MILK PRODUCTION IN BUFFALOES FED RATIONS FORTIFIED WITH EXOGENOUS FIBROLYTIC ENZYMES

THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF

MASTER OF VETERINARY SCIENCE
IN
DAIRYING
(ANIMAL NUTRITION)

BY
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KARNAL- 132001 (HARYANA), INDIA
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Approved by:

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This is to certify that the thesis entitled, **"MILK PRODUCTION IN BUFFALOES FED RATIONS FORTIFIED WITH EXOGENOUS FIBROLYTIC ENZYMES"** submitted by **Dr. CHANDRASHEKHAR** towards the partial fulfilment of the award of the degree of **MASTER OF VETERINARY SCIENCE** in **DAIRYING (ANIMAL NUTRITION)** of the **NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY)**, Karnal (Haryana), India, is a bonafide research work carried out by him under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

Dated: **June , 2009**

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This Thesis is Dedicated to

My Parents

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Date: *(Chandrashekhar)*

ABSTRACT

The present study was conducted to investigate the effect of adding exogenous fibrolytic enzymes (EFE) on nutrient digestibility, milk production, its composition and some blood constituents in lactating Murrah buffaloes. Eighteen Murrah buffaloes were divided into three similar groups on the basis of milk yield (8.48, 8.52 and 8.53kg/day) and days in lactation (68.5, 80.33 and 82.00). The buffaloes were fed a TMR comprising of 45% Wheat straw, 15% Green maize and 40% Concentrate on DM basis (Control group), the same TMR plus (cellulase 4000 µM glucose/g/h + xylanase 7990 µM xylose/g/min; 50:50 w/w) at 1.5g/kg DM through the concentrate (T1 group) and the same TMR plus (cellulase 4000 µM glucose/g/h + xylanase 7990 µM xylose/g/min; 50:50 w/w) supplemented at 3.0 g/kg DM through the concentrate (T2 group) for 90 days. Mean DM intake (kg/d, kg/100kg BW and g/kg $W^{0.75}$) was similar in different groups. No effect on CP intake (g/d, g/100kg BW and g/kg $W^{0.75}$) was recorded. TDN intake (kg/d, kg/100kg BW and g/kg $W^{0.75}$) was higher by ($P<0.05$) 12.53%, 12.59% and 12.60% in T-1 group over that of control, and similar in T-2 and control groups. The average daily milk yield and 4% FCM yield was higher ($P<0.05$) by 12.99 and 15.17% in T-1 group as over that of control and there was no difference between T-2 and control groups. There was no difference in milk composition and milk production efficiency in different experimental groups during six fortnights. The digestibility coefficient of CF, NDF, ADF, cellulose and hemicellulose were higher ($P<0.05$) by 10.65, 7.68, 10.86, 8.90, and 6.72 percent in buffaloes of T-1 group over those of control. There was no ($P>0.05$) difference in blood glucose and blood urea nitrogen concentration in different experimental groups. The results of present study indicated that supplementation of cellulase and xylanase mixture (50:50 w/w) at 1.5g/kg of DM of TMR containing wheat straw (45%), green maize (15%) and concentrate (40%) on DM basis, significantly increased ($P<0.05$) the average daily milk yield and FCM yield in Murrah buffaloes due to improved dietary fiber digestion.

सारांश

इस शोध का मुख्य उदेद्श्य मुर्रा नश्ल की दुधारू भैंसों में पोषक तत्वों की पाचकता, दुग्ध उत्पादन, दुग्ध संघटकों एंव रक्त के कुछ संघटकों पर बाह्य रेशा भंजक एन्जाईमों (बा.रे.ए.) के प्रभाव का अध्ययन करना था। अतः अठारह दुधारू भैंसों को उनके दुग्ध उत्पादन (8.48, 8.52 और 8.53 कि.ग्रा./दिन) एवं दुग्ध उत्पादन की अवधि (68.5, 80.33 और 82.00) के आधार पर तीन वर्गों में बॉटा गाया। नियंत्रक वर्ग के भैंसों को शुष्क पदार्थ आधार पर 45 प्रतिशत गेहूँ का भूसा 15 प्रतिशत हरा मक्का एवं 40 प्रतिशत दाना चारा मिश्रित आहार दिया गया। परिक्षण वर्ग–1 के आहार में नियंत्रक आहार के अतिरिक्त बा.रे.ए., सेल्यूलेज 4000 माईक्रो मोल ग्लूकोज/ग्रा./घंटा तथा जाईलेनेज 7990 माईक्रो मोल जाईलोज/ग्रा./मिनिट, 50:50 (भार प्रति भार), 1.5 ग्रा./कि.ग्रा. शुष्क पदार्थ की दर से एवं वर्ग–2 में (सेल्यूलेज 4000 माईक्रो माल ग्लूकोज/ग्रा./घंटा तथा जाईलेनेज 7990 माईक्रो मोल जाईलोज/ग्रा./मिनिट, 50:50 भार प्रति भार) 3.0 ग्प्र./कि.ग्रा. की दर से शुष्क पदार्थ को 90 दिनों के लिए सम्पूरित किया। औसत शुष्क पदार्थ अन्तर्ग्रहण (कि.ग्रा. प्रति दिन, कि. ग्प्र. प्रति 100 कि.ग्रा. शरीर भार एवं ग्रा./कि. ग्रा.$W^{0.75}$) नियंत्रक एवं दिनों परिक्षण वर्ग में अप्रभावित रहा। कच्ची प्रोटीन अन्तग्रहण (कि.ग्रा./दिन, कि.ग्रा./100 कि.ग्रा. शरीर भार एवं ग्रा./ कि. ग्रा.$W^{0.75}$) भी अप्रभावित रहा। परन्तु सकल पाचक तत्व अन्तर्ग्रहण (कि.ग्रा./दिन, कि.ग्रा./100 कि.ग्रा. शरीर भार एवं ग्रा./कि.ग्रॉ.$W^{0.75}$ नियंत्रक वर्ग की तुलना मे परीक्षण वर्ग–1 में ($P<0.05$) क्रमशः 12.53, 12.59 एवं 12.60 प्रतिशत अधिक पायी गई तथा नियंत्रक एवं परिक्षण वर्ग–2 में अप्रभावित रहा। परिक्षण वर्ग–1 में औसत प्रतिशत दुग्ध उत्पादन एवं एफ.सी.एम. उत्पादन ($P<0.05$) 12.99 एवं 15.17 प्रतिशत नियंत्रण वर्ग से ज्यादा रहा और नियंत्रक एवं परिक्षण वर्ग–2 में दुग्ध में उत्पादन एवं एफ.सी.एम. अप्रभवित रहा, जबकि दुग्ध संघटक एवं दुग्ध उत्पादन क्षमता साभी वर्गो में अप्रभावित रहा। परीक्षण वर्ग–1 में रेशा, एन.डी.एफ., ए.डी.एफ. , सेल्यूलोज एवं हेमीसेल्यूलोज की पाचकता में परीक्षण वर्ग–1 में क्रमाशः 10.65, 7.68, 10.86, 8.90 एवं 6.72 प्रतिशत नियंत्रण वर्ग से अधिक पायी गई। जबकि रक्त ग्लुकोज एवं रक्त युरिया नाईट्रोजन की मात्रा अप्रभावित रहा। उक्त परिणामों से यह विदित होता हैं कि गेहूँ का भूसा, दाना तथा हरा मक्का चारा (45:40:15) युक्त दुधारू मुर्रा भैंसों की आहार को सेल्यूलेज एवं जाईलेनेज (1.5ग्प्र./कि.ग्रा. शुष्क पदार्थ) की संपूरकता से रेशा की पाचकता बढ़ी तथा औसत दुग्ध उत्पादन एवं एफ.सी.एम. उत्पादन में सार्थक बढ़ोतरी ($P<0.05$) हुई।

ABBREVIATIONS

<	=	Less than
>	=	More than
µmol	=	Micromole
^0C	=	Degree Centigrade
ADF	=	Acid detergent fibre
AOAC	=	Association of Official Analytical Chemists
CMC	=	Carboxy methyl cellulose
CMCase	=	Carboxy methyl cellulase
CO_2	=	Carbon dioxide
CP	=	Crude protein
d	=	Day
DCP	=	Digestible Crude Protein
DM	=	Dry matter
DMI	=	Dry matter intake
DNS	=	Dinitrosalicylic acid
EE	=	Ether extract
EFE	=	Exogenous fibrolytic enzyme
FCM	=	Fat corrected milk
Fig	=	Figure
g	=	Gram
h	=	Hour
H_2SO_4	=	Sulphuric acid
IU	=	International Unit
kg	=	Kilogram
L	=	Litre

M	=	Molar
mg	=	Milligram
min	=	Minute
ml	=	Millilitre
mm	=	Millimetre
mM/L	=	Millimole per litre
N	=	Normal
NDF	=	Neutral detergent fibre
NFE	=	Nitrogen free extract
No.	=	Number
NRC	=	National Research Council
O.D.	=	Optical density
OM	=	Organic matter
OMD	=	Organic matter degradability
P	=	Probability
SE	=	Standard error
sec	=	Second
Sl.	=	Serial number
TDN	=	Total digestible nutrient
TMR	=	Total mixed ration
w/w	=	weight by weight
$W^{0.75}$	=	Metabolic body weight

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CHAPTER – 1

Introduction

1. INTRODUCTION

India is endowed with huge and diverse livestock population. It accounts for 57% of world's buffalo population and 14% of cattle population. As per recent estimates, 65-70% of Indian population depends on agriculture, which contributes nearly 18.5 % to India's GDP, out of which about 30% is contributed by animal sector (Economic survey 2007-2008). Crop residues are the backbone of dairy production system in the country. However the efficiency of converting roughages to productive purposes is limited by poor digestibility of forage cell walls.

The agricultural by-products such as crop residues are the most abundant renewable resources of animal feeds. Cereal straws have low protein, low energy, low minerals, and low vitamins content but contain a potential source of digestible energy due to the presence of cell wall carbohydrates, such as cellulose and hemicellulose. These polysaccharides are less available to the ruminants because of high lignin content as the digestion of fibrous substrates in the rumen is slow and incomplete which limits milk production and increases the cost of production. Hence, appropriate treatment of these materials becomes essential to improve their efficiency for this purpose. Various methods have been tried to enhance the digestibility of crop residues such as physical processing, physico-chemical processing, biological treatment and use of feed additives.

The use of exogenous cell wall degrading enzymes is an emerging technology that shows potential in terms of improving the utilization of forages by ruminants. Researchers were discouraged from using enzymes to enhance the utilization of ruminant diets because of perception that the hydrolytic capacity of the rumen could not be enhanced by supplemental enzyme, and concern that such enzyme would be ineffective due to ruminal proteolysis. These concerns have been disproved by several recent studies that have demonstrated that fibrolytic enzyme supplementation enhances the productivity of livestock.

Exogenous fibrolytic enzymes such as cellulase and xylanase have drawn the attention of ruminant nutritionists all over the world due to their large scale production as a result of advances in biotechnology and their positive effect on

digestion. Previously, these were used to improve the nutritive value of feed for non ruminants, particularly in broiler diets. But enzymes have not been routinely used in ruminant diets due to relatively high cost of feed enzymes and variability of response in ruminants. But the availability of newer and relatively cheaper and stable enzyme preparations has prompted a renewed interest in exploring the potential of feed enzymes for ruminants.

Use of enzymes in animal feeding shows tremendous potential to improve production efficiency and minimize nutrients wastage. Fibrolytic enzymes can increase the availability of storage polysaccharides and proteins. Enzymes can break down specific bonds in feedstuffs not usually degraded by endogenous enzymes, thus releasing more nutrients (Beauchemin *et al.,* 2001). Supplementing exogenous enzymes in the diet of growing cattle have also been found to improve average daily gain and feed efficiency and these enhanced the attachment of ruminal micro organism or improved their access to the cell wall matrix and by doing so, accelerated the rate of fibre digestion (Nserko *et al.,* 2000).

The use of exogenous enzymes holds promise as a means of increasing forage utilization and improving the productive efficiency of ruminants. Research has demonstrated that by supplementing fibre-degrading enzymes, primarily cellulases and xylanases, improved the feed utilization of dairy cows and feedlot cattle (Beauchemin *et al.,* 2003). Many studies have reported increased digestion of dry matter and fiber measured *in situ, in vitro* (Nakashima *et al.,* 1988; Yang *et al.,* 1999; Colombatto 2000, Colombatto *et .al.,* 2002, Eun and Beauchemin, 2007) as well as *in vivo* (Krause *et al.,* 1998; Kung *et al.,* 2000a; Pinos and Rodriguez *et.al.,* 2008) and average daily gain (Beauchemin *et al.,* 1999b; McAllister *et al.,* 1999).

Recent studies have shown that adding exogenous fibrolytic enzymes on TMR based diet to ruminant increased the milk production (Kung *et al.,* 2000b; Yang *et.al,* 2000) Addition of exogenous fibrolytic enzymes to ruminant ration containing urea treated wheat straw, increased the milk production in Sahiwal cows (Shojaeian and Thakur 2007). Miachieo and Thakur (2007) also studied the effect of fortifying cellulase and xylanases mixture (50:50 w/w) at 1.5 g/kg DM of the TMR and observed higher daily milk and FCM yield in Sahiwal

cows.

Keeping in view, the potential of exogenous fibrolytic enzymes to improve the nutrient utilization for milk production, the present study has been planned with the following objectives:

1. To investigate the effect of supplementing exogenous fibrolytic enzymes (EFE) on milk yield, its composition and nutrient digestion in buffaloes.
2. To study the effect of supplementing EFE in ration on certain blood metabolites of buffaloes.

