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Generation of fibrolytic enzymes using paddy husk as substrate and identifying corresponding crop residues for the generated fibrolytic enzymes*

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ABSTRACT

A study was carried out to produce cellulase and xylanase using paddy husk as substrate and to match the enzymes produced with sorghum stover, paddy straw and sugarcane tops. For optimization of conditions for growth of *Trichoderma viridae* in paddy husk, the experiment was conducted in a factorial design in nine replicates, with 2 pH levels (5, 7), 2 moisture levels (50 and 70%) and 3 incubation periods (10, 15 and 20 days). Cellulase and xylanase activity declined as the incubation period increased irrespective of pH and moisture level. Based on the results, the optimum conditions identified was 10 days incubation, pH 5 and moisture of 50%. The level of enzymes for sorghum stover, paddy straw and sugarcane tops identified for the *in vitro* degradability study considered practical feasibility of inclusion of *Trichoderma viridae* fermented paddy husk to crop residues. First the level of crude enzyme extract to be supplemented was identified and next the possibility of supplementing *Trichoderma viridae* fermented paddy husk to the crop residues *in lieu* of crude enzyme extracts was examined. It was concluded that 2.03 U (Units) of cellulase and 2.73 U of xylanase was required per gram of paddy straw or sorghum stover and 3.05 U of cellulase and 4.10 U of xylanase was required per gram of sugarcane tops to augment their degradability. Supplementation of *Trichoderma viridae* fermented paddy husk as such to the crop residues was beneficial compared with extracting enzymes and supplementing.

Key words: Degradability, Fibrolytic enzymes, Paddy, Sorghum stover, Sugarcane tops, *Trichoderma viridae*

India being a predominantly agricultural country has abundant agro-industrial fibrous by-products. The use of enzymes to attack the lignocellulose structure of these crop residues for enhancing their feeding value has been attractive. Use of exogenous fibre-degrading enzymes may be a potential means of increasing the nutritive value of crop residues, as enzyme costs are expected to decline in the future with recent developments in fermentation technology and alternative enzyme production systems (Beauchemin *et al.* 2004). The cocktail non - starch polysaccharidase enzyme mixture available in the market are indented to European feed stuffs and do not serve crop residues. Further since they are non-specific, unnecessary or absence of specific enzyme in cocktail enzymes is possible. Unwarranted high 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. However, evolving substrate specific customized enzyme mixture demands availability of individual enzymes in the market. Apart from availability in the market, the prohibitive cost of such individual enzymes is another issue that needs to be addressed to solve the aforesaid problems. The only way out is to produce such enzyme mixture at farms itself. To increase the on-farm efficacy of ruminant enzyme technology, research needs to be carried out to decrease the cost of enzyme production, make enzyme supplementation more practically feasible. With these facts, the study was carried out to explore the possibility of using paddy husk as a substrate to generate fibrolytic enzymes and identify corresponding crop residues for the generated fibrolytic enzymes.

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MATERIALS AND METHODS

Optimization of conditions conducive for Trichoderma viridae *growth in paddy husk:* In this experiment the conditions for growth of *Trichoderma viridae* in paddy husk as substrate were optimized. The experiment had 2 pH levels (5, 7); 2 moisture levels (50 and 70%) and 3 incubation periods (10, 15 and 20 days). Thus the experiment was conducted in a $2 \times 2 \times 3$ factorial design in 3 replicates. The method used in this study for the production of enzymes was the solid state fermentation (Pang *et al.* 2006). Paddy husk

(5 g) were taken in individual flasks and were plugged with non-absorbent cotton and autoclaved at 121ºC for 15 min. Inoculum containing the spore density of 10^6 spores/ml of substrate was aseptically inoculated (1 ml per 5 g substrate) into each flask containing the substrate. The flasks were incubated at 30ºC for 10 days in an incubator. To control the humidity of the incubator an additional flask filled with water was placed inside (Ustoka and Tarib 2007). At the end of the incubation period the fibrolytic enzymes from the respective substrates were extracted by a simple contact method (Krishna and Chandrasekaran 1996). The fermented samples were shaken (150 rpm) with 50 ml of 0.1 M sodium acetate buffer of pH 4.8 at 30ºC for 1h and filtered through Whatman (No1). The filtrates were centrifuged at 10,000 rpm (4ºC) for 15 min to remove spores of the organism and supernatants were used as crude enzyme extracts for enzyme assay. The crude enzyme extracts were assayed for cellulase and xylanase activities as per di nitro salicylic acid (DNSA) reducing sugar method (Miller 1959). Enzyme activity was calculated based on sugar release (glucose/xylose) and was expressed as U/5 g of substrate. The conditions (pH,% moisture and incubation period) that led to maximum production of cellulase and xylanase were selected as the optimum conditions for growth of *Trichoderma viridae* in paddy husk.

