II. REVIEW OF LITERATURE

The sorghum stover is an important crop residue in dry land agriculture that has greater economic significance in farming system. The sorghum is cultivated in Kharif and Rabi seasons in India. Besides the quantum of stover produced, the nutritional quality of stover determines the potential of the genotype in contributing to efficiency of any animal husbandry system. Literature on the nutritional evaluation of sorghum stover assessed by chemical composition, in vitro, in situ and in vivo studies by various workers has been reviewed in this chapter.

2.1 Chemical composition of sorghum stover

Badve et al. (1993) concluded that differences in the nutritive value of sorghum stover occur due to genotype, environmental factors, location and season. Results indicated that improved varieties showed higher yield of stover with better quality than hybrids. Varieties grown in postmonsoon (Rabi) season had higher neutral detergent fibre digestibility (NDFD) than monsoon (Kharif) varieties.

Ramachandra et al. (2002) studied the effect of feeding healthy verses diseased sorghum stover on intake and nutrient digestibility in buffaloes. Healthy sorghum stover contained 90.75, 93.78, 5.40, 0.99, 33.05, 54.34, 6.22, 66.96, 47.59, 19.37, 35.03, 10.17 and 2.39 per cent of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF), nitrogen free extractives (NFE), total ash (TA), neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose, cellulose, lignin and silica, respectively.
Dhore et al. (2005) observed 94.0, 5.6, 30.3, 0.5, 57.6, 6.0, 72.8, 43.3, 32.5 and 8.5 per cent of OM, CP, CF, EE, NFE, TA, NDF, ADF, cellulose and lignin respectively in sorghum stover.

Mahantesh (2006) reported the range of values of 2.55-3.59, 1.09-1.33, 28.89-31.78, 55.75-60.06, 6.85-8.09 per cent for CP, EE, CF, NFE and TA, respectively in four different varieties Solapur Dagadi, CSV-14-R, M 35-1 and SPV-1411.

Sanjay (2007) also studied four different varieties of sorghum stover viz. Maulee, CSV-216-R, CSH-15-R and local. The range of values observed were 3.12-3.78, 1.24-1.34, 29.57-31.65, 55.78-58.02 and 7.52-7.90 per cent for CP, EE, CF, NFE and TA respectively.

An extensive evaluation on stover quality was carried out by Venkatesh et al. (2008) using 15 varieties of sorghum stover grown in three different agroclimatic zones of country. Karnataka was covered under zone 2 and the mean chemical composition of all varieties of sorghum stover were in the range of 2.57-4.58, 9.89-10.90, 67.2-73, 42.5-47.70 and 48.9-52.1 per cent for CP, TA, NDF, ADF and in vitro organic matter digestibility (IVOMD), respectively.

2.1.1 Chemical composition of urea treated sorghum stover

Reddy et al. (1988) used different time and moisture combination to treat sorghum straw with 4 per cent (w/w) ammonia. They concluded that ammoniation increased CP content from 6.3 per cent to 12.0 per cent in treated straw with corresponding decrease in NFE of 41.6 per cent from 45.7 per cent in treated straw.
Further, NDF and hemicellulose percentage decreased but ADF and cellulose percentage were not affected by ammoniation of sorghum straw.

Kanade et al. (1992) reported 4.62, 9.00 and 7.12 per cent CP in untreated, 1 per cent urea and 3 per cent urea treated stover respectively with corresponding NFE values of 50.67, 48.33 and 46.20 per cent. The CP content increased and the NFE content decreased with the increasing percentage of urea used for the treatment. The composition of other constituents was not altered.

Rakesh Kumar and Sharma (2001) reported 3.15 and 8.00 per cent CP, 79.70 and 75.00 percent NDF, 54.20 and 59.30 per cent ADF respectively in untreated and 5 per cent urea treated sorghum stover, respectively.

2.2 Body weight gain

Rajan Gupta et al. (1984) evaluated the effect of ammoniated paddy straw on daily weight gain of growing buffalo calves. They observed that daily weight gain was significantly higher (351 g) in animals fed diet consisting 4 per cent urea ammoniated and concentrate compared with animals fed either 4 per cent fresh urea treated with concentrate (219 g) or untreated straw with concentrate (256 g).

Waghmare et al. (1987) observed in HF x Gir bulls, average daily gain (ADG) of 592 and 565 g when fed with steam treated hybrid sorghum kadbi and untreated hybrid sorghum kadbi respectively. The diets were isonitrogenous with roughage to concentrate ratio maintained at 1.41:1 and 1.10:1, respectively.
Krishnappa (1989) observed mean daily gain of 320, 337 and 317 g when fed with untreated, 2 per cent urea ammoniated and 4 per cent urea ammoniated sorghum stover, respectively in Jersey heifer calves. All the experimental groups were supplemented with concentrates and the diets were isonitrogenous.

Reddy and Reddy (2001) reported a BW gain of 62 g/d in Nellore rams by feeding diet containing urea treated sorghum straw and cowpea fodder fed in the ratio of 70:30. Mahantesh (2006) recorded daily BW gains of 74.56, 103.21, 99.46 and 78.03 g/d gain in sheep by feeding Solapur Dagadi, CSV-14-R, M 35-1 and SPV-1411 varieties of sorghum stover respectively, along with 200g concentrate mixture.