CHAPTER – 2

Review of Literature

Feed costs accounts for the major expense of dairy producers and as a consequence, maximizing utilization of nutrients is essential for the profitability and sustainability of dairy enterprises. The largest fraction of feed energy that is not available to the animal remains entrapped in the undigested fibrous fraction. Methods that reduce this fraction could provide considerable benefit to dairy producers.

The use of exogenous cell wall degrading enzymes is an emerging technology that shows potential in terms of improving the utilization of forages by ruminants. Studies conducted by various workers have demonstrated that supplementing fibrolytic enzymes to the ration of dairy cows and feedlot cattle has significant potential to improve cell wall digestion which can lead to enhanced efficiency of feed utilization by ruminants. The literature on various facets of fibrolytic enzymes supplementation has been presented below.

2.1 SOURCES OF FEED ENZYMES

Feed enzymes are produced by a wide range of bacteria and fungi. Fungal sources include *Trichoderma longibrachiatum*, *Trichoderma reesei*, *Aspergillus niger*, *Aspergillus oryzae* (Pendleton, 2000), whereas; the bacterial sources are *Bacillus subtilis*, *Lactobacillus acidophilus*, *L. plantarum* and *Streptococcus faecium* (Muirhead, 1988).

Enzymes products are produced by a fermentation process, beginning with a seed culture and growth media (usually containing nitrogen, carbohydrates, minerals, and surface active agents). Once the fermentation is complete, the enzymes are separated from the fermentation residues and source organism. The types and activity of enzymes produced can vary widely depending on the strain selected and growth substrate and culture conditions employed for enzyme production (Considine and Coughlan 1989; Gashe 1992; *Lee et al.1998*).

2.2 ENZYME ACTIVITIES INVOLVED IN CELL WALL DIGESTION

Forages are the backbone of ruminant feeding. Even in high concentrate diets, a minimum amount of fiber is required to maintain optimum rumen functioning. However digestibility of plant cell wall in the total tract is still generally less than 65% (Van Soest, 1994). Plant cell walls consist of 40 to 45 percent cellulose, 30 to 35 percent hemicellulose and 20 to 33 percent lignin (Ladisch *et al.*, 1983).

Cellulose is a linear polymer of glucose linked by β-1, 4- glycosidic bonds, having a simple primary structure and complex tertiary structures. Hemicellulose, the second most abundant plant structural polysaccharide, is present in association with cellulose in the walls of most plant species. Based on the main sugar residues present in the polymer backbone, hemicelluloses can be termed xylans, glucomannans, galactans or arabinans. The two main types of hemicelluloses are generally considered to be xylans and glucomannans (Viikari *et al.*, 1993).

The major enzymes involved in cellulose hydrolysis are endocellulases (endoglucanase, endo-β-1, 4-glucanase, carboxymethyl-cellulase or β-1, 4-glucan glucanhydrolase), exocellulase (exoglucanase, exo-β-1, 4-glucanase, cellulose β-1, 4-cellobiosidase) and β-glucosidase (cellobiase or glucohydrolase). In general, endoglucanases hydrolyse cellulose chains at random to produce cellulose oligomers of varying degrees of polymerization; exoglucanases hydrolyze the cellulose chains from the non-reducing end, producing cellobiose, and β-glucosidases hydrolyse short chain oligomers and cellobiose to glucose.

The main enzymes involved in degrading the xylan core polymer to soluble sugars are xylanases and β-1, 4-xylosidase (Bhat and Hazlewood, 2001). The xylanases includes endoxylanases, which yield xyloligomers and β-1, 4-xylosidases, which in turn yields xylose (Bhat and Hazlewood, 2001).

2.3 LEVEL OF ENZYME APPLICATION

The optimal level of enzyme addition to a ration depends on the nature and type of substrate, indicating the need to determine optimum application rates of enzyme preparations for individual feed substrates (Beauchemin *et al.*,

1995). Due to variations in the enzyme activities of different enzyme products, type of feed and method of application, there is a possibility of under-supplementation or over-supplementation both of which may be counterproductive.

Several studies have shown that application of high levels of enzymes to forage or diets produce less desirable responses than low levels.

In a study conducted by Beauchemin *et al.* (1995), average daily gain of growing beef cattle fed alfalfa hay increased by 24 to 30 percent with lower levels of enzyme supplementation (0.25 to 1.0 ml/kg DM); but higher levels of enzyme (2ml and 4 ml/kg DM) were not effective. But when the same enzyme supplementation was done in timothy hay, the higher level (4 ml/kg DM) of exogenous enzyme increased average daily gain of cattle by 36 percent as a result of 17 percent increase in ADF digestibility and 14 percent increase in digestible DM intake. Lewis *et al.* (1999) studied the effect of different level of enzyme (cellulase and xylanases) supplementation on TMR based diet at low (1.25 ml/kg of forage DM), medium (2.5 ml/kg of forage DM) and high (5.0 ml/kg of forage DM) level to dairy cows and concluded that at medium level produces more milk than a low or high level of supplementation. Beauchemin *et al.* (2000) also observed that a high level of enzyme (3.67 L of enzyme supplement/tonne of TMR) application was less effective than a low level (1.22 L of enzyme supplement/tonne of TMR) in increasing total tract digestibility.

In-vivo responses to enzyme supplementation in dairy cattle diets have been shown to be non-linear (Beauchemin *et al.*, 1995; Kung *et al.*, 2000b). Kung *et al.* (2000b) offered forage (60% corn silage and 40% Lucerne hay) treated with increasing levels (0, 1, 2.5 ml/kg TMR) of an enzyme product to dairy cows. Cows fed the low level of enzyme tended ($P<0.10$) to produce more milk (39.5 kg/d) than those fed the control diet (37.0 kg/d) or those fed the higher level of enzyme (36.2 kg/d). Nsereko *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 the diet of dairy cows. They speculated that the application of a moderate level of enzyme to ruminant feeds caused some beneficial disruption of the surface of the feed either before or after ingestion. When excess enzyme was applied, the beneficial disruption of the feed surface

may get diminished because the excess exogenous enzyme attached to feed may have restricted microbial attachment and prevented substrate colonization that leads to limited digestion of feed.

2.4 MODE OF APPLICATION OF FEED ENZYMES TO RUMINANTS

Direct infusion of enzyme preparations into the rumen has been reported to be ineffective (McAllister *et al.*, 1999; Sutton *et al.*, 2001). Application of enzyme preparations on fresh forage have also been found to be less effective (Beauchemin *et al.*, 1995; Feng *et al.*, 1996; Yang *et al.*, 2000) due to increased passage rate (Beauchemin *et al.*, 2003) and inhibitory compounds (Nsereko *et al.*, 2000).

The response of dairy cattle to enzyme supplementation is reported to be the best when the enzyme preparation is applied onto dry feeds including grain and hay (Yang *et al.*, 1999). A pretreatment of feeds with enzymes before feeding has been reported to enhance the beneficial effects of enzymes on ruminal fermentation (Wang et al., 2001; Giraldo et al., 2004).

Yang *et al.* (2000) compared the efficacy of adding enzyme supplement into the concentrate portion of TMR or in to the whole of TMR to supply a similar dose (50 mg/kg DM). They reported that the milk yield was higher when the enzyme was added to the concentrate portion (37.4 kg/d) as compared to TMR (35.2 kg/d). Bowman *et al.* (2002) also reported that the digestibility of DM, NDF and ADF in the total tract of lactating dairy cows was increased when enzyme was supplemented (1 g/cow/day) to the concentrate portion (45% of TMR) as compared to the control. They also reported that when the enzyme was supplemented (1 g/cow/day) to the smaller portion of the diet such as supplement (4% of TMR) or premix (0.2% of TMR), there was only numerical increase in digestibility over the control group.

Shojaeian and Thakur (2007) observed that exogenous fibrolytic enzyme supplement to the concentrate part of the urea treated wheat straw based diet of Sahiwal cows increased the milk yield by 10.12 percent in the treatment group over that of the unsupplemented group.

Miachieo and Thakur (2007) reported that the milk yield and 4% FCM yield increased in Sahiwal cows by 7.66 and 8.58%, respectively in cows supplemented with cellulase and xylanase mixture (50:50w/w) @ 1.5g/kg DM through the concentrate portion of the wheat straw based TMR.

2.5 MODE OF ACTION OF FEED ENZYMES

Exogenous fibrolytic enzymes might be expected to alter feed utilization in ruminants either through their effect on feed prior to consumption, or through enhancement in digestion in the rumen and in the post-ruminal digestive tract.

Pre-ingestive effects of exogenous fibrolytic enzymes with the release of soluble carbohydrates have been reported by Hristov *et al.* (1996). Forsberg *et al* (2000) reported that release of carbohydrates would provide sufficient additional available carbohydrates to encourage rapid microbial growth, shortening lag time required for microbial colonization on substrate. The absorption of enzyme onto the substrate is an important prerequisite for hydrolysis (Pell and Schofield 1993). Partial pre-consumption solubilization of NDF and ADF of feeds has also been reported by Krause *et al.* (1998).

EFE are most effective when applied to feed prior to ingestion by cattle. (Beauchemin et al., 2003). The application of a moderate level of enzyme to ruminant feeds caused some beneficial disruption of the surface of the feed either before or after ingestion (Nsereko *et al.*, 2002). Cell wall hydrolysis in the rumen proceeds in an erosive manner (White *et al.*, 1993), and it is well recognized that a major constraint to digestion is the limited colonization and penetration of cellulolytic microbes and their hydrolytic enzymes onto the exposed surfaces of feed particles. It is most likely that the major portion of the positive production responses resulting from the use of enzyme additives is 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; Wang *et al.*, 2001), stimulation of rumen microbial populations (Wang *et al.*, 2001; Nsereko *et al.*, 2002), and synergistic effects with hydrolases of ruminal microorganisms (Morgavi *et al.*, 2000b).

The net effect is increased enzymatic activity within the rumen, which enhances digestibility of the total diet. Thus, improvements in digestibility are 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. Increased hydrolytic capacity of the rumen can also lead to an increase in digestibility of the nonfiber carbohydrate fraction, in addition to increasing digestibility of the fiber components of a diet, which explains why fibrolytic enzymes can be effective in high-concentrate diets.

2.6 Synergism with Ruminal Microorganisms

The actual increase in hydrolytic capacity in the rumen due to exogenous fibrolytic enzymes may be greater than calculated from microbial and exogenous enzyme activity due to synergism (Morgavi *et al* 2000a). Synergy between exogenous enzymes and rumen microbial enzymes can be defined as the enhanced effect of these two entities acting in coordination. The net effect is the increase in enzymic activity that exceeds the additive effect of each of the individual components. Synergy among cellulases and xylanases has been extensively documented by enzymologists (Bhat and Hazlewood 2001). Morgavi *et al* (2000a) demonstrated substantial synergism between exogenous enzymes and ruminal enzymes such that the net combined hydrolytic effect in the rumen was much greater than estimated from the individual activities. Enzymes from *Trichoderma longibrachiatum* were combined with ruminal enzymes extracted from cattle receiving high fiber or high concentrate diets, hydrolysis of soluble cellulose and xylan increased by up to 35 and 100% respectively. Thus, it is possible that enhanced enzymic activity due to synergy of exogenous enzymes and rumen bacterial enzymes enhance fiber digestion in the rumen.

2.7 EFFECT OF SUPPLEMENTING FIBROLYTIC ENZYMES ON NUTRIENT INTAKE AND DIGESTION

Feng *et al.* (1992) reported that adding fibrolytic enzymes to grass hay improved voluntary intake and digestibility of hay. They also reported that voluntary intake and total tract digestibility of DM and NDF were

increased by treating grass hay with fibrolytic enzymes directly before feeding, but not when applied to fresh or wilted forage. Lewis *et al*. (1999) assigned 30 cows to two dietary treatments which were fed a TMR based on alfalfa hay and silage. The treatment groups received an enzyme solution containing cellulase and xylanase, which was sprayed on the forage component of the ration at a rate of 1.65 ml/kg of forage DM 8 to 24 h prior to feeding. It was reported that increase in DMI by 2.2 kg per cow per day in treatment group over the control.

Yang *et al.* (1999) studied the effect of supplementing fibrolytic enzymes to four diets consisting of 45 percent concentrate, 10 percent barley silage and 45 percent cubed alfalfa hay. The diets contained the following enzyme supplements: 1) control cubes, 2) cubes treated with 1 g of enzyme per kg of hay (LH), 3) cubes treated with 2 g of enzyme per kg of hay (HH), and 4) both concentrates and cubes treated with 1 g of enzyme per kg of DM (HT). They reported that the amount of OM fermented in the rumen was 10 percent higher for cows fed HH or HT than for cows fed the control diet. The digestibility of NDF in the total tract was 12 percent higher for cows fed HH and 9 percent higher ($P<0.10$) for cows fed HT compared to the control diet.

Sutton *et al.* (2001) supplemented fibrolytic enzymes (2 g/kg DM) to the whole TMR, concentrate portion of TMR or infused into the rumen. They reported that there was no significant difference in the DMI between the control group and enzyme supplemented groups.

On the other hand, Beauchemin *et al.* (1999b) reported that enzyme supplementation did not affect the DM and nutrient intake. Yang *et al.* (1999) supplemented different levels of enzymes to the diets of dairy cows and reported that the DMI was similar in cows fed the various diets.

Yang *et al.* (2000) reported that the intake of DM and other nutrients were not affected by enzyme supplementation in the diet of dairy cows.