In vitro *study to match the enzyme produced from paddy husk as substrate at optimised conditions with crop residues:* This *in vitro* study was focused to match the enzyme produced from paddy husk as substrate at the optimized conditions with crop residues so as to enhance the degradability of the crop residues. The crop residues identified for this experiment were sorghum stover, paddy straw and sugarcane tops. Three samples each of these crop residues were collected and estimated for their moisture content (AOAC 2000), dried to constant weight in a hot air oven, ground to pass through 1 mm sieve and stored in air tight containers for further analysis. The samples were analysed for their NDF and ADF content as per Goering and Van Soest (1970). The level of enzymes to be supplemented to these crop residues was identified. Two experiments were carried out in this study. The first experiment was to determine the level of supplementation of the enzyme extract to crop residues and for this, the crude enzyme extract produced from paddy husk as substrate at the optimized conditions was used. The second experiment was to examine the possibility of supplementing the *Trichoderma viridae* fermented paddy husk as such to the crop residues, viz. sorghum stover, paddy straw and sugarcane tops in lieu of crude enzyme extracts.

Experiment 1: To determine the level of supplementation of crude enzyme extract to crop residues: In the first experiment, 3 levels of crude enzyme extract was tested to each of the 3 crop residues (sorghum stover, paddy straw and sugarcane tops) to identify the lowest level of crude enzyme supplementation required to maximise *in vitro* dry matter digestibility (IVDMD). Three levels of crude enzyme extracts were identified keeping in mind the scope for practical feasibility on the inclusion of *Trichoderma viridae* fermented substrates containing crude enzyme extracts to crop residues by farmers. Since farmers feed (on as fed basis) either paddy straw or sorghum stover at 5 kg /day/animal or 20 kg of sugarcane tops/day/animal, it is possible to add about 1 kg of *Trichoderma viridae* fermented substrates containing crude enzymes to these crop residues while feeding their animals. To determine the appropriate level of crude enzyme supplementation, 1 level lower (0.5 kg *Trichoderma viridae* fermented substrate), assumed level (1 kg *Trichoderma viridae* fermented substrate) and 1 level higher (1.5 kg *Trichoderma viridae* fermented substrate) were experimented. These 3 levels will henceforth be referred as level-1, level-2 and level-3. These 3 levels of crude enzyme extract to 3 crop residues was tested for *in vitro* dry matter degradability and compared with the respective control diet that does not contain enzyme.

The *in vitro* dry matter degradability was determined as per the procedure of Tilley and Terry (1963) using rumen liquor from cattle. The experiment was carried out in duplicate using 3 samples of each of the crop residue resulting in 6 measurements for each treatment. Rumen liquor was collected from 3 cows immediately on slaughter from the slaughter house. The pooled contents were transferred to a thermos cud transport container with flushing of carbon dioxide and brought to the laboratory. Ruminal fluid was filtered through 4 layers of muslin cloth and stored in pre warmed thermos container at 39°C till its use.

Accurately weighted (1.5 g) crop residues in plastic tube with lid fitted with a one-way Bunsen gas release valve was incubated with 120ml of McDougall's buffer solution followed by 30ml of the strained rumen liquor and crude enzyme extract (ml) containing the respective levels of enzymes. To determine the quantity of enzyme extracts to be added to 1.5 g of crop residues at 3 levels mentioned earlier, calculations were made for the corresponding levels of enzymes that could be extracted from 3 levels, viz. 0.5 kg, 1.0 kg and 1.5 kg of *Trichoderma viridae* fermented substrate and appropriate quantity of crude enzyme extract were added to the respective crop residues. The method of calculation to derive the quantity of crude enzyme extract to be added in lieu of 0.5 kg, 1.0 kg and 1.5 kg of *Trichoderma viridae* fermented substrate to 1.5 g of crop residues to be incubated in Tilley and Terry (1963) method is furnished in Table 1. The IVDMD was calculated and expressed in terms of dry matter basis. Based on the results of this experiment the level of supplementation of enzymes to the respective crop residues was selected.

