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Effect of exogenous fibrolytic enzyme on *in vitro* digestibility of South African tall maize fodder harvested at different stages

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Abstract
Study was conducted to evaluate *in vitro* digestibility and NDF digestibility of South African Tall maize fodder variety as influenced by advancing maturity. The fodder was harvested at 75, 85, 95 and 105 days after sowing (DAS). The maize fodder was analyzed for their chemical composition and *in vitro* digestibility. The results showed that *in vitro* dry matter digestibility of maize fodder and Neutral detergent fibre digestibility of maize fodder decreased significantly (p<0.05) with advancing stage of maturity, On exogenous fibrolytic enzyme supplementation, the results showed that there was non-significant difference in maize fodder harvested at 75, 85 and 95 DAS. There was a significant difference in digestibility in maize fodder harvested at 105 DAS. Therefore the addition of fibrolytic enzyme showed increase in TDMD and NDFD in later stage of harvesting. It is concluded that maize fodder harvested at early harvesting stage showed better nutrients and digestibility when compared to maize fodder harvested at a later stages. In the current findings it was conferred that the supplementation of fibrolytic enzymes is beneficial in fodders harvested at a later stages, but had no effect on early harvested fodder. The results obtained in the current *in vitro* experiment need to be assessed using *in vivo* trials before concluding with final results.

Keywords: Maize fodder, harvesting stages, fibrolytic enzymes, digestibility

Introduction
Fodder production forms a major component of dairy cattle and sheep production management. The quality and quantity of fodder are influenced by the type of soil and stage of growth (Kim *et al.*, 2001) [22]. Fodders available for feeding livestock differ in their chemical composition depending on factors such as the variety of fodder, composition of soil, type of fertilizer, irrigation pattern, harvesting pattern and stage of maturity at the time of harvest. Forage quality declines with advancing maturity. For examples maize exhibits ideal forage quality when the grains are in the milk stage, afterwards the quality keeps on declining. Maturity at harvest also influence forage consumption by animals. As plant matures and become more fibrous, forage intake drops drastically. Intake potential decreases and Neutral detergent fibre (NDF) concentration increases as plant grow. Enzymes have been used for decades to improve the utilization of swine and poultry diets. For instance phytase, amylase, ß-glucanase and xylanase are added to the cereal-based diets of such monogastrics to increase the utilization of dietary phosphorous, starch, ß-glucans and arabinoxylans, respectively (Adesogan, 2005) [1]. Enzyme application to diets at feeding is attractive because the fermentable substrates released by enzyme action can be directly fermented by ruminal bacteria, there by releasing energy for the host animal. However, care is needed to ensure an even distribution of the small quantity of enzyme that is typically added. Nevertheless, several studies have demonstrated that enzyme application at feeding improves milk production in dairy cows and improves average daily gain in beef cattle.

Materials and Methods
The study was conducted by growing SAT fodder maize in Bangalore rural district as per recommended package of practice with reference to land preparation, irrigation, sowing rate, fertilizer and grain harvesting (Anonymous, 2008) [2]. The fodder was harvested at four different intervals i.e. at 75, 85, 95 and 105 days after sowing (DAS). The representative samples were collected randomly including whole maize plants in a considerably larger quantity of maize fodder harvested at different intervals for laboratory analyses.

The collected samples after moisture estimation were ground in the laboratory by Wiley mill using sieve of 2 mm diameter. The dried and ground samples were stored in clean, well labelled airtight containers for further analysis.

Donor Cow: A crossbred lactating cattle (400 kg Body weight and Milk yield of 3 kg), fitted with a flexible rumen cannula of large diameter (Bar Diamond, Inc. USA) and served as the donor of the rumen inoculums.

Batch Culture *in vitro* Digestibility: To determine the apparent and true DM digestion and NDF digestion, Ankom F57 bags were rinsed in acetone. After acetone rinsing Ankom 57 bags were dried and weight of empty bags was recorded. Air equilibrated enzyme mixed feed samples (400 ± 14 mg) maize fodder were incubated in Erlenmeyer flasks with 80 ml mixed rumen inoculum as per the procedure explained by Tilley and Terry (1963) [34] and Mould *et al.* (2005) [26]. After incubation period of 48 hours, 10 ml of fluid sample from each flask was collected into 15 ml conical tube with cap using disposable pipette. Filled tubes were kept in ice until processed. Ankom F57 bags were removed from flasks and immersed immediately in cold water. All bags were rinsed with cold water until the water is clear.