Patel et al. (2007) fed Gir bullocks with 100:0, 80:20, 70:30 and 60:40 of sorghum stover and groundnut haulms in a switch over design experiment for 30 days. They observed -5.3, -2.3, +3.3 and +10.3 Kg gain respectively. The changes in BW were found to be non significant. Animals maintained on sole feeding of sorghum stover (100:0) lost BW due to low protein intake.

2.3 Dry matter intake (DMI)

Waghmare et al. (1987) observed respective DMI of 2.0, 1.8 and 1.2 per cent of BW; 81.0, 70.0, and 49.8 g of metabolic BW (g/kg BW^{0.75}) in HF x Gir bulls when fed untreated M 35-1 jowar kadbi, steam treated hybrid jowar kadbi and untreated hybrid jowar kadbi. All the groups were supplemented with groundnut meal to meet their protein requirements.
Reddy et al. (1989) compared four different complete diets based on sorghum stover viz, untreated (SS), urea treated (USS), ammonia treated with low moisture (LMASS) and high moisture ammonia treated (HMASS) and fed to Jersey X Ongole bulls. The per cent DMI observed was 2.6, 2.9, 2.9 and 3.2 of BW in SS, USS, LMASS and HMASS diets, respectively.

Kanade et al. (1992) studied the voluntary DMI in crossbred goats by feeding untreated (T-1), 3 per cent urea treated (T-2) and 1 per cent urea treated (T-3) sorghum stover. All animals were supplemented with 115 g wheat bran. The DMI as per cent BW was 2.32, 2.41 and 2.45, while DMI as proportion of metabolic BW (g/kgW^{0.75}) was 49.00, 51.00 and 51.00, respectively in T-1, T-2 and T-3 groups.

Reddy et al. (1993) reported 2.39, 3.09 and 3.21 per cent DMI in Nellore rams by feeding local, SPV 351 and SPV 475 varieties of sorghum straw respectively. MD. Ibrahim et al (1998) fed processed (Chopped, soaked, ground or pelleted) sorghum straw to Deccani rams and studied the nutrient utilization. The per cent DMI recorded was 2.46, 2.83, 2.82 and 3.80 in chopped, soaked, ground and pelleted sorghum stover respectively. Pelleted straw had significantly (P≤ 0.05) higher DMI. Ramachandra et al. (2002) observed DMI of 1.85 and 1.70 per cent of BW when sorghum supplemented with 300 and 500 g groundnut cake respectively to adult buffaloes.

Sheela et al. (2004) observed 2.2 and 2.8 per cent of body weight DMI in Nali rams fed untreated and ureatreated jowar straw respectively without any supplementation of concentrate mixture. Patel et al. (2007) recorded DMI of 1.4, 1.4, 1.5 and 1.5 per cent
of BW in Gir bullocks by feeding with 100 per cent, 80 per cent, 70 per cent and 60 per cent jowar straw, respectively with remaining part of feed met by groundnut haulms.

2.4 Nutrient digestibility and nutritive value

Waghmare et al. (1987) studied the utilization of conventional sorghum (M 35-1) kadbi, untreated hybrid sorghum kadbi and steam treated hybrid sorghum kadbi along with groundnut meal in HF x Gir bulls. The organic matter digestibility (OMD) and NFE digestibility were significantly (P ≤ 0.01) higher in animals receiving M 35-1 and untreated hybrid sorghum groups. The CF digestibility was significantly (P ≤ 0.01) higher in hybrid sorghum fed group whereas NDF digestibility was significantly (P ≤ 0.01) higher in steam treated group. High digestibility of M 35-1 was attributed to better quality of roughage. The observed digestibility per cent were 58.2, 49.5 and 57.7 for OM; 63, 67.8 and 63.7 for CP; 43.1, 61.4 and 65.1 for CF; 62.6, 47.8 and 53.3 for NFE and 35.1, 31.3 and 50.5 for NDF, respectively for conventional sorghum (M 35-1) kadbi, untreated hybrid sorghum kadbi and steam treated hybrid sorghum kadbi diets.

Kanade et al. (1992) conducted a feeding trial in which three groups of crossbred goats were fed with untreated (T-1), 3 per cent urea ammoniated (T-2) and 1 per cent urea ammoniated sorghum straw. T-1 group was alone offered with 115 g wheat bran per day. The digestibility of DM, CP, NFE were significantly (P ≤ 0.05) higher in T-1 and it was due to wheat bran supplementation. The digestibilities of T-2 and T-3 were similar indicating that even 1 per cent urea treatment could improve straw quality. The nutritive value of T-1, T-2 and T-3 diets observed were 5.5, 4.23, 3.31 per cent digestible
crude protein (DCP) and 64.62, 57.43, 55.22 per cent total digestible nutrients (TDN), respectively.