Experiment 2: To examine the possibility of supplementing Trichoderma viridae *fermented paddy husk to crop residues:* This experiment was carried out with the objective of assessing the scope of supplementing *Trichoderma viridae*

Table 1. Method for calculating the crude enzyme extract supplementation to the crop residue for feeding *Trichoderma viridae* fermented substrate

Crop residues	Level of feeding on as fed basis in kg/day/adult cow by the farmers	Corresponding level of feeding on dry matter basis in kg/day/adult cow	Three levels of *Trichoderma viridae* fermented substrate proposed for testing to each crop residue (kg/day/adult cow)	Equivalent amount of *Trichoderma viridae* treated substrate to 1.5 g of respective crop residue	Cellulase activity (U/g) in the corresponding level of *Trichoderma viridae* treated substrate	Volume (ml) of crude enzyme extract required to achieve respective *Cellulase* activity	*Xylanase* activity (U) in the corresponding volume of crude enzyme extracted
Column	1	2	3	4	5	6	7
Sorghum stover	5	(Col 1×DM of crop residue)/100	0.5 (Level-1)	(1.5 × % dry matter in dried and ground crop residues × Col 3) / Col 2	(Col 4 × *cellulase* activity in selected condition)	(Col 5 × volume of filtrate buffer containing crude enzymes extracted from 5 g substrate) / (*Cellulase* activity in selected condition × quantity of substrate fermented)	(Col 6 × X*ylanase* activity in selected condition × quantity of substrate fermented substrate) / volume of filtrate buffer containing crude enzymes extracted from 5 g substrate
Paddy straw	5		1.0 (Level-2)				
Sugarcane tops	20		1.5 (Level-3)				

fermented husk along with crude enzyme instead of extracting the crude enzyme and supplementing to crop residues. The promising results of first experiment formed the basis for fixing the level of enzyme supplementation. According to the level of supplementation of enzyme identified, the respective quantity of fermented substrate was directly used by skipping the enzyme extraction procedure. The experiment was carried out in duplicate using 3 samples of each of the crop residue. The *% in vitro* dry matter degradability and fibre degradability of crop residues was determined by supplementing with the selected level of crude enzyme extract or supplementing with the respective quantity of fermented substrate as per Tilley and Terry, (1963) using rumen liquor from cattle. The procedure adopted was similar to that of experiment I.

RESULTS AND DISCUSSION

Optimization of conditions conducive for Trichoderma viridae *growth in paddy husk:* Table 2 gives details of *cellulase* and *xylanase* production from paddy husk inoculated with *Trichoderma viridae* and incubated at varied intervals (10–20) days at varied moisture (50/70%) and pH (5/7).

Cellulase and xylanase activity was found to decline as the incubation period increased irrespective of pH and moisture level in the paddy husk. The cellulase activity was significantly highest ($P<0.05$) at pH 7 and 70% moisture on the 10th day of incubation. A comparable but slightly lower cellulase production was observed in the 10th day for pH 5 and 50% moisture. The *xylanase* activity was significantly highest ($P<0.05$) at pH 7 and 50% moisture on 15th day of incubation and at pH 5 and 50% moisture, pH 7 and 70% moisture on the 10th day of incubation. For translating from lab to land, the crude enzyme extract production by inoculating with *Trichoderma viridae* at the rate of 10^6 spores per 5 g in paddy husk and incubating for 10 days, the conducive environment considered was pH 5 and moisture of 50%.

In vitro *study to match the enzyme produced from paddy husk as substrate at optimised conditions with crop residues:* The NDF values were 64.37±0.28, 73.73±1.20 and 77.07±1.01, respectively, for sorghum stover, paddy straw and sugarcane tops. The respective values for ADF in sorghum stover, paddy straw and sugarcane tops were 37.72±0.98, 48.29±1.02 and 43.14±2.01.

Experiment 1: To determine the level of supplementation of crude enzyme extract to crop residues: The calculations made for the corresponding levels of enzymes that could be extracted from 3 levels viz. 0.5 kg, 1.0 kg and 1.5 kg of *Trichoderma viridae* fermented substrate and appropriate quantity of crude enzymes extract that were added to 1.5 g of the respective crop residues is presented in Table 3.