Rinsed bags were dried at 101 °C for 4 hours. After drying, weight of bags was recorded. Dried bags were again rinsed with the neutral detergent solution (NDS) for 1h. After rinsing with NDS, bags were washed with distilled water and again dried at 101°C for 4 hours. Then dried weight of bags was recorded. Using following equations TDMD % and NDF % digestibility were calculated.

$$\text{TDMD \% (DM basis)} = \frac{100\text{-}\{W3\text{-}(W1\times C1)\}}{W2 \times DM} \times 100$$

$$\text{NDFD \% (DM basis)} = \frac{100\text{-}\{W3\text{-}(W1\times C1)\}}{W2 \times NDF} \times 100$$

Where, W1 = Bag tare weight
W2 = Sample weight
W3 = Final bag weight after ND treatment
C1 = Blank bag correction (Final oven-dried weight/original blank bag weight)
DM = Dry matter of the fodder
NDF = Neutral detergent fibre

Level of inclusion of enzyme for *in vitro* studies: The levels of the fibrolytic enzyme used during the *in vitro* study were based on Company recommendations of 10-20 g/head/day. The Rumen capacity of ruminant (Cow) stomach is 100-150 litres (Naseri, 2005) [27]. Therefore, according to the recommendations 10 to 20 g of enzyme is required for 100-150 litres of rumen fluid. Hence, 8 to16 mg of enzyme is required for 80 ml of rumen fluid used in *in vitro* dry matter digestibility study. Based on these recommendations the levels, addition of enzymes were fixed as 10 to 20 g per animal per day for *in vitro* evaluation of maize fodder harvested at 75, 85, 95 and 105 days after sowing (DAS) (Meller *et al*., 2019) [25].

Results and Discussion

Chemical composition: The Chemical compositions for South African Tall Maize for both proximate principles and cell wall fractions presented in Table 1.

Proximate principles: The dry matter (DM) content for SAT variety was 17.31±0.19, 21.32±0.16, 25.8±0.29 and 32.2±0.45 percent respectively for 75, 85, 95 and 105 days of harvesting. There was a significant difference in dry matter (p<0.05) contents between different DAS. The dry matter content at the time of harvest showed an increase with increasing stage of maturity. The increase in dry weight with advanced stages of harvesting might be due to peaks of growth period of plants and ultimately attaining maximum photosynthates stage. The findings of the current study were in agreement with the studies of Cone *et al.* (2008) [10] and Ibrahim *et al.* (2014) [19].
`Similarly, the crude protein (CP) and Ether extracts (EE) contents in SAT maize, decreased significantly (p<0.05) with advancing maturity. Khan *et al.* (2011) [21], also reported the decline in Crude protein content in the fodder with advancement of growth. The decrease in CP concentration appears to be the result of continued carbon assimilation during grain maturation, even though nitrogen uptake was probably completed, thereby diluting the plant nitrogen concentration (Wiersma *et al.*, 1993) [36]. Similar results were reported by (Gouri, 2012) [15]. The results obtained are consistent with the findings of Huang *et al.* (2012) [17] who reported that delayed harvest stage produced lower crude protein concentration which might be due to the higher dry matter yield per land. Ettle and Schwarz, (2003) [11] reported decrease in ether extract as increase in maturity. Gouri, (2012) [15] reported similar result when maize stover dried on different days of post-harvest but Serbester *et al.* (2015) [30] reported no significance difference in ether extract in different maturity stages. There was increase in total ash content as increase in maturity. There was a significant difference in total ash (p<0.05) for SAT maize variety harvested at four different DAS. The reason of having maximum total ash with delayed harvesting might be due to more uptakes of minerals for longer period of time in the field. Total Ash composition primarily depends upon soil profile and the harvesting stage (Khan *et al*., 2011) [21]. Similar results were reported by Ibrahim *et al.* (2014) [19]. Similarly, significant increase in ash percentage with delay in the harvesting of forages was observed by Zahid and Bhatti (1994) [37] and Tariq *et al.* (2011) [33] for sorghum and pearl millet, respectively.