Reddy et al. (1993) studied the utilization of local, SPV 351 and SPV 475 varieties of sorghum stover in Nellore rams. The digestibilities of all nutrients were found to be non significant and were in the range of DM 49.11-51.57, OM 50.24-52.87, CP 55.18-57.29, CF 52.7-57.54, EE 60.27-67.56 and NFE 46.28-52.49 per cent. The nutritive value reported was DCP 1.47, 3.30 and 2.94, TDN 49.73, 46.81 and 46.61 per cent respectively in local, SPV 351 and SPV 475 varieties. Significantly higher (P ≤ 0.01) DCP in SPV 351 and SPV 475 varieties was due to higher per cent of protein in these varieties.

Natarajan et al. (1994) conducted an experiment to study the nutritive value of urea treated sorghum stover in dry Surthi buffaloes. The animals were divided into two groups and were offered with untreated or 4 per cent urea ammoniated sorghum stover. Sorghum stover was the sole feed without supplementation of any concentrates. The digestibilities of DM, OM, and CP and the TDN were significantly (P ≤ 0.01) higher in urea treated group. The digestibility per cent were 52.82 and 61.00 for DM, 53.88 and 66.02 for OM, 60.75 and 72.80 for CP, 77.65 and 82.60 for EE, 66.90 and 73.10 for CF, 43.65 and 34.82 for NFE respectively for untreated and urea treated sorghum stover groups. The nutritive value observed was 1.66 and 11.18 of DCP and 50.60 and 56.14 per cent of TDN, for untreated and urea treated sorghum stover groups respectively.

MD. Ibrabahim et al. (1998) used processed sorghum straw to prepare four rations viz T-1 (chopped), T-2 (soaked), T-3 (ground) and T-4 (pelleted) sorghum stover and fed
to Deccani rams to study the nutrients utilization. All rams were offered with 200g concentrate mixture daily. The digestibility of DM, OM, CP, CF, NFE and TDN were significantly (P ≤ 0.01) higher in T-4 group and it was due to increased retention time of diet in the rumen due to increased density of pellets. The nutritive values observed were 4.95, 4.99, 4.57 and 4.58 per cent DCP and 49.99, 43.03, 48.27 and 58.07 per cent TDN for T-1, T-2, T-3 and T-4 rations, respectively.

Ramachandra et al. (2002) studied the digestibility of sorghum stover on intake and nutrient digestibility in buffaloes. The digestibility values (per cent) were 48.32, 52.40, 42.47, 50.69, 47.16, 55.74, 43.94, 40.83, 55.67 and 38.05 for DM, OM, CP, EE, CF, NFE, NDF, ADF, hemicellulose and cellulose, respectively. The DCP and TDN value were 2.29 and 49.30 per cent respectively.

Sheela et al. (2004) fed two groups of Nali rams solely with untreated or 4 per cent urea treated jowar straw to study the nutrients utilization. They observed significantly higher dry matter digestibility (DMD), organic matter digestibility (OMD), ether extract digestibility (EED), crude protein digestibility (CPD), crude fibre digestibility (CFD), neutral detergent fibre digestibility (NDFD), and acid detergent fibre digestibility (ADFD) in 4 per cent urea ammoniated jowar stalk. However, the nitrogen free extract digestibility (NFED) was significantly lower in treated group. The reported digestibility values of treated groups were DM, 51.4; CP, 78.30; EE, 49.20; CF, 56.30; NFE, 44.30; NDF, 57.20; ADF, 53.40; hemicellulose, 65.30 and cellulose, 65.60 per cent. The DCP and TDN (per cent) content were 2.9 and 43.30; 10.8 and 46 respectively for untreated and urea treated groups.
Patel et al. (2007) fed Gir bullocks with sorghum stover and groundnut haulms in the ratio of 100:0, 80:20, 70:30 and 60:40 in a switch over design experiment. The digestibility values of sorghum stover without groundnut haulms were DM, 52.2; OM, 57.8; CP, 54.7; EE, 54.5; CF, 54.1 and NFE, 52.6 per cent. The DCP and TDN content (per cent) was 2.35 and 50 respectively. The digestibility of nutrients improved linearly with increase in the level of groundnut haulms. However, the incorporation beyond 30 per cent did not have any additional benefits.

2.5 Feeding Frequency

Kumar et al. (1987) reported significant improvement in DMI and gain in BW of calves fed on urea supplemented diets at three or four times feeding per day when compared to calves fed two times a day. Robinson (1989) concluded productive benefits like increased efficiency of microbial fermentation, increased intake and milk fat due to increased frequency of feeding.

Madhav and Jaikishan (1990) conducted a feeding trial to determine the effect of feeding frequency on DMI and digestibility of nutrients. Four treatment groups viz. T-1, T-2, T-3 and T-4 were fed diets containing concentrate mixture having 50 per cent of the DCP requirement met through concentrate and remaining 50 per cent by urea. All the groups were fed with ad lib wheat straw. The feeding frequency was 1, 2, 3 and 4 times a day for T-1, T-2, T-3 and T-4 groups respectively. They observed no significant differences in DMI, gain or digestibility coefficients of various nutrients and the nutritive value in terms of DCP and TDN.
Yadav and Mathur (2002) selected twelve healthy growing female animals, six each of sheep (Sonadi) and goats (Devgarhi) and fed them on groundnut straw *ad lib* for 30 days. Thereafter, straw was offered twice a day for 10 days and thrice a day during subsequent period of 10 days. The digestibility coefficients of CP, CF, EE and NFE were significantly higher in goats than sheep. No significant difference was observed in voluntary intake of groundnut straw in both species irrespective of frequency of feeding.