Kung *et al.* (2002) made similar observations when different sources of enzymes were added to the forage portion of TMR and fed to dairy cows. Zheng *et al.* (2000) added a mixture of cellulase and xylanase (1.25 L of enzyme/ton of forage DM) to the forage portion of TMR and fed dairy cows in early lactation. They reported that the DMI was similar ($P>0.25$) among treatment groups during

both the prepartum and postpartum periods.

Pinos-Rodriguez *et al*., (2002) conducted an experiment to study the effect of a directly fed exogenous fibrolytic enzyme on intake and digestion of DM, OM, protein, NDF, ADF, and hemicellulose of alfalfa and ryegrass hay by sheep. Enzyme increased intake of DM ($P < 0.01$), as well as OM and CP ($P < 0.05$) on feeding both hays; however, NDF and ADF intake were not changed.

Bowman *et al.* (2002) and Shojaeian and Thakur (2007) also reported that the DM and other nutrient intake were not significantly altered by inclusion of fibrolytic enzymes into the diet of dairy cows.

Yang *et al.* (2000) applied fibrolytic enzymes (50 mg/kg DM) to three treatments: control, enzyme applied to the total mixed ration (E-TMR), and enzymes added to the barley-based concentrate (E-CON) containing 24% corn silage, 14% chopped alfaalfa hay and 62% concentrate mainly consisting of steam-rolled barley grain. They reported that the digestibility of DM in the total tract was higher for cows fed E-CON than those fed the control diet and intermediate for those fed E-TMR.

Bowman *et al.* (2002) supplemented fibrolytic enzymes to a TMR diet (45% concentrate and 55% of barley silage and alfalfa haylage) in dairy cows so as to supply 1 g enzyme per cow per day through concentrate portion of TMR (CONC), through supplement (4% of TMR; SUPP) or through premix (0.2% of TMR; PREM). It was concluded that cows receiving CONC had a significantly higher DM and OM total tract digestibility as compared to the control group or PREM. Cows receiving SUPP and PREM treatments were reported to show a non-significant increase in nutrient digestibility compared to the control group.

Shojaeian and Thakur (2007) supplemented cellulase (3365 IU/kg DM) plus xylanase (6085 IU/kg DM) to urea treated wheat straw based diet to Sahiwal cows and reported significant ($P<0.01$) increase in DM, OM, NDF and ADF digestibility in the supplemented group.

Miachieo and Thakur (2007) also studied the effect of supplementing a mixture of cellulase and xylanases (50:50 w/w) at 1.5g/kg DM of the wheat straw based TMR, and reported that DM, CF,NDF and TDN intake were higher in treatment group over that of control.

2.8 EFFECT OF SUPPLEMENTING FIBROLYTIC ENZYMES ON MILK PRODUCTION

Most of the research work carried out in America and Europe indicated that animal response to exogenous enzymes appears to be greatest in situations in which fiber digestion is compromised and when energy is limiting in the diet. High producing dairy cows, specially in the peri-parturient period require high levels of available energy to meet the demands of milk production and have shown positive response to enzyme supplementation (Zheng *et al.*, 2000).

Yang *et al.* (1999) studied the effect of supplementing fibrolytic enzymes to a diet consisting of 45 percent concentrate, 10 percent barley silage and 45 percent cubed alfalfa hay. The diets contained the following enzyme supplements: 1) control cubes, 2) cubes treated with 1 g of enzyme per kg of hay (LH), 3) cubes treated with 2 g of enzyme per kg of hay (HH), and 4) both concentrates and cubes treated with 1 g of enzyme per kg of DM (HT). It was reported that milk production increased ($P<0.05$) by 7 percent for cows fed HH or HT compared with cows fed the control diet. Production of milk was intermediate for cows fed LH. Rode *et al.* (1999) supplemented an enzyme mixture containing mainly cellulase and xylanase activities to the concentrate portion (61% of TMR) of a TMR diet to supply 1.3 g/kg of TMR. It was observed that the milk yield tended to increase ($P<0.11$) in treatment group (39.5 kg/d) as compared to the control group (35.9 kg/d). Lewis *et al.* (1999) assigned 30 cows in late lactation to two dietary treatments which were fed a TMR based on alfalfa hay and silage. The treatment groups received an enzyme solution containing cellulase and xylanase, which was sprayed on the forage component of the ration at a rate of 1.65 ml/kg of forage DM 8 to 24 h prior to feeding. Cows consuming the enzyme treated diet produced more milk ($P<0.01$) than those fed the control diet (27.2 vs. 25.9 kg/d). Schingoethe *et al.* (1999) applied enzymes to TMR (33% corn silage, 22% alfalfa hay and 45% concentrate) at three levels viz. 2000 CMCase and 7500 xylanase units, 2850 CMCase and 10700 xylanase units and 4300 CMCase and 16050 xylanase units/kg of forage DM. Milk production was similar ($P>0.05$) among treatments

whereas production of 3.5 percent FCM and energy corrected milk was higher (P<0.05) when cows were fed enzyme-treated diets compared to that of control group.

Kung *et al.* (2000a) supplemented cellulase and xylanase to the forage portion of the diet (whole plant corn silage 45%, alfalfa hay 5% and concentrate 50%) to dairy cows at two levels EA2 (3500 CMCase units and 16000 xylanase units) and EA5 (8800 CMCase units and 40000 xylanase units/kg of forage DM). They reported that milk production from cows fed the diet containing forage treated with EA2 (39.5 kg/d) was greater than that from cows fed the control diet (37.0 kg/d). In addition, cows fed the diet with EA2 treated forage tended to have greater (P<0.15) 3.5 percent FCM production than cows fed the control diet and E45.

Zheng *et al.* (2000) supplemented a mixture containing 3500 units cellulase and 13350 units/kg xylanase of forage DM to a TMR (60% corn silage and 40% alfalfa hay) and fed them to dairy cows either in the close-up dry period, at calving or at peak milk production. The results showed that 3.5 percent FCM was higher (P=0.04 to P=0.07) for cows fed enzyme treated diet as compared to the control diet. Milk production was similar (P>0.20) in cows fed enzyme treated diet at different periods, although numerically, it was highest for cows that started receiving enzyme-treated forages right after parturition. Yang *et al.* (2000) applied fibrolytic enzymes (50 mg/kg DM) to the whole TMR (E-TMR) or to the concentrate portion of TMR (E-CONC). They reported that the milk yield (kg/d) was higher for cows fed E-CONC (37.4) than for cows fed the control diet (35.3) or E-TMR (35.2).

Bowman *et al.* (2002) supplemented fibrolytic enzymes to the TMR diet (45% concentrate and 55% of barley silage and alfalfa haylage) of dairy cows so as to supply 1 g enzyme per cow per day. Enzyme was added to the concentrate portion of TMR (CONC), to the supplement (4% of TMR; SUPP) or to the premix (0.2% of TMR; PREM). They reported that cows receiving CONC had numerically higher FCM than the control, SUPP and PREM groups. Kung *et al.* (2002) studied the effect of supplementing two different fibrolytic enzyme combinations into the forage portion of a TMR (30% corn silage, 15% alfalfa hay and 55% concentrate) on the nutritive value for lactating cows. They

reported that milk production was not affected by treatment, but cows fed the enzyme mixture treated forage produced 2 to 5 kg more 3.5 percent FCM (P<0.12) than those fed untreated forages.

Sutton *et al.* (2003) sprayed an enzyme product (2 g/kg DM) either on the TMR (TMR-E) or the concentrate portion of the TMR (CONC-E) or infused into the rumen (RUMEN-E). There was no significant effect on milk yield but it was numerically higher for the enzyme treated groups as compared to the control group. Titi (2003) supplemented exogenous fibrolytic enzymes to the concentrate diet of dairy cows in early lactation and reported that the enzyme supplemented group had higher (P<0.05) milk yield and 3.5 percent FCM as compared to the control group. Bala (2005) reported improved milk production and milk production efficiency (P<0.05) in goats fed concentrate and wheat straw (2.5:1 ratio, DM basis) supplemented with 8000 IU cellulase and 18750 IU xylanase per kg feed dry matter.

Miachieo and Thakur (2007) reported that daily milk yield and 4% FCM yield increased in sahiwal cows by 7.66 and 8.58% respectively over that of unsupplemented group on fortifying wheat straw based TMR with cellulase (4,470 IU/g) and xylanases (7,800IU/g) mixture (50:50 w/w) supplemented @1.5g/kg DM of the TMR.

Rana and Singh (2007), studied the effect of EFE supplementation in the diet of buffaloes on milk yield. Murrah buffaloes of control group were offered conventional diet of berseem and concentrate mixture, while those in the experimental group were supplemented with 15g fibrolytic enzyme for 10 weeks. The milk yield and fat corrected milk yield increased by 7.1 and 10.9% respectively, in treatment over the control group.

Shojaeian and Thakur (2007) observed that supplementation of cellulase (4486 I.U/g) plus xylanase (8113 I.U/g) mixture (50:50 w/w) @ 1.5 g/kg DM of a ration containing 60% urea treated wheat straw and 40% concentrate to sahiwal cows, increased the milk yield by 10.12 percent over that of control group.

However, some workers have reported no positive effect of fibrolytic enzyme supplementation on milk production in dairy cows.

Shepherd and Kung (1996) conducted an experiment to see the effect of supplementing cellulase

and hemicellulase to corn silage and reported no increase in milk production in cows. Chen *et al.* (1994) also reported no benefit from the addition of enzyme complex to corn silage on the lactation performance of dairy cows. Luchini *et al.* (1997) supplemented an enzyme combination of cellulase and xylanase to the diet of dairy cows and reported no difference in milk yield. Phipps *et al.* (2000) also reported no increase in milk production in dairy cows fed enzyme supplemented diet. Vicini *et al.* (2003) supplemented fibrolytic enzymes or soluble sugars and mallic acid to the ration of dairy cows. Enzyme solutions were sprayed either on the forage component of the TMR or on the whole TMR while mixing. There was no significant increase in milk production on supplementing these additives. Mandebvu *et al.* (2003) supplemented an enzyme preparation (1 g/kg non-forage DM), containing primarily cellulase, xylanase and protease activities, as dry powder form into the concentrate portion of TMR and reported no significant increase in milk production in dairy cows. The dry enzyme mixture had no effect on milk production (Reddish and Kung 2007)

In general, results of experiments on supplementing exogenous fibrolytic enzymes to the diet of dairy cows have indicated a positive response, but variable results have also been obtained. The variability in the response of dairy cows to exogenous fibrolytic enzyme supplementation have been attributed to factors such as enzyme type, level of supplementation, method of application and the energy balance of the test animals (Beauchemin *et al.*, 2003. Dean *et al.,* 2006). The composition of the diet has also been cited as a potential factor for inconsistent results (Yang *et al.*, 1998).

Most of the studies on the use of fibrolytic enzymes in developed countries have been conducted on high concentrate and green fodder/ hay based diets. There is very limited information available on the efficacy of these fibrolytic enzymes on wheat/ paddy straw based rations.

2.9 EFFECT OF ENZYME SUPPLEMENTATION ON MILK COMPOSITION AND COMPONENT YIELD

The effect of diet on yield of milk protein has been reported to be greater than the milk protein percent itself. Likewise, yields of total solids tended to be

higher when cows were fed enzyme treated forages, reflecting higher fat and protein yields (Beauchemin *et al.*, 2003). Lewis *et al.* (1999) reported that milk components like fat, protein and lactose tended to be higher in cows fed enzyme treated diets as compared to control. They reported that yield of fat and protein was greater ($P<0.05$) for cows fed forage treated with enzymes because of increase in milk production. Beauchemin *et al.* (1999a) also reported higher fat content of milk when cows were fed barley based diet supplemented with fibrolytic enzymes.

Schingoethe *et al.* (1999) reported a higher ($P<0.05$) production of 3.5 percent FCM when cows were fed enzyme supplemented diet. This increase reflected slightly higher milk production ($P=0.12$) as well as concentration of milk fat and protein as compared to the control group. They also reported that the differences in milk production and composition occurred within the first few weeks of the study and continued till the end of the study.

Rode *et al.* (1999) reported that the percentage of milk fat was lower ($P=0.02$) for cows fed enzyme treated ration than for cows fed the control diet. They also reported that the percentage of milk protein ($P=0.13$) and lactose ($P=0.09$) also tended to be lower for cows fed the enzyme supplemented diet, which may be due to marked increase in milk yield (10%) in the treatment group which was not accompanied by an increase in DMI.

Zheng *et al.* (2000) reported that milk composition was unaffected by enzyme supplementation to dairy cattle diets, but the protein percentage tended ($P=0.06$) to be higher for cows fed enzyme treated forages. As a result, they found that the yields of total solids tended to be higher ($P=0.06$) in enzyme supplemented groups.

Use of enzyme supplement in the diet of dairy cows tended to increase both fat and protein percentage and decrease lactose percentage of milk, although it was not significantly different from the control group (Bowman *et al.*, 2002). In contrast, Yang *et al.* (1999) reported that milk lactose content was higher for cows fed enzyme supplemented diet ($P<0.05$) than those fed control diets. They also reported that the milk protein content was higher ($P<0.09$) for cows fed enzyme supplemented diet while there was no treatment effect on milk fat content.

Sutton *et al.* (2003) applied an enzyme product (2 g/kg DM) to dairy cattle diet either on the TMR (TMR-E) or the concentrate portion of the TMR (CONC-E) or infused into the rumen. They reported that the milk protein content was higher ($P<0.05$) in cows fed CONC-E as compared to other groups and control. They found no significant treatment effect on milk fat or lactose contents.