Cell wall fractions: The Neutral detergent fibre, Acid detergent fibre (ADF) and Acid detergent lignin (ADL) increased significantly (p<0.05) with advancing maturity. There was a significant difference in NDF (p<0.05) for SAT maize variety harvested at 75, 85 and 95 DAS but there was no significant difference in the same variety harvested at 95 and 105 DAS. NDF measures most of the structural components in plant cells (*i.e.* lignin, hemicellulose and cellulose). The results obtained are similar to the Rehman *et al.* (2017) [29] and Khan *et al*., (2011) [21]. The reason of having maximum fibre content of maize with delayed harvesting may be due to the maturity of stem and dilution factor. Results were also similar with those of Bukhari *et al.* (2011) [6], who reported the increase in fibre of pearl millet with delayed harvesting. There was no significant difference in ADF (p<0.05) for SAT maize variety harvested at 85 and 95 DAS. Rehman *et al.* (2017) [29] reported similar ADF content for maize fodder harvested at 80, 90 and 100 DAS. Similar results were also reported by Kruse *et al.* (2008) [23]. Lignin accumulation and synthesis occur at the stage of secondary cell wall development (Carmi *et al.*, 2006) [7]. The reason might be that at early harvest, the moisture contents in the plant were high and the concentration of dry matter was less as the plant matures dry matter accumulation increased in the plant, which resulted in maximum content of ADF, NDF and lignin (Carmi *et al.*, 2006) [7]. The results were in agreement with findings Rehman *et al.* (2017) [29]. Gouri, (2012) [15] also reported increase in ADL content with increase in maturity.

True dry matter digestibility: The True Dry Matter Digestibility (TDMD %) for South African tall (SAT) maize variety, when harvested at different (75, 85, 95 and 105) DAS, supplemented with fibrolytic enzyme (g/ head/ day) mixture at 0, 10 and 20 levels are presented in Table 2 and 3, respectively and graphically depicted in Figure 2.
The True Dry Matter Digestibility (TDMD %) of South African Tall maize (SAT) variety, harvested at 75, 85, 95 and 105 DAS was 77.09±0.009, 75.76±0.005, 73.03±0.008 and 69.90±0.004, respectively. The TDMD values decreased significantly (p<0.05) with increase in maturity. Similar results were reported by Bilal *et al.* (2007) [9]. The higher DM digestibility of fodder harvested at 75 DAS compared to others could be due to lower NDF, ADF and lignin concentrations (Firdous *et al.*, 1996) [13]. Similarly Chaudhary *et al.* (2016) [8] and Made (2016) [24] stated that a significantly negative correlation was observed between IVDMD and fibre components (NDF, ADF and lignin) implying that higher lignin concentrations might be responsible for lower IVDMD with increase in maturity. Azim *et al.* (1989) [3] also reported a decline in dry matter digestibility (DMD) of whole maize plant due to decline in leaves and increase in stem as increase in maturity.

True dry matter digestibility (TDMD) of South African Tall maize (SAT) variety harvested at different days after sowing (DAS) and supplemented with different level of fibrolytic enzyme
On enzyme supplementation at 0, 10 and 20 g/head/day at different harvesting days showed no significant difference in TDMD (p<0.05) in 75, 85 and 95 DAS. There was an increase in digestibility at 105 DAS after supplementing enzyme at 10 g and 20 g. This is in agreement with, Colombatto *et al.* (2007) [9] who reported a linear increase in the *in vitro* DM and OM digestibility with increasing enzyme levels. Beauchemin *et al.* (2004) [3] suggested that the addition of enzyme improved digestion and the colonization of ruminal microorganism of cell wall which promoted the utilization of cellulose by microorganism and increased the DM digestibility. Tang *et al.* (2008) [32] also reported that increase in IVDMD in 4 cereal straw with addition of exogenous fibrolytic enzymes. Spoelstra *et al.* (1992) [31] reported that corn silage harvested at the dough stage of maturity and treated with cellulase and hemicellulase enzymes had as much as 25% digested by the enzymes.