Teller *et al.* (2004) used five ruminally cannulated crossbred steers (474 ± 30 kg) to feed diets containing 70 per cent barley straw and 30 per cent concentrate in an unbalanced 5 x 5 Latin square design experiment. They investigated into the effects of frequency of feeding barley grain-based concentrates (daily, alternate days or every third day) with different dietary protein (7.9 and 11.5 per cent). Voluntary intake of straw, ruminal disappearance of straw, apparent digestibility and heat production were observed. Frequency of feeding did not influence voluntary intake of straw or digestibility of nutrients. Apparent digestibilities of DM, gross energy, ADF and CP were 5, 6, 8 and 33 per cent higher (*P* < 0.05), respectively, in diets containing high-protein concentrate, but were not affected by frequency of concentrate feeding.

Drennan *et al.* (2006) studied the effect of concentrate cereal type diets *viz* rolled barley-based vs. rolled wheat-based diet with concentrate at feeding frequency of 6 kg feed at once a day or 6 kg feed at two times per day on intake, rumen fermentation, diet digestibility and performance of finishing steers. Dietary DMI and *in vivo* digestibility, final live weight, kill-out proportion, carcass weight, carcass conformation score, carcass fat score and daily live weight and estimated carcass gain were not affected by cereal
type or feeding frequency. Cereal type or feeding frequency had no effect on feed conversion efficiency (FCR) expressed as either live-weight or carcass gain per unit feed DMI. Estimated carcass weight gain and unit of feed intake per unit carcass yield were similar for wheat based and barley-based concentrate offered either as one feed or two equal portions daily.

Zali and Ganjkhanlou (2007) conducted an experiment to evaluate the effect of feeding frequency on non carcass components and wholesale cuts of Iranian fat tailed lambs. Three groups of lambs were fed total mixed ration. First group fed once daily, second group fed twice and third group fed four times daily. The final BW, gain and ADG and other carcass characteristics did not differ significantly (P ≥ 0.05).

Keskin et al. (2007) conducted a trial to determine to what extent the frequency of supplementary feeding would affect the production of sheep while grazing wheat stubble in the winter rainfall region of South Africa. Two groups received no supplementary feed, two groups received 200 g/ewe/day, two groups received 400 g/ewe every second day, and two groups received 600 g/ewe every third day. During the feeding period, no significant differences were observed between 200 g and 400 g supplemented groups. Lambing percent, weaning percent, birth weight, 42-day weight and survival rate of the lambs were not affected significantly. This implies that supplying this type of supplementary feed to ewes only every third day or at least every second day is a viable option, whereby production is not harmed but a reduces the labour and transport costs.
2.6 Use of exogenous fibrolytic enzymes to ruminants

Over the years, significant improvement in forage digestibility has been achieved through breeding programme and production in agronomic advances. Despite this, forage digestibility continues to limit intake of available energy by ruminants and contribute to excessive nutrient excretion by livestock. The use of fibrolytic enzymes holds promise as means of increasing forage utilization and improving productive efficiency of ruminants (Beauchemin et al., 2003).

2.6.1 Feed enzymes for ruminants

Feed enzymes are produced by a batch fermentation process, beginning with seed culture and growth media. Once the fermentation is complete, the enzyme protein is separated from the fermentation residues and source of organism (Cowan, 1994; Lee et al., 1998). Enzyme products for ruminant diets are of fungal (Trichoderma longibrachiatum, Aspergillus niger, A.oryzae) and bacterial (Bacillus species) origin (Pendleton, 2000). Furthermore, most of the commercially available enzyme products that have been evaluated as ruminant feed additives are produced for non feed applications; cellulases and xylanases are used extensively in food, pulp, paper, textile, fuel and chemical industries (Bhat and Hazlewood, 2001).

2.6.2 Enzyme activities involved in cell wall digestion

The focus of most enzyme related research for ruminants has been plant cell wall degrading enzymes. Cellulose and hemicellulloses, the major structural polysaccharides in plants are converted to soluble sugars by enzymes collectively referred as cellulases and hemicellulases. Cellulose is hydrolyzed through a complex process involving cellulases
and numerous specific enzymes contribute to cellulase activity. The major enzymes involved are endocellulase which includes endoflucanase, endo β 1,4 glucanase, carboxymethyl cellulase, β 1,4 glucanohydrolase, exocellulase which includes exoglucanase, exo β 1,4 glucanase, β 1,4 cellobiosidase and β glucosidase. The main enzymes involved in degrading the xylan core polymer to soluble sugars are xylanases which include endoxylanases and β 1,4 xylosidases. The hemicellulolytic enzymes include β mannosidase, α L-arbinofuranosidase, α D-glucuronidase, α D-galactosidase, acetyl xylan esterase and ferulic acid esterases. A temperature of approximately 60°C and pH of 4-5 are optimal for most of commercial enzymes. However, the optimal temperature and pH for assessing enzyme activity are not representative of the conditions of rumen, which are closer to a pH of 6 - 6.7 and 39°C. Thus, the activities quoted for commercial enzyme products are considerably higher than for those that would be measured at a pH and temperature similar to that of the rumen (Coughlan, 1985; Bhat and Hazelwood, 2001; McCleary, 2001).

