantly highest (P<0.05) sugar release with regard to groundnut haulm. Further increase in enzyme level caused no significant increase in sugar release. Hence for pectinase 700 IU/g in case of sorghum stover and 600 IU/g in case of groundnut haulm were the selected concentrations.

IN VITRO TRIAL III

In this trial, NSPase enzyme mixture constituting cellulase, xylanase and pectinase were tested at their selected levels, 10% lower than selected level and 10% higher than selected level for both sorghum stover and groundnut haulm. Significantly highest (P<0.05) sugar release was observed at the selected level of enzyme mixture, for both sorghum stover as well as for groundnut haulm (Table -5). Low addition of enzyme did not bring about maximum hydrolysis as more substrate was available that was not acted upon by enzyme. High level of enzyme inclusion also did not result in maximum hydrolysis owing to the fact that substrate was not available for the enzyme to act. The reason may be partly attributed to negative feedback inhibition wherein, the enzyme action is inhibited by production of a critical concentration of a product of the enzyme substrate interaction; also excessive enzyme application blocks binding sites for enzymes Andesogan (2005).The selected level of enzyme mixture for sorghum stover of cellulase is 1200 IU/g, xylanase 120 IU/g and pectinase 700 IU/g, whereas die selected level of enzyme mixture for groundnut haulm consists of cellulase 1600 IU/g, xylanase 100 IU/g and pectinase 600 IU/g.

CONCLUSION

From this study it can be concluded that for the effective utilization of sorghum stover an enzyme mixture containing cellulase, xylanase and pectinase at a concentration of 1200 IU/g, 100 IU/g and 700 IU/g would be very beneficial. A non-starch polysaccharidase mixture containing cellulase, xylanase and pectinase at a concentration of 1600 IU/g, 100 IU/g and 600 IU/g respectively would be very beneficial for effectively utilizing groundnut haulm.

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TABLE 1

Total sugar (µg/g) released (glucose/xylose/galacturonic acid) on incubation (39ºC for 24 hours) of sorghum stover or groundnut haulms with respective enzymes (cellulase/xylanase/pectinase) at graded concentrations in wide range to determine the approximate enzyme activity (IU/g) required for maximum hydrolysis (Mean ± SE)

Cellulase (IU/g)	Glucose released (µg/g)		Xylanase (IU/g)	Xylose released (µg/g)		Pectinase (IU/g)	Galacturonic acid (µg/g)	
	Sorghum stover	Groundnut haulm		Sorghum stover	Groundnut haulm		Sorghum stover	Groundnut haulm
400	1779.25 ± 84.76 ^{a}	483.50 ± 54.50 ^{a}	50	210.25 ± 25.25 ^{a}	183.5 ± 12.00 ^{a}	200	2502.25 ± 116.26 ^{a}	1129.00 ± 56.50 ^{a}
800	1971.5 ± 39.50 ^{ab}	739.75 ± 54.75 ^{b}	100	287.85 ± 7.65 ^{b}	204.05 ± 6.45 ^{ab}	400	2625.00 ± 127.51 ^{ab}	1489.00 ± 103.01 ^{b}
1200	2318.75 ± 77.76 ^{c}	924.25 ± 61.29 ^{b}	150	303.50 ± 6.50 ^{b}	213.5 ± 3.00 ^{ab}	600	2777.25 ± 117.76 ^{abc}	1902.00 ± 81.01 ^{c}
1600	2305.00 ± 85.0 ^{c}	1473.50 ± 55.50 ^{c}	200	300.40 ± 4.90 ^{b}	216.10 ± 4.10 ^{ab}	800	2976.75 ± 125.76 ^{bc}	1913.25 ± 76.76 ^{c}
2000	2330.00 ± 30.00 ^{c}	1585.00 ± 65.00 ^{c}	250	300.20 ± 10.00 ^{b}	209.35 ± 9.15 ^{ab}	1000	3107.25 ± 79.26 ^{c}	1942.85 ± 52.65 ^{c}
2400	2230.00 ± 180.02 ^{bc}	1555.5 ± 34.50 ^{c}	300	305.50 ± 9.50 ^{b}	210.3 ± 15.30 ^{ab}			
2800	2242.00 ± 92.51 ^{bc}	1436.50 ± 60.50 ^{c}	350	302.85 ± 7.35 ^{b}	217.25 ± 7.25 ^{b}			

Mean of four samples $^{a\,b\,c}$ Mean values bearing different superscripts differ significantly (P<0.05).