Table 2. Cellulase and xylanase activity (U) per gram of paddy husk on inoculation with 10^6 spores of *Trichoderma viridae*

Treatment	Incubation period in days					
	Cellulase			*Xylanase*		
	10	15	20	10	15	20
pH 5 and 50% Moisture	13.56 $^{ab\pm}$0.69	9.65 $^{cd\pm}$1.23	7.49 $^{de\pm}$0.96	18.26 $^{a\pm}$0.54	14.55 $^{cd\pm}$1.31	12.07 $^{de\pm}$1.20
pH 5 and 70% Moisture	12.95 $^{ab\pm}$0.50	7.25 $^{de\pm}$1.16	5.94 $^{e\pm}$1.23	15.13 $^{bc\pm}$0.42	9.95 $^{ef\pm}$1.17	7.77 $^{f\pm}$0.96
pH 7 and 50% Moisture	11.54 $^{bc\pm}$1.03	13.15 $^{ab}\pm$1.19	8.84 $^{cde\pm}$1.06	15.42 $^{abc\pm}$0.82	18.33 $^{a}\pm$1.12	17.44 $^{ab\pm}$1.13
pH 7 and 70% Moisture	15.63 $^{a\pm}$1.05	6.21 $^{e}\pm$0.82	0.19 $^{f\pm}$0.11	18.25 $^{a\pm}$1.06	8.33 $^{f}\pm$0.41	3.15 $^{g\pm}$0.52

*Mean of 9 observations. Mean values bearing different superscripts in rows within cellulase or xylanase differ significantly (P<0.05).

Table 3. Calculation of the crude enzyme extract supplementation with crop residue for feeding *Trichoderma viridae* fermented substrate

crop residues Column Sorghum stover	Feeding level on as fed basis in kg/ day/adult cow by the farmers	Corresponding Feeding level on dry matter basis in kg/day/ adult cow	Three levels of *Trichoderma viridae* fermented substrate proposed for testing to each crop residue (kg/day/adult cow)	Equivalent amount of *Trichoderma viridae* treated substrate to 1.35* g of respective crop residue (1.35* × Col 3) / Col 2	*Cellulase* activity (U) in the corresponding level of *Trichoderma viridae* treated substrate (Col 4 × 13.56**)	Volume (ml) of crude enzyme extract required to achieve respective *Cellulase* activity (Col 5 × 30***) / (13.56** × 5****)	*Xylanase* activity (U) in the corresponding volume of enzyme extracted (Col 6 × 18.26 *****×5****) / 30***
Column	1	2	3	4	5	6	7
Sorghum	5	4.5	0.5 (Level-1)	0.15	2.03	0.90	2.73
straw	5	4.5	1.0 (Level-2)	0.30	4.02	1.80	5.41
	5	4.5	1.5 (Level-3)	0.45	6.10	2.70	8.21
Paddy	5	4.5	0.5 (Level-1)	0.15	2.03	0.90	2.73
straw	5	4.5	1.0 (Level-2)	0.30	4.02	1.80	5.41
	5	4.5	1.5 (Level-3)	0.45	6.10	2.70	8.21
Sugarcane	20	6	0.5 (Level-1)	0.11	1.52	0.68	2.05
tops	20	6	1.0 (Level-2)	0.22	3.05	1.35	4.10
	20	6	1.5 (Level-3)	0.34	4.58	2.03	6.14

Dry matter was considered 90% in paddy straw and sorghum stover and 30% in sugarcane top; *quantity of crop residue tested for IVDMD was 1.5g, which is equivalent to 1.35g on dry matter basis;** cellulase activity in crude enzyme extracted from per g of paddy husk vide Table 6, first row second column;*** volume of filtrate buffer containing extracted enzymes from 5 g substrate vide chapter 4.1;****quantity of paddy husk inoculated; ***** xylanase activity in crude enzyme extracted from per g of paddy husk vide Table 7, first row second column.

The column 6 of Table 3 indicated the volume of crude enzyme extract to be supplemented to the respective crop residues. It is evident that in a given volume of crude enzyme extract, the *xylanase* activity is proportionately higher than *cellulase* and therefore crop residues higher in xylan are expected to be degraded relatively higher than those crop residues having higher level of cellulose.

The *% in vitro* dry matter degradability of crop residues (sorghum stover, paddy straw and sugarcane tops) supplemented with varying levels of crude enzyme extract is presented in Table 4.