2.6.3 Enzyme activity

Wallace et al. (2001) used six enzyme products to examine the relationship between enzyme activities and in vitro gas production using grass and corn silage. A significant positive correlation was reported between cellulase activity and gas production from grass silage. It was observed that preparations relatively high in cellulase activity increased the rate of gas production from corn silage compared with the control (no added enzyme). In contrast, products with relatively high xylanase activity did not increase gas production when glucanase activity was low. Based on the results of this study, one may assume that it would be possible to improve the effectiveness of enzyme
preparations by increasing cellulase activity. However, it should be noted that the levels of enzymes used in these studies were 20 to 40 times higher than the levels normally (0.5 to 2 mg/g of total mixed ration (TMR) DM used as ruminant feed additives. The authors were unable to document any positive effects of exogenous enzymes on rate of gas production when using lower enzyme levels, which may have been a reflection of the techniques used. Caution must be applied when extrapolating from in vitro studies where relatively high levels of enzyme addition are used.

Colombatto et al. (2002a) used 23 commercial enzyme products to determine the relationship between enzyme activity and the in vitro degradation of feeds. The enzyme products were assayed for 16 different activities using pH and temperature conditions similar to those of the rumen (39°C and pH 6.0), and the level of enzyme product added per unit of feed was similar to that used in vivo (1.5 mg/g of DM). For incubations performed in buffer without ruminal fluid, there was a strong relationship between the release of reducing sugars from alfalfa hay or corn silage and the biochemical characteristics of the enzyme products.

Protein content of the enzyme product explained about 60 per cent of the variation in reducing sugars released, indicating that concentrated products with higher protein content were most effective. This can be explained by the fact that the same amount of each enzyme product was used even though the enzyme activity varied tremendously among products. β-glucanase activity explained a further 24 per cent of the variation in reducing sugars released from alfalfa hay, whereas, endoglucanase, exoglucanase, β-glucosidase, xylanase, and amylase explained a further 37 per cent of the variation for
corn silage. Thus, for alfalfa hay, about 84 per cent of the variation and for corn silage about 97 per cent of the variation was explained by these factors.

Colombatto et al. (2002b) evaluated same enzyme products used in Colombatto et al. (2000a) for their effects on in vitro DM degradation. Five of the 23 products significantly improved the 18 hour degradation of alfalfa hay and nine of the 23 products improved the degradation of corn silage. There was a significant relationship between xylanase activity and feed digestion. However, the relationship was positive with alfalfa hay, but negative with corn silage. The correlations between xylanase activity and digestion were significant, the xylanase activity alone explained less than 30 per cent of the variation in degradation. No other significant relationship was observed between enzyme activities and feed degradation.

2.6.4 Enzyme level

Some of the variability associated with the use of exogenous enzyme products in ruminant diets was due to supplementation with insufficient or excessive enzyme activity. In vivo responses to enzyme addition were typically nonlinear (Beauchemin et al. 1995; Kung et al. 2000).

Kung et al. (2000) offered forage (60 per cent corn silage and 40 per cent lucerne hay on DM basis) treated with increasing levels (0, 1, 2.5 ml/kg of total mixed ration) of an enzyme product to cows. Cows fed the low level of enzyme tended ($P \leq 0.10$) to produce more milk (39.5kg/d) than those fed the control diet (37.0 kg/d) or those fed the high level of enzyme (36.2 kg/d).
Beauchemin et al. (1995) also reported nonlinear responses for growing beef cattle. The ADG of cattle fed alfalfa hay increased by 24 to 30 per cent with lower levels of added enzyme (0.25 to 1 mL/kg DM) as a result of increased intake and digestible DM, but higher levels of enzyme (2 and 4 mL/kg of DM) were not effective. With timothy hay, a high level (4 mL/kg of DM) of exogenous enzymes increased ADG of cattle by 36 per cent as a result of a 17 per cent increase in ADF digestibility and a 14 per cent increase in digestible DMI.

These studies demonstrated that high levels of enzyme addition can be less effective than low levels, and the optimal level of enzyme supplementation may depend on the diet. Lack of response to low levels of enzyme addition may indicate an insufficient supply of enzyme activity. However, the rationale for reduced efficacy of added enzymes at high levels of incorporation is not clear.