TABLE 2

Total glucose (µg/g) released from sorghum stover and groundnut haulm on incubation (39 ºC for 24 hours) with cellulase at narrow graded concentrations to determine the accurate enzyme activity (IU/g) required for maximum hydrolysis of respective crop residue (Mean * ± SE).

Sorghum stover		Groundnut haulm	
Cellulase (IU/g)	Glucose	Cellulase (IU/g)	Glucose
1000	2030.75 ± 83.69 ^{a}	1400	1075± 60.69 ^{a}
1100	2031.75 ± 55.54 ^{a}	1500	1480 ± 76.51 ^{b}
1200	2322.25 ± 78.59 ^{b}	1600	1720 ± 128.85 ^{c}
1300	2221.75 ± 54.26 ^{b}	1700	1617± 128.85 ^{c}
1400	2232.50 ± 56.77 ^{b}	1800	1615 ± 60.33 ^{c}

*Mean of six samples $^{a\,b\,c}$ Mean values in column bearing different superscripts differ significantly (P<0.05).

TABLE 3

Total xylose (µg/g) released from sorghum stover and groundnut haulm on incubation (39ºC for 24 hours) with xylanase at narrow graded concentrations to determine the accurate enzyme activity (IU/g) required for maximum hydrolysis of respective crop residue (Mean ± SE)

Xylanase (IU/g)	Sorghum stover	Groundnut haulm
60	218.00 ± 15.95 [a]	141.00 ± 18.91 [a]
80	225.75 ± 15.22 [a]	145.75 ± 11.57 [a]
100	268.25 ± 7.72 [ab]	223.12± 15.38 [b]
120	321.25 ± 21.32 [c]	218.75 ± 10.48 [b]
140	316.37 ± 21.01 [c]	211.25 ± 10.90 [b]

Mean of six samples.

[a b c] Mean values in column bearing different superscripts differ significantly (P<0.05).

TABLE 4

Total galacturonic acid (µg/g) released from sorghum stover and groundnut haulm on incubation (39ºC for 24 hours) with pectinase at narrow graded concentrations to determine the accurate enzyme activity (IU/g) required for maximum hydrolysis of respective crop residue (Mean * ± SE).

Sorghum stover		Groundnut haulm	
Pectinase (IU/g)	Galacturonic acid	Pectinase (IU/g)	Galacturonic acid
400	1605.7 ± 148.43 [a]	400	1432.68 ± 42.65 [a]
500	2754.99 ± 90.47 [b]	500	1513.72 ± 35.56 [a]
600	2839.61 ± 63.59 [b]	600	1887.01 ± 57.627 [b]
700	3147.68 ± 68.66 [c]	700	1659.15± 51.02 [b]
800	3055.44 ± 49.66 [c]	800	1795.32 ± 49.46 [b]

*Mean of six samples.

[a b c] Mean values in column bearing different superscripts differs significantly (P<0.05).

TABLE 5

Total sugars (reducing sugars in µg/g) released from sorghum stover or groundnut haulm on incubation (39^{0}C for 24 hours) with enzyme mixture at three levels (Mean * ± SE).

Treatment group	Sorghum stover	Groundnut haulm
10 % Lower than selected level	770.83 ± 80.18 [a]	995.25 ± 12.72 [a]
Selected	1328.22 ± 60.2 [b]	1408.12 ± 103.8 [b]
10 % Higher than selected level	1290.52± 91.13 [b]	1477.36± 66.67 [b]

* Mean of six samples

[a b] Mean values in column bearing different superscripts differs significantly (P<0.05).