Beauchemin *et al*. (2000) carried out an experiment in which cows received a diet consisting of 45% forage containing 0, 1.22, or 3.67 litre of enzyme product/tonne of total mixed ration (DM basis). Milk fat and lactose per cent was found to be same in all the treatments. However, cows received enzyme treatments had significantly higher protein per cent than control cows. Milk protein percentage increased due to addition of a low or high level of enzyme, but protein yield was not affected by enzyme. Yield and percentage of milk fat or lactose were not affected by the treatment.

Kung *et al.* (2000a) reported that cows fed forage treated with higher level (8800 CMCase units and 40000 xylanase units/kg of forage DM) of enzyme had lower milk fat ($P<0.10$), protein, protein yield ($P<0.10$) and fat yield ($P<0.10$) as compared to cows fed forage treated with lower level (3500 CMCase units and 16000 xylanase units/kg of forage DM) or control group. This was because cows fed the lower level of enzyme produced more ($P<0.10$) milk (39.5 kg/d) as compared to the cows fed the higher level of enzyme (36.2 kg/d) or the control group (37.0 kg/d).

Yang *et al.* (2000) reported a decline in the milk fat percentage, while milk protein and lactose were not affected on supplementing fibrolytic enzymes through the concentrate portion of the diet. Kung *et al.* (2002) reported that the milk fat and protein (percentage and yield) were not affected by enzyme supplementation in the diet.

Mandebvu *et al*. (2003) conducted an experiment on 42 multiparous Holstein cows (60-80 days in milk) randomly assigned to two dietary groups viz. control and TMR containing fibrolytic enzymes. The enzymes contained primarily cellulase, xylanase and neutral protease activities, and were added as dry powder to the concentrates prior to addition of forages in the mixture wagon. Enzymes were applied at 1 g/kg of non-forage dry matter. Addition of

fibrolytic enzymes had no effect on milk composition. Reddish and Kung (2007) examined the effect of supplementation 10 g (cellulase and xylanase) mixture /cow per day on TMR based diet consisting of 26% (DM basis) corn silage, 17% alfalfa silage, 7% chopped alfalfa hay, and 50% concentrate and reported that the dry enzyme mixture had no effect on DM intake, milk production, milk composition. Similarly Miachieo and Thakur (2007) and Shojaeian and Thakur (2007) did not observe any difference on milk composition of Sahiwal feed EFE supplemented wheat straw based TMR.

2.10 EFFECT OF ENZYME SUPPLEMENTATION ON BODY WEIGHT CHANGES

During the peri-parturient period, the DMI of dairy cows decreases and negative energy balance sets in, especially in high yielders. In this period, there may be a decrease in the body weight due to decreased DMI and onset of lactation. If enzyme supplementation causes an increase in DMI and / or nutrient digestibility, it may be expected that there may be body weight gain or body weight may be maintained in early lactation due to enzyme supplementation in the diet. But there have been no findings in this respect, which may be because the nutrients are diverted for milk synthesis.

Yang *et al.* (2000) used 43 Holstein cows in early lactation to investigate the effects of method of adding fibrolytic enzymes to diets on body weight. The enzyme product used contained relatively high xylanase and low cellulase activities. An enzyme solution (50 mg of enzyme powder dissolved into 20 ml of water per kg) was sprayed onto total mixed ration before feeding. Alternatively, 7.3 g of enzyme powder, dissolved in 20 litres of water, was added per tonne of concentrate (50 mg of enzyme/kg of diet dry matter). There was no effect on body weight and body weight changes in cows.

Knowlton *et al.* (2002) reported a numerical increase in the body weight in early lactation cows fed diets containing enzyme supplement compared to those fed the control diet, while there was no treatment effect in late lactation groups. Titi (2003) used twenty-eight multiparous lactating cows to evaluate the effect of exogenous fibrolytic enzyme supplementation on body weight change during early lactation. He found that there was no treatment effect on body

weight change, which is consistent with the findings of Rode *et al.* (1999), Schingoethe *et al.* (1999), Beauchemin *et al.* (2000), Kung *et al.* (2000a) and Sutton *et al.* (2003). These findings suggest that supplementation of fibrolytic enzymes in the ration of dairy cows has no positive effect on body weight.

CHAPTER – 3

Materials and Methods

Different experiments were conducted to study the effects of exogenous fibrolytic enzyme supplementation on milk production and nutrients utilization in Murrah buffaloes.

3.1 ESTIMATION OF ENZYME ACTIVITY

The activities of cellulase and xylanase enzymes were estimated by measuring the rate of release of reducing sugars from pure substrates by colorimetry method using the dinitrosalicylic acid (Miller, 1959).

Definition: One international unit (IU) of cellulase corresponds to 1 µmol of reducing sugar released as glucose in 1 min under the standard assay conditions.

The principle of the above method of activity estimation is that the enzyme catalyses the hydrolysis of cellulose releasing glucose by breaking the beta 1-4 linkages. The amount of glucose released is measured colorimetrically to measure enzyme activity.

3.1.1 Cellulase assay

A. Reagents used:

a) 0.1 M phosphate buffer, pH 6.8

Solution 1: 17.799g $Na_2HPO_4.2H_20$ was dissolved in about 600 ml distilled water and the volume made to 1 L.

Solution 2: 15.601g $NaH_2PO_4.2H_20$ was dissolved in about 600 ml distilled water and the volume made to 1 L.

Take solution 1. In a beaker and add Solution 2 till the pH reaches 6.8.

b) Carboxymethyl cellulose (1%):

Dissolved 1 gm of carboxymethyl cellulose in distilled water and diluted it to100ml.

c) Dinitrosalicylic acid solution (DNS):

Composition: NaOH - 10 gm, DNS - 10 gm and Phenol- 2 gm

Dissolved 10 gm NaOH pellets in about 500 ml of distilled water. Added 10 gm of DNS and 2 gm phenol to solution. Made up the volume to 1 litre with distilled water. Sodium sulphite (Na_2SO_3) 0.05% was added just before use.

d) Rochelle salt solution:

Dissolved 40 gm of Rochelle salt (Potassium Sodium Tartarate) in distilled water and made volume to 100 ml.

e) Standard solution of glucose (0.1%):

Dissolved 100 mg of glucose in 100 ml distilled water.

f) Cellulase enzyme solution (0.1%); Dissolved 100 mg of cellulase in 100 ml of phosphate buffer of pH- 6.8.

B. Procedure:

i) For standard curve:

Following protocol was used for preparing standard curve:

Sl.	Blank	1	2	3	4	5	6	7	8	9	10
Distilled water (ml)	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.0
Standard Glucose (0.1%) ml	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
DNS (ml)	3	3	3	3	3	3	3	3	3	3	3
Kept in boiling water bath for 10 min											
Rochelle salt (ml)	1	1	1	1	1	1	1	1	1	1	1
Distilled water (ml)	15	15	15	15	15	15	15	15	15	15	15
	Measured O.D. at 575 nm										

ii) For control:

Following protocol was used:

	1	2	3	4
Enzyme (ml)	0.1	0.2	0.3	0.4
Buffer (ml)	0.4	0.3	0.2	0.1
DNS (ml)	3	3	3	3
Incubated at 40^0C for 20 min.				
CMC (ml)	0.5	0.5	0.5	0.5
Kept in boiling water bath for 10 min				
Rochelle salt (ml)	1	1	1	1
Distilled water (ml)	15	15	15	15
	Measured O.D. at 575 nm			

For test samples:

Following protocol was used:

S. No.	1	2	3	4
Enzyme (ml)	0.2	0.3	0.4	0.5
Buffer (ml)	0.3	0.2	0.1	0.0
CMC (ml)	0.5	0.5	0.5	0.5
Incubated at 40^0 C for 20 min.				
DNS (ml)	3	3	3	3
Kept in boiling water bath for 10 min.				
Rochelle salt (ml)	1	1	1	1
Distilled water (ml)	15	15	15	15
Measured O.D. at 575 nm				

C: Calculations

Change in O.D. = O.D. of Test – O.D. of Control

Enzyme activity (units/ml) = µmol glucose/h/ml

$$= \frac{\mu g \text{ glucose}}{T \times S \times 180}$$

Where,

T = Time of incubation (in h)

S = Volume of sample taken

180 = Molecular weight of glucose

D. Standard curve reading for glucose

Concentration of glucose/mg	Optical Density at 575 nm
0.1	0.0149
0.2	0.0422
0.3	0.0933
0.4	0.1306
0.5	0.1741
0.6	0.2106
0.7	0.2537
0.8	0.2830
0.9	0.3193
1.0	0.3256

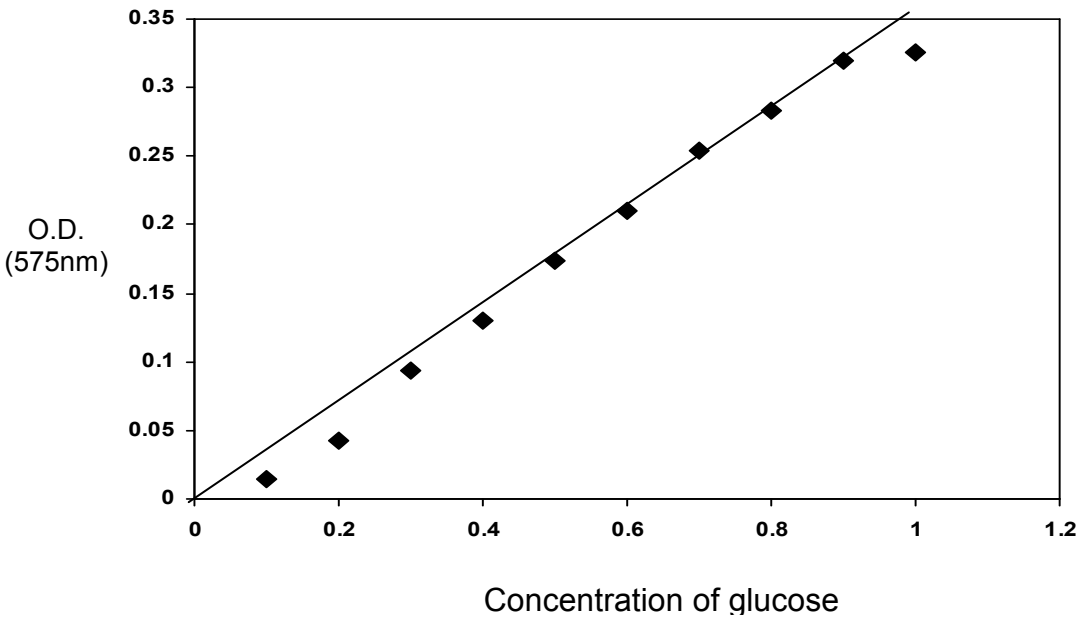

Figure 3.1 Standard curve for glucose

3.1.2 Xylanase assay:

Enzyme assay for Xylanase was also done using the procedure of Miller (1959). In this method, the principle of estimation is that xylanase catalyses

hydrolysis of xylan and releases its structural units, D-xylose, by breaking β 1-4 linkages. The activity of enzyme is determined by estimating colorimetrically the amount of D-xylose released during incubation of enzyme with substrate.

A. Reagents used:

a.) 0.1 M phosphate buffer, pH 6.8 (As given in 3.1.1. a)

b.) 0.5% Xylan substrate

Weighed 0.5 gm of xylan (from oat spelts), added 60 ml of distilled water. Heated beaker directly till the first bubble appeared. Added 10 ml of 1M acetate buffer of PH- 5.0 to the stirring substrate. Made up the volume to 100 ml with distilled water. Transferred the substrate to the beaker and let it stir for approx. 5 minutes

c.) Dinitrosalicylic acid (DNS) solution: (As given in 3.1.1. c)

d.) Rochelle salt solution (40%): (As given in 3.1.1.1 d)

e.) Standard solution of Xylose (0.1%):

Dissolved 100 mg of xylose in 100 ml distilled water.