Nserekp et al. (2002) reported a quadratic response in total bacterial numbers in ruminal fluid with increasing levels of an enzyme product from Trichoderma longibrachiatum added to a dairy cow diet. These authors speculated that the application of a moderate level of enzyme to ruminant feeds caused some beneficial disruption of the surface structure of the feed either before or after ingestion. When excess enzyme was applied, the beneficial disruption of the feed surface structure was diminished because the excess exogenous enzyme attached to feed could have restricted microbial attachment and limited digestion of feed.
2.6.5 Methods of providing enzyme to animals

Enzymes have been applied to total mixed rations (TMR), hay, ensiled forages, concentrate, supplement or premix. Exogenous enzymes may be expected to be more effective when applied to high moisture feeds (such as silages) compared to dry feeds due to higher moisture content. The requirement for water in the hydrolysis of complex polymers to soluble sugars is a fundamental biochemical principle. Furthermore, silage pH values are usually at, or around, the optimal pH for most fungal enzymes. However, in practice, some exogenous enzymes are more effective when applied in a liquid form to dry forage as opposed to wet forage (Beauchemin et al. 2003).

Feng et al. (1996) applied an enzyme solution directly to grass and observed no effect when added to fresh or wilted forage. However, when it was applied to dried grass, enzymes increased DM and fibre digestibility. However, Yang et al. (1999) observed no difference between applying an enzyme product to dry forage or to both dry forage and concentrate.

Studies conducted by Lewis et al. (1996) and McAllister et al. (1999) demonstrated that infusion of enzymes into the rumen has not been effective. In contrast, applying fibrolytic exogenous enzymes in a liquid form onto the feeds prior to consumption can have a positive effect on animal performance (Rode et al., 1999; Schingoethe et al., 1999; Kung et al., 2000; Yang et al., 2000). It may therefore be inferred that association of enzymes with feed may enable some form of preingestive attack of the enzymes upon the plant fibre and/or enhance binding of the enzymes to proteolysis in the rumen.
Nsereko et al. (2000) reported the presence of compounds in whole-crop barley silage that inhibited endo-1, 4-β-xylanase activity of an enzyme product from *T. longibrachiatum* by 23 to 50 per cent, although there was no effect on cellulase activity. The growth of the epiphytic microbiota was stimulated by soluble sugars released by enzyme treatment, which lead to a decrease in the silage feed value when the time elapsed between enzyme application and consumption was sufficiently long.

Yang et al. (2000) reported increased milk production and digestibility of the diet when enzymes were added to the concentrate portion of a dairy cow diet, but not when they were added directly to TMR.

2.6.6 Mode of action

In light of the exceptionally high starch and fibre digesting capacity of the rumen, it is difficult to explain why treatment of grain or forage with enzymes prior to consumption further improves feed utilization. The mode of action of exogenous enzymes in improving digestion of plant cell wall is a fairly complex mechanism.

Exogenous enzymes in the rumen are generally more stable (Hristov et al., 1998; Morgavi et al., 2000b and 2001), particularly when applied to feed, prior to ingestion. Application of enzymes to feed enhances the binding of the enzyme with the substrate, which increases the resistance of the enzymes to proteolysis and prolongs their residence time within the rumen. In the rumen, the close association between digestive bacteria and substrate concentrates the digestive enzymes close to their specific substrates. However, some ensiled feeds contain compounds that could be inhibitory to xylanases (Nsereko et al., 2000); therefore, applying enzymes to dry feeds decreases the variability in response.
Beauchemin et al. (1999) observed that applying enzymes to feed also provides a slow-release mechanism for enzymes in the rumen. Thus, the greater the proportion of the diet treated with enzymes, the higher the chances that enzymes endure in the rumen. Without this stable feed-enzyme complex, the enzymes are solubilized in ruminal fluid and flow rapidly from the rumen.

There is ample evidence for preconsumptive effects of exogenous enzymes causing the release of soluble carbohydrates (Hristov et al. 1996), and in some cases, partial solubilization of NDF and ADF (Gwayumba and Christensen, 1997; Krause et al. 1998). Nsereko et al. (2000) demonstrated compelling evidence that applying enzymes to feed causes structural changes to occur, thereby making feed more amenable to degradation. It is most likely that the positive production responses resulting from the use of enzyme additives was due to ruminal effects.

Adding exogenous enzymes to the diet increases the hydrolytic capacity of the rumen mainly due to increased bacterial attachment (Yang et al., 1999; Morgavi et al., 2000a; Morgavi et al., 2000c; Wang et al., 2001), stimulation of rumen microbial populations (Wang et al., 2002; Nsereko et al., 2002), and synergistic effects with hydrolases of ruminal microorganisms. The net effect was increased enzymatic activity within the rumen, which enhanced digestibility of the total diet fed. Thus, improvements in digestibility were not limited to the dietary component to which the enzymes are applied, which explains why fibrolytic enzymes can be effective when added to the concentrate portion of a diet.
2.6.7 Animal response to feed enzymes

Iwassa et al. (1997) compared adding two levels of enzyme at 1x and 2x to feed lot finishing diets consisting barley grain and barley silage. They observed significantly higher DMD and improved feed efficiency in enzyme supplemented groups.

Grasses which tend to have greater concentration of fibre as well as lignin and phenolic acids impede digestion. Kruger et al. (2003) stated that there was greater scope for digestibility enhancement with enzymes in C4 plants containing high lignin content.

Dean et al. (2003) tested the ammonia and enzyme application to Bermuda grass hay. The enzyme application did not affect the DMD of hay but did increase the NDFD, while, ammonia application increased (P ≤ 0.05) both these values. They concluded that the enzymes were not as effective as ammonia at increasing DM or NDFD in the hay, suggesting that more appropriate enzyme mixtures need to be used for enhancing the nutritive value of mature grasses.

