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Short Communication

In vitro Evaluation of Total Mixed Rations Supplemented with Exogenous Fibrolytic Enzymes and Live Yeast Culture

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ABSTRACT

An *in vitro* experiment was conducted to study the effect of supplementation of exogenous fibrolytic enzyme (EFE) or live yeast culture or both in total mixed rations on digestibility of nutrients. The dietary treatments included a groundnut haulms based total mixed ration (TMR) with R: C ratio of 70: 30 (T_1), T_1 supplemented with EFE @ 15 g/animal/d (T_2), T_1 supplemented with live yeast culture @ 10 g/animal/d (T_3) and T_1 supplemented with EFE @ 15 g/animal/d and live yeast culture @ 10 g/animal/d (T_4). Results indicated that the *in vitro* digestibility (%) of DM, CP, NDF and ADF were lower ($P<0.05$) in T_1 as compared to other treatments. Further, the *in vitro* digestibility (%) of DM, CP, NDF and ADF increased linearly from T_2 to T_4, but the differences were not significant ($P>0.05$). It is concluded that supplementation of either EFE or yeast culture or both increased the *in vitro* digestibility of nutrients.

Key words: Exogenous fibrolytic enzymes, Yeast culture, *In vitro* digestibility

INTRODUCTION

Leguminous crop residues like groundnut haulms (GNH) left after harvesting the nuts are rich in protein, calcium and phosphorous content (Murthy *et al*., 2001). However, the high fibre content limits its use as sole source of roughage for ruminants. In recent years, either yeast culture or fibrolytic enzymes have been tried to improve the nutritive value of poor quality roughages. The benefits associated with yeast culture (*Saccharomyces cerevisiae*) include increased DMI (Kishan Kumar and Ramana, 2008), increased growth rates (Srinivas Kumar *et al*., 2010) and improved DM and NDF digestibility (Raj Kiran *et al*., 2014). The use of fibrolytic enzymes as feed additives have been shown to improve fibre degradation under *in vitro* (Rajamma *et al*., 2015), *in sacco* (Bassiouni *et al*., 2011; Rajamma *et al*., 2014) and *in vivo* (Gaafar *et al*., 2010) conditions. However, very little information is available on the additive effect of fibrolytic enzymes and yeast culture on poor quality roughages (Can *et al*., 2007; Tang *et al*., 2008). Hence, the current experiment was designed to investigate the effect of supplementation of fibrolytic enzymes and/or live yeast culture in GNH based total mixed rations on *in vitro* digestibility of nutrients.

MATERIALS AND METHODS

Groundnut haulms used as basal roughage was oven dried at 70°C and then ground in Willey mill using 1 mm sieve. Total mixed ration (around 12% CP) containing roughage: concentrate ratio of 70:30 was prepared using groundnut haulms as roughage source. The percent composition of concentrate mixture is presented in table 1.

The four dietary treatments comprise of atotal mixed ration (TMR) supplemented with exogenous fibrolytic enzyme (EFE) and/or live yeast culture*viz*., TMR with R: C ratio of 70: 30 (T_1), T_1 supplemented with EFE @ 15 g/animal/d (T_2), T_1 supplemented with live yeast culture @ 10 g/animal/d (T_3) and T_1 supplemented with EFE @ 15 g/animal/d and live yeast culture @ 10 g/animal/d (T_4). The exogenous fibrolytic enzyme (Fibrozyme) used in the present study was procured from M/s Alltech Inc., Nicholasville, USA. The fibrozyme (fermentation extracts of *Aspergillus niger* and *Trichoderma viride* containing cellulases and hemicellulases; 100 IU as xylanase/g) was supplemented at the rate of 2.5 g of enzyme /kg TMR (on DM basis). The live yeast culture (Levucell SC 20 diluted) (*Saccharomyces cerevisiae* 1- 1077 containing 4 x 10^9 CFU / 10 g) used in the present study was procured from Lallemand, France.

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The TMR samples were analyzed for proximate constituents (AOAC, 2007) and fibre fractions (Van Soest *et al*., 1991). The four dietary treatments were evaluated for *in vitro* DM, CP, NDF and ADF digestibility (Tilley and Terry, 1963) by incubating the feed samples for 72 h at 30^{o}C in buffered rumen liquor collected from two rumen fistulated buffalo bulls maintained on TMR comprising of GN haulms and concentrate mixture in 70: 30 ratio to meet the nutrient requirements (ICAR, 1998).

The data was analyzed statistically (Snedecor and Cochran, 1994) and tested for significance by Duncan's multiple range test (Duncan, 1955) using SPSS 17.0 version.

RESULTS AND DISCUSSION

The chemical composition of GN straw and GN straw based complete ration was presented in Table 2. Chemical composition revealed that the CP content was 9.3 and 12.25 per cent in GN straw and GN straw based complete ration, respectively on DM basis.

The *in vitro* digestibility (%) of total mixed rations supplemented with exogenous fibrolytic enzyme (EFE) and/or live yeast culture studied using rumen liquor collected from buffalo bulls maintained on a standard basal diet is presented in Table 3. Results indicated that the % IVDMD varied between 50.97 to 57.02, % IVCPD between 53.98 to 60.57, % IVNDFD between 49.15 to 57.28 while % IVADFD between 46.42 to 55.0 percent among the different dietary treatments. The *in vitro* digestibility (%) of DM, CP, NDF and ADF were lower ($P<0.05$) in T_1 as compared to other treatments.