B. Procedure:

For standard curve:

Following protocol was uses for preparing standard curve:

Sl. No	**Blank**	**1**	**2**	**3**	**4**	**5**
Distilled water (ml)	1.0	0.8	0.6	0.4	0.2	0.0
Standard Xylose (0.1%), ml	0	0.2	0.4	0.6	0.8	1.0
DNS (ml)	3	3	3	3	3	3
Kept in boiling water bath for 10 min						
Rochelle salt (ml)	1	1	1	1	1	1
Distilled water (ml)	15	15	15	15	15	15
Measured O.D. at 575 nm						

For control:

Following protocol was used:

Sl. No.	1	2	3	4	5
Enzyme (ml)	0.1	0.2	0.3	0.4	0.5
Buffer (ml)	0.4	0.3	0.2	0.1	0.0
DNS (ml)	3	3	3	3	3
Incubated at 40° C for 15 min.					
Xylan (ml)	0.5	0.5	0.5	0.5	0.5
Kept in boiling water bath for 10 min.					
Rochelle salt (ml)	1	1	1	1	1
Distilled water (ml)	15	15	15	15	15
Measured O.D. at 575 nm					

For test samples:

Following protocol was used:

Sl. no.	1	2	3	4	5
Enzyme (ml)	0.1	0.2	0.3	0.4	0.5
Buffer (ml)	0.4	0.3	0.2	0.1	0.0
Xylan (ml)	0.5	0.5	0.5	0.5	0.5
Incubated at 40^{0} C for 15 min.					
DNS (ml)	3	3	3	3	3
Kept in boiling water bath for 10 min.					
Rochelle salt (ml)	1	1	1	1	1
Distilled water (ml)	15	15	15	15	15
Measured OD at 575 nm					

C. Calculations:

Change in O.D. = O.D. of Test – O.D. of Control

Enzyme activity (units/ml) = µmol xylose/min/ml

$$= \frac{\mu g \text{ xylose}}{T \times S \times 150}$$

Where,

T = Time of incubation (in min)

S = Volume of sample taken

150 = Molecular weight of xylose

D. Standard curve reading for xylose

Concentration of xylose/mg	Optical Density at 575 nm
0.2	0.0057
0.4	0.0195
0.6	0.0304
0.8	0.0419
1.0	0.0501

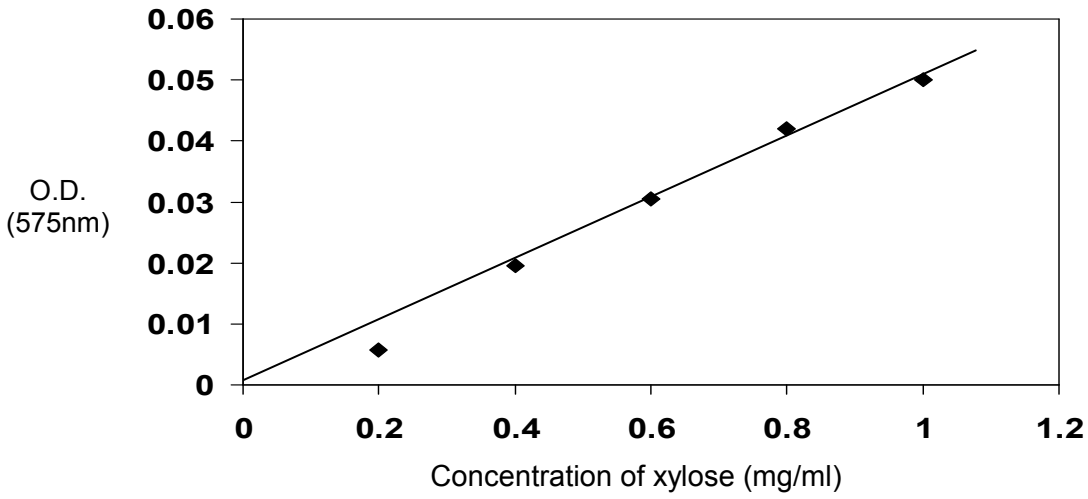

Figure 3.2 Standard curve for xylose

3.2 EXPERIMENTAL ANIMALS AND THEIR MANAGEMENT

3.2.1 Selection and Distribution of Animals

Eighteen lactating Murrah buffaloes, in early to mid lactation, were selected from the herd maintained at NDRI, Karnal. After deworming with broad spectrum anthelmentics, all the eighteen animals were kept on control ration and daily milk yield of all the animals was recorded for one week. The body weight of all the animals was recorded before feeding for two consecutive days at the start of the experiment. After one week, the animals were divided into three groups of six each on the basis of their milk yield and days of lactation, so that the average milk yield of the three groups was similar at the start of the lactation trial. Details and distribution of animals in different treatments are given in Table 3.1.

3.2.2 Housing and Management of Animals

All the Murrah buffaloes were housed in well-ventilated shed in cattle yard of NDRI, Karnal, having the arrangement for individual animal feeding without having access to the other animal's diet. The animals shed were washed twice daily and thoroughly cleaned to remove faeces and dirt. All the animals were maintained under clean and hygienic conditions. Antiseptic solution containing phenyl was applied at regular intervals on the floor of the shed to keep the animals away from infection. Arrangement was also made to protect the animals from extreme winter climate.

3.2.3 Feeding of experimental animals

All the eighteen Murrah buffaloes were fed as follows:

a) Group I (Control)

Animals were fed TMR diet containing concentrate, wheat straw and green maize in the ratio of 40:45:15,on DM basis respectively.

b) Group II (Treatment 1)

Animals were fed same TMR as in control group plus 1.5 g enzyme mixture (Cellulase and Xylanase, 50:50 proportion w/w) per kg dry matter intake.

c) Group III (Treatment 2)

Animals were fed same TMR as in control group plus 3 g enzyme mixture (Cellulase and Xylanase, 50:50 proportion w/w) per kg dry matter intake.

Supplementation of Enzyme

Cellulase (µM glucose/g/h) and Xylanase (µM xylose/g/min)mixed in 50:50 proportion (w/w) and mixed in the concentrate portion of TMR at two levels; 1.5 and 3.0 g/kg DM intake were supplemented.

Table 3.1: Details of lactating Murrah buffaloes and their distribution in different treatment groups

Sr. No.	Animal No.	Milk Yield (kg)	Days in Lactation	Lactation Number	Initial body weight (kg)
Group: I. Control					
1	409	7.00	79	5	593.45
2	437	9.80	31	3	598.45
3	5261	10.76	35	4	509.40
4	4918	9.40	23	4	498.95
5	5029	7.20	28	5	566.25
6	5331	6.70	215	3	620.25
Mean ±SE		**8.48±0.70**	**68.5±30.44**	**4±0.37**	**564.41±20.33**
Group: II T-1 (Exogenous Fibrolytic Enzymes @ 1.5 g/kg DMI)					
7	438	9.03	31	2	560.85
8	5253	8.40	108	2	575.75
9	5389	10.10	24	2	600.85
10	5262	9.30	58	4	594.10
11	5426	6.50	107	2	513.20
12	5433	7.80	154	2	538.60
Mean ±SE		**8.52±0.52**	**80.33±20.82**	**2.33±0.33**	**563.89±13.71**
Group: III T-2 (Exogenous Fibrolytic Enzymes @ 3.0g/kg DMI)					
13	441	6.40	62	2	585.35
14	5042	7.00	55	5	553.10
15	5308	10.17	78	2	541.00
16	5326	7.33	95	3	516.75
17	5358	9.90	124	2	551.50
18	5321	10.40	78	2	507.65
Mean ±SE		**8.53±0.74**	**82.00±10.15**	**2.67±0.49**	**543.38±11.57**

3.2.4 Feeding Schedule

Feeding was done once in a day; in the morning at 9.00 AM and Water was offered *ad lib* thrice daily @ 5.30 hrs. 13.30 and 17.30 hrs.

3.2.5 Milking of Animals

Animals were hand milked twice a day, i.e., in the morning at 5.30 AM, and in the evening at 5.30 PM.

3.2.6 Observations Recorded During the Lactation Trial

The lactation trial was conducted for a period of 90 days and the following observations were recorded during the course of the study:

3.2.6.1 Feed intake

Daily DM intake was calculated by recording the daily feed offered and the residual DM throughout the experimental period.

3.2.6.2 Milk yield

Daily milk yield was recorded for individual animal by using a circular dial type spring balance with a capacity of 20 kg and an accuracy of ±0.05 kg.

3.2.6.3 Milk composition

Milk samples from individual animals were collected and analysed for milk composition at fortnightly intervals throughout the experimental period. The samples collected twice from milking of the day for each animal were proportionately pooled to represent milk sample of that animal. Representative samples were analysed for its composition (fat, Protein, lactose and SNF) using Funke gerber lactostare.

The total solids in milk were calculated as follows:

$$\text{Total Solid} = \text{Milk fat \%} + \text{Milk SNF \%}$$

3.2.6.4 Body weights

The body weights of all animals were recorded at fortnightly intervals by using Atlas platform balance of 1500 kg capacity with an accuracy of 10 kg. Animals were weighed after the morning milking before offering feed and water.

The body weight was recorded on consecutive days and the average was considered as the body weight.

3.2.7 Digestibility Trial

Digestibility trial was conducted in the mid of the lactation trial to determine the digestibility of nutrients in the animals. The digestibility trial was conducted for seven days during which proper record of feed intake and refusals by individual animals were maintained. Faecal collection was done throughout 24 h for all the animals during the digestion trial. Animals were also weighed on two consecutive days before and after the trial.

3.2.8 Collection of Biological Samples

3.2.8.1 Collection of feed samples

Representative samples of feed offered and residues were taken each morning for dry matter estimation and pooled samples of 7 days were analysed after grinding to pass through 1 mm sieve for proximate principles and fibre fractions.

3.2.8.2 Collection of faecal samples

The total quantity of faeces voided by each animal during 24 h was recorded individually and a composite sample of faeces was taken separately for each animal in clean dry bottles fitted with lid to the laboratory everyday for aliquoting.

3.2.8.3 Aliquoting of faeces

For estimation of nitrogen, 1/400 aliquot of the total faeces voided each day was preserved with 25 percent H_2SO_4 in pre-weighed plastic bottles. At the end of the collection period, the bottles were again weighed; mixed thoroughly and about 5 g sample was taken for acid digestion. For dry matter, proximate principles, NDF and ADF, 1/150 aliquot of the total faecal voided each day was dried daily at 100°C and pooled for 7 days for each animal. At the end of the collection period, the pooled samples were ground in a laboratory Willey mill for estimation of proximate principles and fibre components.

3.2.9 Analytical Procedures

3.2.9.1 Analysis of blood

Blood collection

Blood samples from all the animals were collected individually at the starting and at the end of the experiment. The blood samples from individual animals were collected by jugular vein puncture in the clean, numbered centrifuge tubes containing EDTA as anticoagulant in each tube. The serum was separated by centrifugation of the blood samples at 5000 RPM for 10 min and decanted in another numbered clean, dried plastic vials and stored in deep freeze for subsequent analysis of glucose and blood urea nitrogen.

a) Estimation of blood glucose

Estimation of blood glucose was done by using GOD-POD liquid gold kit purchased from cogent span diagnostic Ltd, Surat, India.

Principle - Glucose Oxidase (GOD) oxidizes glucose to gluconic acid and hydrogen peroxide. In presence of enzyme peroxidise, released hydrogen peroxide is coupled with phenol and 4-Aminoantipyrine (-AAP) to form coloured quinoneimie dye. Absorption of coloured dye is measured at 525 nm and is directly proportional to glucose concentration in the sample.

Reagents used –

1) Glucose Reagent
2) Glucose Diluent
3) Glucose Standard

PROCEDURE – For Spectrophotometers

Pipetted into tube marked	Blank	Standard	Test
Serum	--	--	--
Glucose Standard	--	20µL	--
Working Glucose Reagent	1500µL	1500µL	1500µL
Mixed Well, Incubated at 37°c for 10 minutes			
Purified Water	1500µL	1500µL	1500µL

1) Blanked the spectrophotometer with reagent Blank
2) Measured absorbance of standard followed by the Test (Read absorbance at 525 nm)
3) Calculated results as per given calculation formula.

$$\text{Serum Glucose (mg/dl)} = \frac{\text{Absorbance of Test}}{\textit{Absorbance of Standard}} \times 100$$

B) Estimation of blood urea nitrogen

Estimation of blood urea nitrogen was done by using NED-dye liquid gold kit purchased from cogent span diagnostic Ltd, Surat, India.

Principle- Urea condenses with o-phthaldehyde and Naphthyl Ethylene Diamine (NED) to form coloured complex. The rate of formation of this complex is directly proportional to urea concentration in the sample and is measured in an Initial Rate (fixed time) mode at 525 nm.

Reagents used –

1) o-phthaldehyde Reagent
2) NED Reagent
3) Urea Standard

PROCEDURE – For Spectrophotometers

Pipetted into tube marked	Blank	Standard	Test
Purified Water	500µL	-	-
Reagent 1	-	500µL	500µL
Standard	-	-	50µL
Serum	-	-	-
Mixed Properly			
Reagent 2	-	500µL	500µL

Mixed well.

1) Blanked the spectrophotometer with reagent Blank
2) Measured absorbance of standard followed by the Test (Read absorbance at 525 nm)
3) Calculated results as per given calculation formula.

$$\text{Serum urea concentration (mg/dl)} = \frac{AT2 - AT1}{AS2 - AS1} \times 100$$

Where-

1) AT1= Initial reading of test (OD).
2) AT2= Reading after 60 seconds of AT1.
3) AS1=Initial reading of standard (OD).
4) AS2= Reading after 60 seconds of AS1.

3.2.9.2 Estimation of Proximate Principles

Ground samples of concentrate mixture, wheat straw, green maize fodder, feed refusal and faces were analyzed for proximate principles; viz., dry matter (DM),organic matter (OM).crude protein (CP), ether extract (EE), crude fibre (CF) and total ash as per standard procedures of Association of Official Analytical Chemists 1992 as follows:

a) Dry Matter (DM)

A known quantity of ground sample was taken in a pre-weighed moisture cup. These cups were placed in a hot air oven at 100 ± 3°C for 24 h. The loss in moisture content after drying was estimated and dry matter was calculated as follows:

$$\text{DM (\%)} = \frac{\text{Weight of sample after drying}}{\text{Weight of the fresh sample}} \times 100$$

b) Organic Matter (OM)

It was determined by subtracting the total ash content from 100.