Nowak et al. (2003) conducted an experiment using two non-lactating cannulated Jersey cows in a 2x2 Latin square design to determine the effect of fibrolytic enzyme supplementation on DM, NDF and ADF ruminal disappearance from wheat straw and total mixed ration (TMR). An enzyme complex containing 8000 carboxymethyl cellulase and 20,000 xylanase units per 1 kg DM was added in powder form to a diet consisting of barley based concentrate and meadow hay. Ruminal disappearance was measured by in situ method and intestinal digestibility by a mobile bag technique. The addition of exogenous enzymes had minimal effect on the effective degradability of DM, NDF and ADF from wheat straw and TMR. Fibrolytic enzymes increased DM, NDF and ADF
disappearance after 4 and 6 hours of incubation, but decreased after a longer incubation of 12 and 24 hours. The use of enzymes improved the intestinal digestibility of TMR and wheat straw DM, but no statistical differences were observed.

Deepika et al. (2007) determined the effect of enzyme-treated wheat straw on the rumen fermentation pattern in buffaloes. Adult rumen-fistulated buffaloes were divided into 2 groups and offered untreated wheat straw (T1) or fibrolytic enzyme-treated wheat straw (T2) along with concentrate mixture. The rumen liquor samples were collected at 2 hours post-feeding from each animal for 2 consecutive days. The CP and fibre contents of untreated and enzyme-treated wheat straws were comparable. The rumen pH, ammonia nitrogen and total nitrogen (N) concentrations were unaffected by the enzyme treatment. The total volatile fatty acid (TVFA) concentration was significantly higher in T2 than in T1. Application of enzymes to the roughage resulted in higher ruminal digestion. Carboxy methyl cellulase and xylanase activities were higher in T2 than in T1. The total rumen protozoal count was comparable between T1 and T2. It was concluded that the application of fibrolytic enzyme in the ruminant diet significantly increased the xylanase and VFA concentrations without affecting the rumen protozoa population.

Balci et al. (2007) used two groups of steers to investigate the effect of a fibrolytic enzyme on fattening performance. The animals were randomly allocated to control or enzyme-treated groups. They were fed for a period of 80 days with normal ration or normal ration along with 60 g/day of fibrolytic enzyme. Both groups were given wheat straw as roughage source. During the last week of the 80 days experimental period, for 3 consecutive days, rumen fluid samples were collected from control and treatment
groups through the esophageal tube, 3 hours after morning feeding. They were used for
analysis of pH and in vitro DM, OM and NDF digestibility. During the fattening period
live weight gain of control and treatment groups were determined and the difference was
found to be statistically significant (P≤0.05). Total BW gain and FCR were better in
treated than in control steers (P≤0.05). Rumen pH was found to be 6.19 and 6.20 for the
control and treated groups, respectively. The results obtained from this study suggested
that steers fed with enzyme supplemented feed had better daily weight gain, total weight
gain and FCR.

Giraldo et al. (2008) tested effect of exogenous fibrolytic enzyme preparation
delivered directly into the rumen on fistulated Merino sheep. The supplementation of
enzyme did not affect DMI, TVFA concentration and diet digestibility in sheep fed with
grass hay based diet. However, both the ruminally insoluble but potentially degradable
fraction of grass hay DM and its fractional rate of degradation were increased (P≤ 0.05)
by enzyme treatment. Supplementation with enzyme increased (P≤0.01) effective and
potential degradability of grass hay DM and NDF by increase in number of cellulolytic
bacteria at 4 hours after feeding.

Singh and Das (2009) studied the effect of fibrolytic enzyme treated wheat straw
on rumen fermentation and nutrient utilization in Bhadawari buffalo calves. They
concluded that rumen pH and total N concentration were not affected by the enzyme
treatment. The TVFA concentration was significantly (P≤ 0.05) higher (84.83 m mol/l) in
enzyme treated group as compared (74.50 m mol/l) to control. Enzyme supplementation
did not alter DMI. The digestibility coefficient of DM, OM, NDF, ADF and CF were significantly (P≤0.05) higher for enzyme treated diet.