Supplementation of EFE in TMR (T_2) increased ($P<0.01$) the *in vitro* digestibility (%) of DM, CP, NDF and ADF as compared to the control. This may be attributed to the ability of EFE to degrade complex substrates to simpler ones making them more amenable to rumen microorganisms (Azzaz *et al.*, 2013). Morgavi *et al.* (2000) demonstrated synergism between EFE and ruminal enzymes such that the net combined hydrolytic effect in the rumen was greater than estimated from the individual activities. Further, EFE enhance the attachment of rumen microbes to feed particles (Yang *et al.*, 1999) and also stimulate the rumen microbial population (Nsereko *et al.*, 2002). These factors in combination might have resulted in increased *in vitro* digestibility of nutrients in the enzyme supplemented TMR as compared to the control. Similarly, increased IVDMD (Balci *et al.*, 2007; Gado *et al.*, 2007; Shojaeian and Thakur, 2007; Ganai *et al.*, 2011; Issac *et al.*, 2011; Bhasker *et al.*, 2012), IVCPD (Rajamma *et al*, 2015), IVNDFD (Eun *et al.*, 2006; Balci *et al.*, 2007; Shojaeian and Thakur, 2007; Miachieo and Thakur, 2007; Thakur *et al.*, 2008; Thakur and Shelke, 2011) and IVADFD (Thakur and Shelke, 2011) with EFE supplementation in the diet were also reported earlier.

Yeast culture supplementation in TMR (T_3) resulted in increased ($P<0.01$) *in vitro* digestibility (%) of DM, CP, NDF and ADF as compared to the control. These results indicate that addition of yeast culture stimulated microbial metabolism and the content of fermentable carbohydrates and available nitrogen leading to better nutrient availability for rumen microorganisms (Nehra *et al*., 2013). Further, the increased nutrient digestibility observed upon yeast culture supplementation might be attributed to the increased population of fibre degrading bacteria and/or their activity (Harrison *et al*., 1988) and due to supply of soluble growth factors (i.e. malate, organic acids, B-complex vitamins and amino acids) that are required by rumen bacteria for growth (Yoon and Stern, 1996). Similarly, increased IVDMD (Malik and Singh, 2009; Nehra *et al*., 2013; Elghandour *et al*., 2014), IVCPD (Fortina *et al*., 2011) and IVNDFD (Fortina *et al*., 2011; Elghandour *et al*., 2014) with yeast culture supplementation in the diet were also reported earlier.

Supplementation of both EFE and live yeast culture in TMR (T_4) also resulted in increased ($P<0.01$) *in vitro* digestibility (%) of DM, CP, NDF and ADF when compared to T_1. Tang *et al*. (2008) conducted an *in* vitro study with a mixture of live yeast culture and exogenous fibrolytic enzymes and reported that the best results for the degradation of DM and organic matter of incubated maize silage, wheat straw and rice straw were obtained for a mixture in which the ratio of yeast and enzyme was 5 and 7.5 g, respectively in kg DM of the tested roughage. Similarly, Can *et al*. (2007) also reported that supplementation of both yeast culture and EFE in the diet increased ($P<0.01$) the IVDMD and IVNDFD as compared to the control, whereas, no synergistic effect of using a mixture of enzyme and yeast preparations in the diet was also reported earlier (Lopuszanska-Rusek and Bilik, 2011).

Table 1: Ingredient composition of Concentrate mixture

Ingredient	Concentrate mixture
Maize grain	27.0
De Oiled Rice Bran	35.0
Cotton seed cake	25.0
Sunflower cake	10.0
Mineral mixture	2.0
Salt	1.0
Total	100.0

Table 2: Chemical composition of Groundnut haulms and Total mixed rations

Nutrient	Groundnut haulms	TMR
Dry matter	88.36	92.82
Organic matter	90.90	90.88
Total ash	9.10	9.12
Crude protein	9.30	12.25
Ether extract	1.56	1.54
Crude fibre	37.80	27.62
Nitrogen free extract	42.24	49.47
Neutral Detergent Fibre	56.42	53.22
Acid Detergent Fibre	48.89	43.24
Acid Detergent Lignin	10.49	9.77
Hemi-cellulose	7.53	9.98
Cellulose	36.94	31.59

Table 3: *In vitro* digestibility (%) of Total Mixed Rations supplemented with Exogenous Fibrolytic Enzymes and / or live yeast culture

Dietary treatment	IVDMD	IVCPD	IVNDFD	IVADFD
T_1	50.97^{a}	53.98^{a}	49.15^{a}	46.42^{a}
T_2	55.69^{b}	58.43^{b}	54.41^{b}	51.94^{b}
T_3	56.63^{b}	59.64^{b}	55.28^{b}	53.56^{b}
T_4	57.02^{b}	60.57^{b}	57.28^{b}	55.00^{b}
SEM	0.97	1.01	1.21	1.33
Significance	**	**	**	**

Values in the columns with different superscripts differ significantly; ** ($P<0.01$)

Conclusion

It is concluded that supplementation of either exogenous fibrolytic enzymes or live yeast culture or combination of both increased the digestibility of nutrients *in vitro*. However, the study indicated that supplementation of both exogenous fibrolytic enzymes and live yeast culture in combination had no added effect on the digestibility of nutrients.

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