Neutral detergent fibre digestibility: The Neutral Detergent Fiber Digestibility (NDFD %) for South African tall (SAT) maize variety, when harvested different (75, 85, 95 and 105) DAS, supplemented with fibrolytic enzyme (g/ head/ day) mixture at 0, 10 and 20 levels are presented in Table 2 and 3, respectively and graphically depicted in Figure 2.
The values for NDFD contents harvested at 75, 85, 95 and 105 were 43.56±0.22, 41.73±1.35, 39.56±0.17 and 38.25±0.89, respectively. The NDFD values decreased significantly (p<0.05) with increase in maturity. Maize parenchyma cells are thin walled, but much of this tissue becomes lignified by physiological maturity (Jung and Casler, 2006) [20]. Hoffman *et al.* (2001) [16] reported that with advancing maturity all types of fodder plants develop xylem tissue for water transport, accumulate cellulose and other complex carbohydrates, and these tissues become bound together by a process known as lignification. The combined effect of physiological changes results in plant cell walls (NDF), which are more difficult for rumen bacteria to attach to and to digest. This lead to decrease in NDF digestibility as plant matures. Similar results were also noticed in Firdous *et al.* (1996) [13].

Neutral detergent fibre digestibility (NDFD) of South African Tall maize (SAT) variety harvested at different days after sowing (DAS) and supplemented with different level of fibrolytic enzyme
On enzyme supplementation at 0, 10 and 20 g/head/day at different harvesting days showed there was increase in NDFD (p<0.05) at 10 and 20 g of enzyme level harvested at 95 and 105 DAS. The fodder harvested at 105 DAS has significance difference in NDFD (p<0.05) at different level of enzyme supplementation S AT maize variety. Newbold, (1997) [28] reported that exogenous enzymes have shown to stimulate fibrolytic bacteria and increase their attachment to fibre particles. These bacteria increase the digestibility of fibre. Feng *et al.* (1996) [12] reported improved DM, NDF and ADF digestibility when fibrolytic enzymes were applied to grass hay before feeding to cattle. Similarly, Gallardo *et al.* (2010) [14] reported that there is increase in NDF digestibility in Alfa Alfa hay when exogenous fibrolytic enzyme was applied at highest dose of 3g/kg DM. Baloyi, (2008) [4] reported similar results. In Contrary, Wallace *et al.* (2001) [35] found that supplementation of enzymes did not increase digestibility of corn silage or grass silages.

Table 1: Proximate principles and Fibre fractions of South African Tall Maize Fodder harvested at 75, 85, 95 and 105 Days after sowing (DAS).

Parameter (%)	Harvesting Stages (DAS)				
	75	85	95	105	P value
DM	17.31±0.19^a	21.32±0.00^b	25.8±0.10^c	32.2±0.90^d	0.001
CP	7.98±0.01^a	7.73±0.11^a	7.42±0.00^b	7.20±0.10^b	0.006
EE	2.00±0.02^a	1.82±0.06^b	1.62±0.01^c	1.50±0.01^c	0.002
TA	5.30±0.10^d	5.71±0.03^c	6.57±0.00^b	6.98±0.01^a	0.001
NDF	40.60±0.73^c	41.59±0.52^c	44.62±0.09^b	48.73±0.04^a	0.001
ADF	19.93±0.11^c	22.85±0.56^b	23.24±0.63^b	24.87±0.00^a	0.005
ADL	2.79±0.02^d	3.12±0.06^c	3.65±0.06^b	3.92±0.02^a	0.001

Note: Means bearing different superscript in rows differ significantly (p<0.05).

Table 2: True dry matter digestibility and Neutral detergent Fiber digestibility of South African Tall Maize Fodder Variety harvested at 75, 85, 95 and 105 Days after sowing (DAS).