2.6.8 Effect of Tween 80 and enzymes

Tween 80 (Poly oxyethylene sorbitan monoolate) is a surfactant and appears to have some effect on protozoa, gram negative bacteria and non cellulolytic bacteria. Jack (2002) investigated the effect of Tween 80 and monensin on rumen fermentation of the diet containing 70 per cent wheat straw treated by white-rot fungus *Pleurotus tuberregium* (TWS-PT) and 30 per cent barley in artificial rumen (RUSITEC). The RUSITEC consisted of four fermentation vessels (V1, V2, V3, V4): V1 was without additives (control), V2 received daily 10 mg of monensin, V3 received daily 0.5 per cent Tween 80 (v/w) and V4 involved the combination of 10 mg of monensin with 0.5 per cent Tween 80 (v/w). After an adaptation period (6 days) the fermentation parameters were determined for six consecutive days. Tween 80 did not affect the rumen fermentation of the diet consisting 70 per cent TWS-PT and 30 per cent barley in RUSITEC. Monensin affected the rumen fermentation of the diet by the decreased degradability of DM, NDF, ADF, hemicellulose, cellulose (P≤0.001), the decrease of methane production (P≤0.001) and the higher proportion of propionate in comparison to control. Tween 80 did not improve the potency of monensin. Only some indices of the increased molar per cent of propionate (about 3.4 per cent) and the decreased methane production (about 0.47 mmol) were found by using Tween 80 plus monensin in comparison to use of monensin alone.
Wanjae et al. (2005) studied in vitro rumen DM degradability, volatile fatty acid (VFA) and methane production in a total mixed ration pretreated with different levels of either commercial cellulytic enzyme (CCE), the surfactant poly oxyethylene sorbitan monooate (Tween 80) or both. Comparisons were made with (i) no pretreatment (Control); (ii) with 1 per cent CCE alone; (iii) with 2 per cent CCE alone; (iv) with 1 per cent Tween 80 alone; (v) with 1 per cent Tween 80 + 1 per cent CCE; (vi) with 1 per cent Tween 80 + 2 per cent CCE; (vii) with 2 per cent Tween 80 alone; (viii) with 2 per cent Tween 80 + 1 per cent CCE; and (ix) with 2 per cent Tween 80 + 2 per cent CCE. Activities of xylanase and cellulase were significantly \( (P < 0.001) \) increased with pretreatment of CCE alone, Tween 80 alone and both combined. Within the same treatment of no CCE and 1 per cent CCE levels, the total VFA concentration was significantly \( (P < 0.001) \) higher with 2 per cent Tween 80 than with no or 1 per cent Tween 80 treatments. The molar percentage of propionate significantly \( (P < 0.001) \) increased only with Tween 80. Throughout the in vitro incubation, the average value of methane concentration decrease was greater \( (P \leq 0.001) \) with 1 per cent CCE treatment than with no CCE or 2 per cent CCE treatments. Accumulative methane production was significantly lower with 1 per cent CCE treatment than with no CCE and 2 per cent CCE treatments. A ratio of VFA : methane production was significantly \( (P \leq 0.001) \) higher with 2 per cent Tween 80 treatment than with no Tween 80 and 1 per cent Tween 80 treatments, showing the average values of 7.28, 5.33 and 6.69, respectively.

A series of in vitro and in vivo experiments were conducted by Hwang et al. (2008) to investigate the effects of the mixture of Tween 80 and cellulytic enzymes (xylanase and cellulase) on total tract nutrient digestibility and rumen cellulytic
bacterial adhesion rates in Holstein steers. Ground timothy hay sprayed with various levels of Tween 80 and cellulolytic enzymes was used as substrates in an in vitro experiment to find out the best combinations for DM degradation. The application level of 2.5 per cent (v/w) Tween 80 and the combination of 5 U xylanase and 2.5 U cellulase per gram of ground timothy hay (DM basis) resulted in the highest in vitro dry matter degradation rate (P ≤ 0.05). Feeding the same timothy hay to Holstein steers also improved in vivo nutrient (DM, CP, CF, NDF and ADF) digestibilities compared to non-treated hay (P ≤ 0.05). Moreover, Tween 80 and enzyme combination treatment increased total ruminal VFA and concentrations of propionic acid and isovaleric acid with decreased acetate to propionate ratio (P ≤ 0.001). The results indicated that a mixture of Tween 80 and cellulolytic enzymes can improve rumen environment and feed digestibility with variable influence on cellulolytic bacterial adhesion on feed.

2.7 Effect of grain supplementation

Lusby et al. (1976) and Kartchner (1980) reported that small quantities of available carbohydrates can stimulate cellulose digestion in vitro, while larger quantities (200-800g/d) decrease in vivo cellulose digestibility and forage intake.

Chase and Hibberd (1987) studied four levels consisting 0, 1, 2 or 3 kg/d of ground corn fed to beef cows maintained on low quality native grass hay. They observed linear (P ≤ 0.001) decrease in digestibility of cellulose and hemicelluloses as the amount of supplemental corn increased. Hay intake was also decreased linearly (P ≤ 0.001). The digestibility of organic matter was also showed the same response.
Krause et al. (1998) conducted a study to determine the effects of treating barley grain with a fibrolytic enzyme mixture on chewing activities, ruminal fermentation, and total tract digestibility in cattle. Steers were given *ad libitum* access to one of four diets that consisted of 95 per cent barley-based concentrate and 5 per cent forage (DM basis). The concentrate was either control or enzyme treated and the forage was either barley silage or barley straw. Applying the enzyme mixture on to the barley silage lowered the concentrations of dietary ADF and NDF. Enzyme treatment of barley increased total tract ADF digestibility by 28 per cent (*P*≤0.05). Acetate-to-propionate ratio tended to decrease, which suggested that enzymes may have increased ruminal starch digestion as a result of enhanced digestion of barley hulls. The study demonstrated that using a fibrolytic enzyme mixture in high-grain diets that contain mainly barley grain can improve fibre digestion and grain utilization, but the mode of action was unclear.