The *in vitro* dry matter degradability of sorghum stover was significantly (P<0.05) highest at level-2 of crude enzyme extract supplementation (cellulase 4.02 and *xylanase* 5.41 U), however level-1 of crude enzyme extract supplementation (cellulase 2.03 and xylanase 2.73 U) also showed comparable *in vitro* dry matter degradability. Level-3 crude enzyme extract supplementation improved *in vitro* dry matter degradability over control but not the extent of level-1 of supplementation. The % improvement in *in vitro* dry matter degradability over that of control were calculated for the different crop residues supplemented with varying levels of crude enzyme extract. For sorghum stover the % improvement in *in vitro* dry matter degradability over control was 22.96, 17.73 and 6.5, respectively, for level-2, level-1 and level-3 supplementation of crude enzyme extract. Overall

Table 4. *In vitro* dry matter degradability (% DMB) of crop residues on supplementation with different levels of crude enzyme extract

Crop Residues	Level of enzyme supplementation	Enzyme	Enzyme activity (U)	% IVDMD
Sorghum stover	Control	Cellulase	0.00	44.17 ^{c}±1.47
		Xylanase	0.00	
	Level-1	Cellulase	2.03	61.90 ^{ab}±7.85
		Xylanase	2.73	
	Level-2	Cellulase	4.02	67.13 ^{a}±1.29
		Xylanase	5.41	
	Level-3	Cellulase	6.10	50.67 ^{b}±1.45
		Xylanase	8.21	
Paddy straw	Control	Cellulase	0.00	39.35 ^{b}±1.33
		Xylanase	0.00	
	Level-1	Cellulase	2.03	52.48 ^{a}±2.99
		Xylanase	2.73	
	Level-2	Cellulase	4.02	43.32 ^{b}±2.96
		Xylanase	5.41	
	Level-3	Cellulase	6.10	45.54 ^{b}±3.60
		Xylanase	8.21	
Sugarcane tops	Control	Cellulase	0.00	47.41 ^{b}±1.32
		Xylanase	0.00	
	Level-1	Cellulase	1.52	51.54 ^{b}±1.47
		Xylanase	2.05	
	Level-2	Cellulase	3.05	70.33 ^{a}±1.37
		Xylanase	4.10	
	Level-3	Cellulase	4.57	52.87 ^{b}±3.25
		Xylanase	6.14	

*Mean of 6 observations. Mean values bearing different superscripts in columns differ significantly (P<0.05) within the respective crop residue.

crude enzyme extract supplementation improved *in vitro* dry matter degradability of sorghum stover at significant (P<0.05) level over control. Since supplementation at lower level with comparable improvement in *in vitro* dry matter degradability to higher level of crude enzyme extract supplementation is desirable from the economic point of view, supplementation of level-1 with cellulase 2.01 and xylanase 2.73 U is recommended.

In paddy straw *in vitro* dry matter degradability was significantly (P<0.05) highest at level-1 (cellulase 2.03 and xylanase 2.73 U). The % improvement in *in vitro* dry matter degradability over control was 13.13, 6.19 and 3.97, respectively, for level-1, level-3 and level-2 supplementation of crude enzyme extract. Level-3 and level-2 supplementation of crude enzyme extract did not improve *in vitro* dry matter degradability of paddy straw compared to control.

Sugarcane tops revealed significantly (P<0.05) highest *in vitro* dry matter degradability at level-2 (cellulase 3.05 and *xylanase* 4.10 U) of crude enzyme extract supplementation. The % improvement in *in vitro* dry matter degradability over control for sugarcane tops was 22.92, 5.46 and 4.13, respectively, for level-2, level-3 and level-1 supplementation of crude enzyme extract. Level-3 and level-2 supplementation of crude enzyme extract did not improve *in vitro* dry matter degradability of sugarcane tops compared to control.

Similar to the findings in this study Wang (1998) observed that treatment of maize stover with an enzyme product, prepared from *Trichoderma viride*, reduced the contents of some cell wall components and enhanced the ruminal digestibility in sheep. Tricarico and Dawson (1999) also reported that the addition of xylanase and cellulase enzyme preparations improved the *in vitro* ruminal digestion of fescue hay. Tang *et al*. (2008) also reported that fibrolytic enzyme supplementation enhanced IVDMD and IVOMD for 4 types of cereal straws, with the significance of this effect being dependent on the level of supplemented enzymes. Mohammadabadi and Chaji (2010) also reported that enzyme treatment increased *in vitro* digestibility of DM, NDF and OM for sugarcane tops.