Harvesting DAS	TDMD	NDFD
75	77.09±0.009^a	43.56±0.22^a
85	75.76±0.005^a	41.73±1.35^{ab}
95	73.03±0.008^b	39.56±0.17^{bc}
105	69.90±0.004^c	38.25±0.89^c
P value	0.06	0.03

Note: Means bearing different superscript in rows differ significantly (p<0.05).

Table 3: True Dry Matter Digestibility of South African Tall Maize and Neutral Detergent Fiber Digestibility Fodder Variety harvested at 75, 85, 95 and 105 Days after sowing (DAS), supplemented with different levels of fibrolytic enzymes

Enzyme level	Harvesting DAS – True dry matter digestibility (TDMD)			
	75	85	95	105
0	77.09±0.009	75.76±0.005	73.03±0.0008	69.90±0.004^{b}
10	77.62±0.003	76.34±0.002	73.95±0.0264	71.04±0.0008^{ab}
20	77.87±0.004	76.91±0.006	74.88±0.0002	72.07±0.001^{a}
P Value	0.162	0.22	0.718	0.024
Neutral Detergent Fiber Digestibility (NDFD)				
0	43.56±0.22	41.73±1.35	39.56±0.17	38.25±0.89^{b}
10	44.88±0.86	43.10±1.54	41.63±5.9	40.58±0.16^{ab}
20	45.49±0.11	44.47±0.14	43.72±0.05	42.70±0.25^{a}
P Value	0.162	0.22	0.718	0.024

Note: Means bearing different superscript in rows differ significantly (p<0.05).

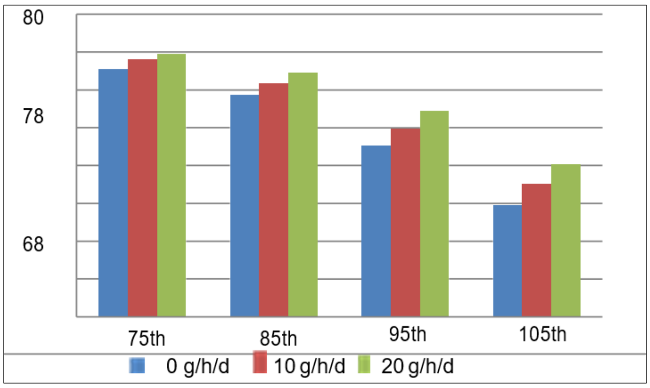

Fig 1: True Dry Matter Digestibility (TDMD) of South African Tall maize fodder Variety harvested at 75, 85, 95 and 105 DAS and supplemented with different level of fibrolytic enzyme

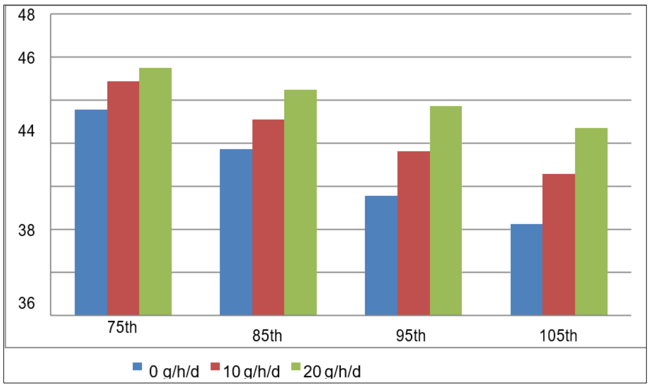

Fig 2: Neutral detergent fiber Digestibility NDFD of South African Tall maize fodder variety harvested at 75, 85, 95 and 105 DAS supplemented with different level of fibrolytic enzyme

Conclusion

Proximate analyses of maize fodder showed significant (p<0.05) decrease in crude protein and ether extract contents. The DM, total ash and all the cell wall fractions *viz.*, NDF, ADF and ADL resulted in significant (p<0.05) decreasing trend. On enzyme supplementation the TDMD and NDFD increased significantly (p<0.05) in at 95 and 105 DAS in DAS. In the current findings it was conferred that the supplementation of fibrolytic enzymes is beneficial in fodders harvested at a later stages, but had no effect on early harvested fodder. The results obtained in the current *in vitro* experiment need to be assessed using *in vivo* trials before concluding with final results.

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