OM (%) = 100 – Total ash (%)

c) Crude Protein (CP)

Crude protein was estimated as per Kjeldahl's method. A known quantity of sample was taken in Kjeldahl flask and digested with concentrated H_2SO_4 and 2-3 g of digestion mixture (Na_2SO_4 and $CuSO_4$ in the ratio of 9:1) till the solution became colourless. After digestion, the contents were cooled and volume was

made to 100 ml. Ten ml of aliquot was distilled in Kjeldahl Distillation apparatus. About 100 ml of distillate was collected into a conical flask containing 10 ml of 2 per cent boric acid solution having mixed indicator (0.1% methyl red and 0.1% bromocresol green in the ratio of 2:1 in absolute alcohol). The distillate was then titrated against standard sulphuric acid solution (N/10).

$$N\ (\%) = \frac{0.0014 \times \text{Volume of N/10 } H_2SO_4 \text{ used} \times \text{Volume made (ml)}}{\text{Aliquot taken (ml)} \times \text{Sample taken (g)}} \times 100$$

The crude protein (%) of sample was calculated by multiplying the N-content with factor 6.25.

d) Total Ash

A known quantity of sample was taken in pre-weighed china crucible. After charring the sample (till the smoke disappeared), the crucible was kept in muffle furnace for ignition at 550°C for 2-3 h. the crucible was removed on cooling and kept in a desiccator and weighed again to find out weight of ash. The ash content was calculated as given below:

$$\text{Total ash } (\%) = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

e) Ether Extract (EE)

A known quantity of ground sample was taken in a Whatman's thimble and extracted for 10-12 hrs with petroleum ether (40-60°C) in Soxhlet's Extraction apparatus attached to a pre-weighed oil flask. The oil flask was removed and after evaporating the excess of ether, it was dried overnight in an oven for. The flask was cooled in a dessicator and weighed. The difference in two weights gave the amount of ether extract in the sample.

$$EE\ (\%) = \frac{\text{Weight of ether extract}}{\text{Weight of sample}} \times 100$$

f) Crude Fibre (CF)

A known quantity of moisture and fat free sample (after ether extraction) was taken in spoutless beaker of 1 Lit. capacity previously marked to 200 ml. Then 25 ml of 10 per cent H_2SO_4 (v/v) was added and volume was made up to 200 ml with water from the sides of the beaker to have 1.25 per cent acid

solution in the beaker. The contents of beaker were refluxed for 30 minutes and then filtered through muslin cloth using Buchner funnel with the help of vacuum pump. After repeated hot water washings, the residue left on muslin cloth was transferred to the same spout less beaker with smooth steel spatula, followed by little washing of muslin cloth. Took 25 ml of 10 per cent NaOH (w/v) and volume was made to 200 ml with water to have 1.25 per cent alkali solution. Contents were refluxed for 30 minutes and filtered through muslin cloth and washed with hot water. The residue left on muslin cloth was transferred to a clean silica crucible with the help of steel spatula. The contents were dried in hot air oven at 100 ± 5°C and weighed. Silica crucible containing dried residue was kept in a muffle furnace at 550°C for 2 h for ashing and weighed after cooling. The crude fibre content of sample was estimated as follows:

$$\text{Crude fibre (\%)} = \frac{\text{Weight of dried residue} - \text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

g) Nitrogen Free Extract (NFE)

The nitrogen free extracts (NFE) of sample was estimated as follows:

NFE (%) = 100 – [CP% + EE% + CF% + %Total ash]

h) Cell wall components and cell soluble

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were estimated as per Goering and Van Soest (1970).

i) Neutral detergent fibre

Preparation of neutral detergent solution

Reagents

Sodium lauryl sulphate	-	30.00 g
Disodium ethylene diamino tetra acetate (EDTA) decahydrate	-	18.61 g

Sodium borate decahydrate	-	6.81 g
Disodium hydrogen phosphate (anhydrous)	-	4.56 g
Triethylene glycol	-	10 ml
Distilled water	-	1lit

Solution Preparation

EDTA and sodium borate decahydrate were put together in a large beaker with some distilled water and heated on hot plate until dissolved. The solution containing Sodium lauryl sulphate and Triethylene glycol was added. Disodium hydrogen phosphate was taken in another beaker and some amount of distilled water was added and the contents were heated until dissolved. Then, it was added to solution containing other ingredients and volume was made up to one litre.

Procedure

Weighed 0.5 to 1 g of sample which was taken in 1 L spout less beaker. To this, 100 ml neutral detergent solution was added. Decalin was avoided because the fat contents of samples were very low. The contents of spoutless beaker were refluxed for one hour. After refluxing, the sample was filtered through pre-weighed 50 ml capacity sintered glass crucible grade-I using oil-free vacuum pump. Material was washed with hot boiling water and then acetone to remove all salts. The sintered crucible containing residue was dried in hot air oven (100 ± 5°C) and weighed again.

The NDF was calculated as follows:

$$NDF\ (\%) = \frac{\textit{Weight of dry residue}}{\textit{Weight of sample taken}} \times 100$$

ii) Acid detergent fibre (ADF)

Acid detergent solution: 20 g cetyl-trimethyl-ammonium bromide (CTAB) was dissolved in one litre of 1 N H_2SO_4.

Procedure

Approximately 1 g of sample was taken in a spoutless beaker of 1 L capacity. To this, 100 ml acid detergent solution was added and the contents were refluxed for exactly 1 hour. After refluxing the residue was filtered through pre-weighed sintered glass crucible grade-I using vacuum pump, washed with hot water and followed by aceton to remove all salts.

The ADF was determined as follows:

$$ADF\ (\%) = \frac{Weight\ of\ dry\ residue}{Weight\ of\ sample\ taken} \times 100$$

iii) Cellulose

Principles:

For estimation of cellulose the acid detergent fibre (ADF) procedure is used as a preparatory step. The ADF residue consists of cellulose, lignin, cutin and acid insoluble ash (mainly silica) treatment with sulphuric acid dissolves cellulose.

Procedure:

Sintered crucible grade G-I containing ADF contents was placed in enamel tray in such a manner that one end of the enamel tray was at about 2 cm height than the other end, so that acid could drain away from the crucible. The crucible was then filled with 72 percent H_2SO_4 (w/w basis) and the contents were stirred with glass rod to break all the lumps. The crucible was refilled with 72 percent H_2SO_4 after 1 hour interval. After 8-10 hours, the crucible was removed from the tray and filtration of acid was done by using vacuum pump. The material was washed with hot water until free form acid and kept in oven (100 ± 5°C) overnight and weighed.

$$Cellulose\ (\%) = \frac{W1-W2}{Y} \times 100$$

Where,

W1= wt of crucible + wt of sample (before acid extraction)

W2= wt after extraction

Y = wt of initial sample (DM)

iv) Hemicellulose

Hemicellulose was calculated by subtraction of ADF from NDF as follows:

$$\textit{Hemicellulose (\%)} = \textit{NDF (\%)} - \textit{ADF (\%)}$$

v) Acid detergent lignin (ADL)

Sintered crucible grade G-I containing ADF contents was placed in enamel tray in such a manner that one end of the enamel tray was at about 2 cm height than the other end, so that acid could drain away from the crucible. The crucible was then filled with 72 percent H_2SO_4 (w/w basis) and the contents were stirred with glass rod to break all the lumps. The crucible was refilled with 72 percent H_2SO_4 after 1 hour interval. After 3 hours, the crucible was removed from the tray and filtration of acid was done by using vacuum pump. The material was washed with hot water until free form acid and kept in oven (100 ± 5°C) overnight and weighed. Crucible was then kept in muffle furnace for ashing. The acid detergent lignin was calculated as follows:

$$\textit{ADL (\%)} = \frac{\textit{Loss of Weight on ashing}}{\textit{Weight of sample}} \times 100$$

3.2.10. Calculations

3.2.10. 1 Intake of nutrients

Intake of different nutrients and other dietary components was calculated as follows:

Intake of nutrients = Total quantity of nutrients offered through feed – Quantity of nutrients left in residue

3.2.10. 2 Digestibility of nutrients:

Digestibility of nutrients =   DMI × % of nutrient in feed – Dung DM outgo X % of nutrient in dung × 100

DM intake × % of nutrient in feed

3.2.10. 3 Total digestible nutrients

Total digestible nutrient percentage was calculated using the following formula:

TDN = Digestible crude fibre **+** Digestible CP + Digestible NFE
+ (Digestible ether extract x 2.25)

3.2.10. 4 FAT CORRECTED MILK YIELD (4% FCM)

The fat corrected milk (FCM) was calculated by the following equation given by ARC (1980):

$$FCM\ (kg) = 0.4\ M + 15\ F$$

Where,

M = Milk yield (kg), and

F = Weight of fat contained in milk (kg).

3.3 Statistical Analysis

Data was analysed as one way ANOVA using standard statistical procedures as described by Snedecor and Cochran (1986).

CHAPTER – 4

Results and Discussion

4. RESULTS AND DISCUSSION

4.1 Enzyme Activity

The commercial fibrolytic enzymes namely, cellulase and xylanase, were procured from M/s Biocon Ltd. Bangalore. These enzymes were in powder form and on testing exhibited activities of 4,000 µM glucose/g/h and 7,990 µM xylose /g/min of cellulase and xylanase, respectively. In the present study, both enzymes were mixed in the ratio of 50:50 (w/w) and used at three levels, i.e., 0.0 g (no enzyme), 1.5 g (cellulase and xylanase) and 3.0 g (cellulase and xylanase) per kg DM of the ration. The enzyme mixtures were added to the concentrate portion of the TMR. The standard curves of cellulase and xylanase estimation are given in Figures 3.1.1 and 3.1.2 respectively, in Materials and Methods.

4.2 EFFECT OF SUPPLEMENTING EXOGENOUS FIBROLYTIC ENZYMES (EFE) ON NUTRIENT INTAKE, DIGESTIBILITY, MILK PRODUCTION AND COMPOSITION IN LACTATING MURRAH BUFFALOES.

To investigate the effect of supplementing fibrolytic enzyme mixture at two levels viz. 1.5 and 3.0 g/kg DM (Cellulase 4000 µM glucose/g/h + Xylanase 7990 µM xylose/g/min; 50:50 w/w) to the concentrate portion of the TMR on milk production, nutrient intake and digestibility, eighteen Murrah buffaloes were allotted to three dietary treatments on the basis of milk yield (8.48, 8.52 and 8.53 kg/d) and stage of lactation (68.5, 80.33 and 82 days in milk). The details of experimental animal are given in Table 3.1 in the Materials and Methods. The TMR consisted of 45 percent wheat straw, 15 percent green maize and 40 percent concentrate, on DM basis. First group being control, was fed this diet without any enzyme supplementation whereas, T-1 was fed the same TMR as to the control group and supplemented with 1.5 g enzyme mixture/kg DMI of the ration and T-2 was fed same TMR as to the control group and supplemented with 3.0 g enzyme mixture/kg DM of the ration. Lactation trial was conducted for 12 weeks and a digestibility trial was carried out to

estimate the nutrient digestibility in the middle of the lactation trial. Observations were recorded with respect to daily milk yield, DM intake, fortnightly body weights and milk composition. The chemical composition of feed stuffs and TMR used during lactation trial is presented in Table 4.1 and Fig. 4.1.

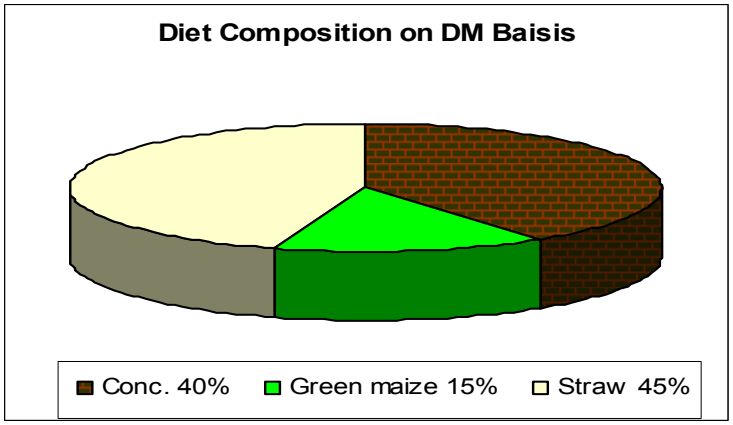

Fig. 4.1 Composition of TMR on Dry Matter basis

4.2.1 EFFECT OF SUPPLEMENTING EXOGENOUS FIBROLYTIC ENZYMES ON BODY WEIGHT CHANGES

The effect of supplementation of fibrolytic enzymes on body weight changes of lactating buffaloes is presented in Table 4.2. Body weights at the start of experiment were 564.41, 563.89 and 550.21kg in control, T-1 and T-2 groups, respectively. After 12 weeks of experimental feeding, the average body weights of six fortnights were 568.72, 570.06 and 561.17 kg in control, T-1 and T-2 groups respectively. Body weights ranged between 566.25 to 574.36 in control, 565.35 to 576.43 in T-1 and 554.34 to 567.53 kg in T-2 groups, respectively over different fortnights. No significant difference in body weights was recorded between different groups during different fortnights.

Similar trend was observed in case of metabolic body weights ($W^{0.75}$) as given in Table 4.3. Mean metabolic body weights at the beginning of the trial were 115.72, 115.68 and 113.58 kg in control, T-1 and T-2 respectively. After 12 weeks of experimental feeding the average metabolic body weights ($W^{0.75}$) of six fortnights were 116.35, 116.65 and 115.26 kg in control, T-1 and T-2

Table 4.1: Chemical composition (on % DM basis) of feed ingredients used during the lactation trial

Parameters	Concentrate mixture	Green Maize	Wheat Straw	TMR
DM	89.11	12.98	90.07	41.13
OM	92.25	88.94	91.56	91.44
CP	19.93	9.98	4.43	11.44
EE	3.82	1.97	1.35	2.41
CF	8.82	28.19	42.28	26.81
NFE	60.38	48.80	43.50	50.78
Total ash	7.75	11.06	8.44	8.56
NDF	30.23	52.47	77.78	55.83
ADF	13.48	32.80	50.43	32.66
Hemi cellulose	16.75	19.67	27.35	23.17
Cellulose	8.69	25.18	38.77	24.73
ADL	4.25	5.26	8.53	5.90
Cell contents	69.77	47.53	22.22	44.17
Silica	1.05	2.23	3.52	2.14

respectively and these ranged between 115.84 to 117.22 in control, 115.93 to 117.63 in T-1 and 114.21 to 116.22 kg in T-2 groups respectively, during the experimental period. There was no difference in the three groups over different fortnights.