From the results of this experiment level-1 (cellulase 2.03 and xylanase 2.73 U) was the selected level of supplementation of crude enzyme extract to both paddy straw and sorghum stover and level-2 (cellulase 3.05 and xylanase 4.10 U) was the selected level of supplementation of crude enzyme extract to sugarcane tops.

Experiment 2: To examine the possibility of supplementing Trichoderma viridae *fermented substrate to crop residues:* This experiment was carried out based on the results of experiment 1 wherein level-1 (cellulase 2.03 and xylanase 2.73 U) supplementation of crude enzyme extract to either paddy straw or sorghum stover and level-2 (cellulase 3.05 and xylanase 4.10 U) supplementation of crude enzyme extract to sugarcane tops were identified as the optimum inclusion level. Hence in this experiment the crop residues were tested again by supplementing with the respective level of crude enzyme extract and compared with equivalent quantity of *Trichoderma viridae* fermented substrate through *in vitro* degradability of dry matter, NDF and ADF (Table 5).

There existed no significant (P>0.05) variation between *in vitro* degradability of dry matter on supplementation with crude enzyme extract or *Trichoderma viridae* fermented paddy husk in sorghum stover and paddy straw. However in sugarcane tops crude enzyme extract supplementation had significantly (P<0.05) higher *in vitro* degradability of dry matter compared to supplementation with *Trichoderma viridae* fermented paddy husk.

Similarly no significant (P>0.05) difference existed in *in vitro* degradability of NDF and ADF on supplementation with crude enzyme extract or *Trichoderma viridae* fermented paddy husk in all crop residues, viz. sorghum stover, paddy straw and sugarcane tops. However, *in vitro* degradability of NDF and ADF of sugarcane tops was relatively higher in crude enzyme extract supplementation compared to

Table 5. Comparative *in vitro* degradability of dry matter (% DMB) of crop residues on supplementation with crude enzyme extract or *Trichoderma viridae* fermented paddy husk (mean*±SE)

Crop residues Level of crude enzyme extract supplementation	Sorghum stover Level-1		Paddy straw Level-1		Sugarcane tops Level-2	
Enzyme	Cellulase	Xylanase	Cellulase	Xylanase	Cellulase	Xylanase
Enzyme activity (U)	2.03	2.73	2.03	2.73	3.05	4.10
Equivalent amount of *Trichoderma viridae* treated substrate to 1.5 g of respective crop residue (vide Table 8, Column 4)	0.15		0.15		0.225	
% IVDMD						
Form of supplementation : Crude enzyme extract	61.90±7.85		52.48±2.99		70.33±1.37 [a]	
Form of supplementation : *Trichoderma viridae* fermented paddy husk	64.78±3.96		51.56±2.01		61.87±3.01 [b]	
% in vitro degradability of NDF						
Form of supplementation : Crude enzyme extract [NS]	41.62±1.59		47.77±2.11		50.50±1.74	
Form of supplementation : *Trichoderma viridae* fermented paddy husk [NS]	39.93±0.56		48.21±0.44		47.37±0.68	
% in vitro degradability of ADF						
Form of supplementation : Crude enzyme extract [NS]	20.69±1.93		28.92±1.03		29.48±1.34	
Form of supplementation : *Trichoderma viridae* fermented paddy husk [NS]	21.41±6.70		30.00±0.32		25.17±0.84	

*Mean of 6 samples. Mean values bearing different superscripts in rows differ significantly (P<0.05) within the respective crop residue and between form of supplementation. NS: Nonsignificant (P>0.05).

supplementation with *Trichoderma viridae* fermented paddy husk.

The findings of this study concur with that of Salman *et al*. (2008) who reported *Trichoderma viride* treated sugar beet pulp as a supplement in goat rations at 0.9% (w/w) to complete feed mixture improved the digestion coefficients percentage of dry matter, moisture, organic matter and crude fibre. Similar to the finding of this study, Zinn and Salinas (1999) also reported that a rumen-stable fibrolytic enzyme supplement increased the ruminal digestion of NDF by 23% in a cattle fed a 35% forage diet.

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