Feeding of fibrolytic enzymes did not show any effect on body weight gains in the present investigation. Various workers have reported variable effects of fibrolytic enzyme supplementation on body weight changes in lactating ruminants. Yang *et al.* (2000) reported no effect on body weight changes in cows fed the control diet, enzyme treated TMR or enzyme treated barley based concentrate. Shojaeian and Thakur, (2007) also reported no effect on body weight changes due to enzyme supplementation to the urea treated wheat straw based TMR fed to dairy cows.

Knowlton *et al.* (2002) reported a numerical increase in the body weight in early lactation cows fed diets containing enzyme supplements. But as the buffaloes used in the current study were both in early and mid lactation, no clear-cut effect on body weight change was observed. Similar findings were also reported by several other workers (Rode *et al.,* 1999; Beauchemin *et al.,* 2000 and Titi 2003).

The present findings suggested that enzyme supplementation in concentrate mixture of lactating buffaloes did not affect the body weight change.

4.2.2 Effect on Dry Matter Intake

The average DM intake (Table 4.4) over different fortnights varied from 12.43 to 13.07 kg/d in control group, 13.17 to 14.02 kg/d in T-1 and from 12.79 to 13.21 kg/d in T-2 group. There was no significant difference in DM intake between the control and the two treatment groups throughout the experimental period. Similar trend was observed in the overall average DMI in three groups, the values being 12.77, 13.61, and 12.97 kg/d in control, T-1 and T-2 groups, respectively.

The average DM intake per 100 kg BW (Table 4.5) ranged from 2.19 to 2.29 kg/d in the control group while it ranged from 2.32 to 2.45 in T-1 and 2.30 to 2.36 kg/d in T-2 groups, respectively, over the entire experimental period. The average DM intake (kg/100 kg body weight) was 2.24, 2.39 and 2.33 kg for the control, T-1 and T-2 respectively, which was statistically similar.

The DM intake g/kg metabolic body weight ($W^{0.75}$) ranged from 106.85 to 111.93 in control while it ranged from 113.11 to 119.76 and from 111.74 to 114.62 g/d in T-1 and T-2 groups, respectively in different fortnights (Table 4.6). The overall average DM intake g/kg metabolic body weight was 109.36 in control group and 116.50 and 112.86 g/d in T-1 and T-2 groups, respectively.

Thus, the results of the present study indicate that supplementation of exogenous fibrolytic enzymes (Cellulase 4000 µM glucose/g/h + Xylanase 7990 µM xylose/g/min; 50:50 w/w) @ 1.5 g/kg and 3.0 g/kg DMI did not affect the DMI. The results obtained in present study are in agreement with those of Rode *et al.,* (1999), who observed no effect on feed intake when cows were fed on enzyme-treated grain mix. Zheng *et al*. (2000) applied a mixture of cellulase and xylanase enzymes @ of 1.25 Litre of enzyme concentrate/tonne of forage DM and found no change in feed intake of cows. Shojaeian and Thakur, (2007) reported there was no effect on DM intake when sahiwal cows were supplemented with fibrolytic enzyme mixture @ 1.5 g (3365+6085 I.U of cellulase+ xylanase)/ kg of feed. Similarly, Kung *et al*. (2000a) also did not observe any effect of exogenous fibrolytic enzyme supplementation on DMI. However, enzyme supplementation had been reported to increase DMI in some other studies by Stokes and Zheng, (1995) and Lewis *et al*., (1999), Beauchemin *et al*., (2001) reported that across 16 dairy cow studies, the average increase in dry matter intake due to enzyme supplementation was 1.6 kg/day. The reason for this effect in this may be due to the fact that cows used were high yielder and in early lactation.

4.2.3 Effect on Nutrient Intake

4.2.3.1 Effect on CP intake

Table 4.2: Fortnightly body weights (kg) changes in Murrah buffaloes

Fortnights	Control	T-1	T-2
0	564.41±20.33	563.89±13.71	550.21±12.47
1	566.25±25.12	567.75±12.66	554.34±13.40
2	569.36±25.41	572.83±9.66	562.83±16.10
3	565.38±24.31	565.35±8.78	560.87±16.95
4	567.84±29.11	573.38±9.04	566.30±17.55
5	574.45±26.16	576.43±8.71	567.53±17.59
6	573.36±27.34	570.78±8.82	566.13±17.29
Average	**568.72 ± 25.40**	**570.06 ± 10.20**	**561.17 ± 15.91**

Table 4.3: Fortnightly metabolic body weight ($w^{0.75}$kg) changes in Murrah buffaloes

Fortnights	Control	T-1	T-2
0	115.72±3.14	115.68±2.12	113.58±1.93
1	115.97±3.88	116.28±1.95	114.21±2.07
2	116.45±3.92	117.07±1.48	115.51±2.48
3	115.84±3.74	115.93±1.35	115.20±2.61
4	116.18±4.50	117.16±1.38	116.03±2.70
5	117.22±4.01	117.63±1.33	116.22±2.71
6	117.05±4.19	116.76±1.35	116.01±2.66
Average	**116.35 ± 3.88**	**116.65 ± 1.49**	**115.26 ± 2.43**

Table 4.4: Fortnightly total DMI (kg/day) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	12.89±0.74	13.17±0.62	13.03±0.56
2	13.07±0.77	14.02±0.46	12.93±0.61
3	12.65±0.87	13.66±0.42	12.79±0.62
4	12.43±0.89	13.53±0.44	12.90±0.52
5	12.73±0.91	13.59±0.36	12.94±0.60
6	12.83±0.90	13.67±0.40	13.21±0.67
Average	**12.77 ± 0.82**	**13.61 ± 0.44**	**12.97 ± 0.57**

Table 4.5: Fortnightly dry matter intake (kg/100 kg BW/day) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	2.27±0.06	2.32±0.09	2.36±0.14
2	2.29±0.07	2.45±0.07	2.31±0.15
3	2.23±0.10	2.42±0.08	2.30±0.17
4	2.19±0.11	2.36±0.08	2.30±0.15
5	2.22±0.11	2.36±0.07	2.30±0.16
6	2.24±0.12	2.40±0.09	2.36±0.18
Average	**2.24 ± 0.09**	**2.39 ± 0.08**	**2.33 ± 0.15**

Table 4.6: Fortnightly dry matter intake (g/kg $w^{0.75}$) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	110.74±3.45	113.11±4.52	114.46±6.12
2	111.93±3.91	119.76±3.61	112.49±6.73
3	108.81±5.17	117.86±3.87	111.74±7.48
4	106.85±5.61	115.54±3.76	111.80±6.46
5	108.40±5.75	115.55±3.12	112.07±7.09
6	109.46±5.94	117.20±3.94	114.62±7.84
Average	**109.36 ± 4.97**	**116.50 ± 3.80**	**112.86 ± 6.95**

Table 4.7: Fortnightly CP intake (g/d) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	1466.46±84.43	1498.39±70.74	1482.83±63.57
2	1487.76±75.45	1595.71±52.51	1471.98±69.18
3	1439.69±65.20	1553.95±48.32	1455.08±70.51
4	1414.91±68.84	1540.01±59.62	1467.60±58.98
5	1449.23±78.24	1546.25±50.46	1473.08±68.70
6	1459.52±86.48	1555.79±45.35	1502.82±76.14
Average	**1452.93 ± 93.06**	**1548.35 ± 49.53**	**1475.57 ± 65.16**

Table 4.8: Fortnightly CP intake (g/100 kg BW) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	262.28±7.67	281.21±8.06	267.00±16.61
2	252.24±10.30	271.60±9.35	260.91±18.68
3	249.96±12.62	272.58±9.05	264.06±17.12
4	255.58±12.94	269.86±7.34	262.68±18.51
5	254.26±13.32	270.12±8.47	267.54±19.92
6	253.39±10.34	271.70±10.47	262.92±17.51
Average	**254.62 ± 10.73**	**272.85 ± 8.60**	**264.18 ± 17.81**

Table 4.9: Fortnightly CP intake (g/kg $W^{0.75}$) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	12.60±0.39	12.87±0.51	13.03±0.70
2	12.74±0.44	13.63±0.41	12.80±0.77
3	12.38±0.59	13.41±0.44	12.72±0.85
4	12.16±0.64	13.15±0.43	12.72±0.73
5	12.34±0.65	13.15±0.35	12.75±0.81
6	12.46±0.68	13.34±0.45	13.04±0.89
Average	**12.45 ± 0.53**	**13.26 ± 0.42**	**12.84 ± 0.77**

Overall average CP intakes (Table 4.7) were 1452.93, 1548.35 and 1475.57 g/d in control, T-1 and T-2 groups, respectively. CP intakes varied from 1414.91 to 1487.76 g/d in the control, 1498.39 to 1595.71 and 1455.08 to 1502.82 in T-1, T-2 groups respectively, in different fortnights. Overall average CP intake g/ per 100 kg body weight (Table 4.8) was 254.62, 272.85 and 264.18 g/d for control, T-1 and T-2 groups, respectively. Mean CP intakes (Table 4.9) per kg $W^{0.75}$ were 12.45, 13.26, and 12.84 for control, T-1 and T-2 groups, respectively. There was no difference in the CPI in the three treatments groups.

4.2.3.2 Effect on TDN intake

Average TDN intakes (Table 4.10, Fig. 4.2) were 7.26, 8.17, and 7.55 kg/day in control, T-1 and T-2 groups, respectively, which was significantly higher ($P<0.05$) by 12.53 percent in the T-1 over that of control group .TDN intakes ranged from 7.07 to 7.43, 7.90 to 8.42 and 7.45 to 7.69 kg/d in the control, T-1 and T-2 groups, respectively, in different fortnights. TDN intake (kg/day) was significantly higher ($P<0.05$) in the first, second and fourth fortnight in T-1 than that of control group.

Average TDN intake, kg/100 kg BW (Table 4.11) were 1.27, 1.43, and 1.36 in control, T-1 and T-2 respectively, which were significantly higher ($P<0.05$) by 12.59 percent in T-1 over that of control group. There was no difference between control and T-2. Average TDN intakes (Table 4.12) , g / kg $W^{0.75}$ were 62.20, 70.04 and 65.89 in control T-1 and T-2 groups respectively, which was also significantly higher ($P<0.05$) by 12.60 percent in T-1 over that of control. However, there was no difference between control and T-2. It was evident from the results obtained that there was significant ($p<0.05$) improvement in TDN intake in buffaloes on supplementation of fibrolytic enzymes mixture @1.5g/kg DM of TMR.

The above findings further corroborate the finding of the similar reports that average TDN intake was significantly higher ($p<0.01$) by 10.27 percent in experimental group over that of control in Sahiwal cows fed urea treated wheat

straw (Shojaeian and Thakur 2007). Miachieo and Thakur (2007), also recorded higher TDN intake in sahiwal cows fed wheat straw based ration fortified with exogenous fibrolytic enzymes. The overall average nutrient intakes are presented in a condensed form in Table 4.13.

4.2.4 Effect on Plane of nutrition

Nutrient requirements as per Kearl (1982) and actual intakes of DM, CP and TDN by the buffaloes of control and two treatment groups have been presented in Table 4.14. Against the requirement of 13.08, 13.60 and 13.13 kg/day, the actual DM intake was 12.77, 13.61 and 12.97, kg/day in control, T-1 and T-2 groups, respectively. Similarly, against the requirement of 1692.5g, 1832.5 and 1475.57 g/day CP intake the actual intake was 1542.93, 1548.35 and 1475.57 g/day showing deficit of 14.15, 15.51 and 16.55 in T-1, T-2 and T-3 groups, respectively. TDN intake was lower than requirement by 14.59, 9.92 and 14.98 in control, T-1, and T-2 groups respectively.

4.2.5 Effect of Supplementation of Fibrolytic Enzymes on Milk Yield and Composition

The milk production and composition on supplementing cellulase and xylanase at two levels (50:50 w/w) at 1.5 g/kg and 3.0 g/kg of DM intake to the concentrate portion of the TMR, fed to lactating Murrah buffaloes have been presented in this section.

4.2.5.1 Effect on milk production

The average milk yield (kg/d) of 90 days lactation trial (Table 4.15, Fig.4.3) was 8.62, 9.74, and 9.01 kg in control T-1 and T-2 groups respectively. The milk yield ranged between 8.56 to 9.23, 8.90 to 10.31 and 8.36 to 9.42 kg/d in control, T-1 and T-2 groups, respectively in different fortnights. Average daily milk yield was numerically higher for all fortnights in the treatment groups as compared to that of the control group. Though milk yield (kg/d) was similar at the start of the experiment (8.48 in control, 8.52 in T-1 and 8.53 kg/d in T-2 group), the average milk yield during the entire experimental period was higher

Table 4.10: Fortnightly TDN intake (kg/d) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	7.32^{a}±0.9	7.90^{b}±0.11	7.59^{ab}±0.10
2	7.43^{a}±0.10	8.42^{b}±0.13	7.54^{ab}±0.09
3	7.19±0.12	8.20±0.13	7.45±0.10
4	7.07^{a}±0.12	8.12^{b}±0.11	7.51^{ab}±0.11
5	7.24±0.12	8.16±0.13	7.54±0.12
6	7.29±0.14	8.21±0.13	7.69±0.12
Average*	**7.26^{a} ± 0.25**	**8.17^{b} ± 0.12**	**7.55^{a} ± 0.11**

* Figures with different superscripts in a row differ significantly (P<0.05)

Table 4.11: Fortnightly TDN intakes (kg/100 kg BW) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	1.29±0.03	1.39±0.05	1.38±0.08
2	1.30±0.04	1.47±0.04	1.35±0.09
3	1.27±0.05	1.45±0.05	1.34±0.10
4	1.25±0.06	1.42±0.05	1.34±0.09
5	1.26±0.06	1.42±0.04	1.34±0.09
6	1.27±0.07	1.44±0.05	1.37±0.10
Average*	**1.27^{a} ± 0.03**	**1.43^{b} ± 0.03**	**1.36^{a} ± 0.04**

* Figures with different superscripts in a row differ significantly (P<0.05)

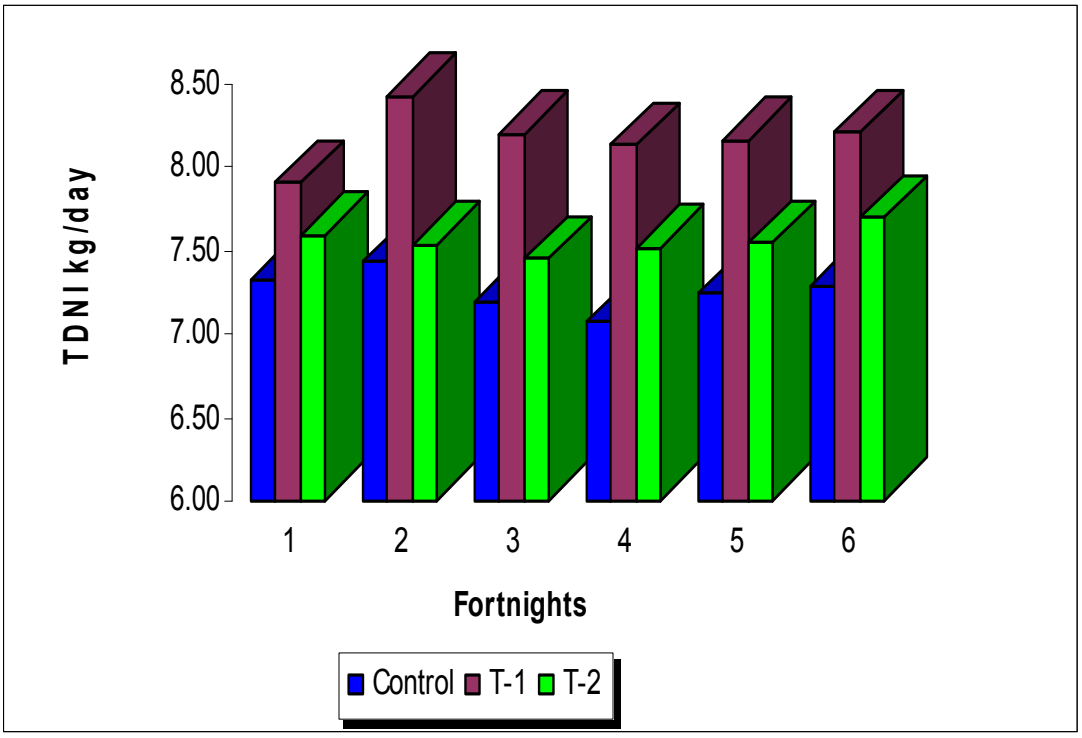

Fig. 4.2: Fortnightly average TDNI kg/day

Table 4.12: Fortnightly TDN intakes (g/kg $W^{0.75}$) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	62.94±1.96	67.90±2.71	66.69±3.57
2	63.62±2.22	71.89±2.17	65.54±3.92
3	61.85±2.94	70.75±2.32	65.10±3.36
4	60.73±3.19	69.36±2.26	65.14±3.76
5	61.62±3.27	69.37±1.87	65.29±3.13
6	62.22±3.37	70.35±2.36	66.78±4.57
Average*	**62.20^{a} ± 1.11**	**70.04^{b} ± 0.89**	**65.89^{a} ± 1.37**

* Figures with different superscripts in a row differ significantly (P<0.05)

Table 4.13: Overall mean body weights and nutrient intakes in Murrah buffaloes fed control and enzyme supplemented diets

Particulars	Groups		
	Control	T-1	T-2
Body weight (kg)	568.72±25.40	570.06±10.20	561.17±15.91
Metabolic BW (kg)	116.35±3.88	116.65±1.49	115.26±2.43
DMI, kg/day	12.77±0.82	13.61±0.44	12.97±0.57
DMI, kg/100 kg BW	2.24±0.09	2.39±0.08	2.33±0.15
DMI,g/$W^{0.75}$	109.36±4.97	116.50±3.80	112.86±6.95
CP intake, g/day	1452.93±93.06	1548.35±49.53	1475.57±65.16
CP intake g/100 kg BW	254.62±10.73	272.85±8.60	264.18±17.81
CP intake, g/$W^{0.75}$	12.45±0.53	13.26±0.42	12.84±0.77
TDN intake, kg/day	7.26^{a}±0.25	8.17^{b}±0.12	7.55^{a}±0.11
TDN intake/100 kg BW	1.27^{a}±0.03	1.43^{b}±0.03	1.36^{a}±0.04
TDN intake, g/kg $W^{0.75}$	62.20^{a}±1.11	70.04^{b}±0.89	65.89^{a}±1.37

*Figures with different superscripts in a row differ significantly (P<0.05)

Table 4.14: Comparison of plane of nutrition of buffaloes with feeding standard

Particulars	Groups		
	Control	T-1	T-2
DMI (kg/day)	12.77±0.82	13.61±0.44	12.97±0.57
Requirement as per Kearl (1982)	13.08	13.60	13.13
CP Intake (g/day)	1452.93±93.06	1548.35±49.53	1475.57±65.16
Requirement as per Kearl (1982)	1692.5	1832.5	1768.28
Deficit in CP intake (%)	14.15	15.50	16.55
TDN Intake (g/day)	7.26^{a}±0.25	8.17^{b}±0.12	7.55^{a}±0.11
Requirement as per Kearl (1982)	8.50	9.07	8.88
Deficit in TDN Intake (%)	14.59	9.92	14.98

Table 4.15: Fortnightly milk yield (kg/day) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
0	8.48±0.70	8.52±0.52	8.53±0.56
1	8.56^{a}±0.16	9.33^{b}±0.14	8.95^{ab}±0.17
2	8.95^{a}±0.17	10.31^{b}±0.11	9.26^{a}±0.18
3	9.23^{a}±0.19	10.24^{b}±0.11	9.42^{a}±0.18
4	9.02^{a}±0.21	9.96^{b}±0.09	9.22^{a}±0.17
5	8.52^{a}±0.28	9.68^{b}±0.09	8.86^{a}±0.17
6	7.44^{a}±0.24	8.90^{b}±0.09	8.36^{c}±0.16
Average*	**8.62^{a} ± 0.19**	**9.74^{b} ± 0.12**	**9.01^{a} ± 0.17**

* Figures with different superscripts in a row differ significantly (P<0.05)

(P<0.05) by 12.99 % in group T-1 over that of control and 8.10 % in T-1 over that of T-2 whereas, there was no difference between milk yield of T-2 and control.

The findings of the present study are in agreement with the results of other workers who have reported increased milk production in cows fed fibrolytic enzymes supplemented rations (Lewis *et al*., 1999; Rode *et al.*, 1999; Yang *et al.*, 2000). Yang *et al.* (1999) studied the effect of supplementing fibrolytic enzymes to a diet consisting of 45 percent concentrate, 10 percent barley silage and 45 percent cubed alfalfa hay and found that milk production increased (P<0.05) by 7 percent for cows fed enzyme supplemented diets as compared with those fed the control diet. Titi (2003) also reported that cows fed enzyme supplemented diet produced 37% more milk than the cows fed control diet. Similarly, Shojaeian and Thakur (2007), reported 9.98% increase in milk yield of Sahiwal cows supplemented cellulase and xylanase @ 1.5g/kg DM to urea treated wheat straw based ration. Average milk yield and 4% FCM yield increased in sahiwal cows by 7.66 and 8.58%, respectively in cows supplemented with cellulase and xylanase mixture (50:50w/w) @ 1.5g/kg DM of the wheat straw based TMR (Miachieo and Thakur, 2007). Similarly Rana and Singh (2007), reported increased milk yield and fat corrected milk by 7.1% and 10.9% in Murrah buffaloes, ration supplemented with 15g/d of fibrozyme (cellulase and hemicellulase; 100 IU as xylanases/g) for 10 weeks.

4.2.5.2 Effect on 4 percent FCM production

Average fat corrected milk (4% FCM) yields over the entire experimental period have been presented in Table 4.16 and Fig.4.4. Mean 4% FCM yield (kg/d) ranged from 11.69 to 14.21, 14.23 to 16.21, and 13.55 to 15.07 kg/d in control, T-1 and T-2 groups respectively in different fortnights. Average FCM yields were 13.32, 15.34 and 14.43 kg/d in control, T-1 and T-2 groups, respectively, showing significantly higher FCM yield (P<0.05) by 15.17% in T-1 over that of control whereas, there was no difference in that of T-2 and control.

Several researchers have reported similar findings to the present one. Kung *et al.,* (2000a) supplemented cellulase and xylanase to the forage portion of the diet (whole plant corn silage 45%, alfalfa hay 5% and concentrate 50%) to dairy cows and reported higher (P<0.15) 3.5 percent FCM production than cows fed the control diet. Titi (2003) supplemented exogenous fibrolytic enzymes to the concentrate diet of dairy cows in early lactation and reported that the enzyme supplemented group had higher (P<0.05) milk yield and 3.5 percent FCM yield as compared to the control group. Bowman *et al.*, (2002) supplemented fibrolytic enzymes to the TMR (45% concentrate and 55% of barley silage and alfalfa haylage) of dairy cows through the concentrate portion of TMR and reported higher FCM yield in the treatment group, Shojaeian and Thakur (2007), fortified cellulase and xylanase enzymes to a TMR (40% wheat straw, 40% concentrate and 20% green) and reported 8.58 percent higher (P<0.05) FCM yield in supplemented cows over that of control group. Similarly Rana and Singh (2007) reported 10.9% increased FCM yield on feeding fibrozyme to Murrah buffaloes.

The results of the present study indicate that supplementation of cellulase and xylanase mixture (50:50 ratio w/w) @1.5g/kg feed DM so as to provide about 4000 µM glucose/g/h cellulase and 7990 µM xylose/g/min xylanase by adding the enzyme mixture in powder form to the concentrate portion of the TMR (45% wheat straw, 40% concentrate and 15% green maize) increased milk yield and FCM yield significantly.

4.2.5.3 Effect on milk composition

A) Total solids (%):

The average total solids (%) content of milk (Table 4.17) was 18.16, 18.34, and 18.50 in the control and T-1 and T-2 groups, respectively, which were statistically similar. Mean total solids contents (%) of milk at different fortnights were also similar and ranged from 18.05 to 18.25, 18.13 to 18.57 and 18.28 to 18.67 in control, T-1 and T-2 groups, respectively. Several workers have also reported no effect on milk total solids contents on fortifying fibrolytic

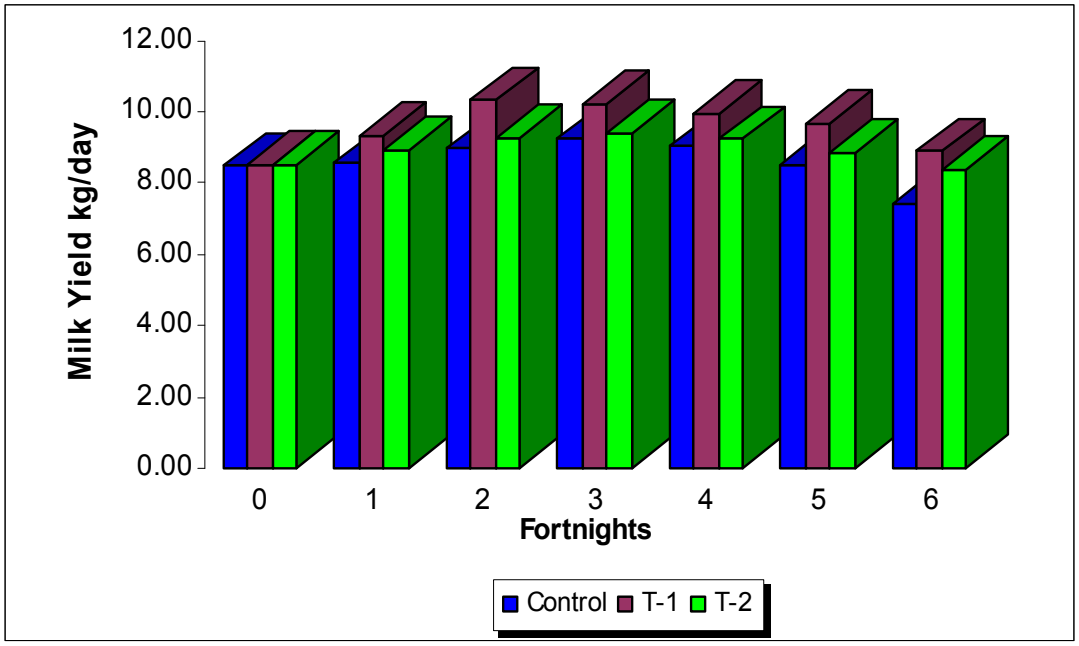

Fig. 4.3 (a): Fortnightly milk yield kg/day

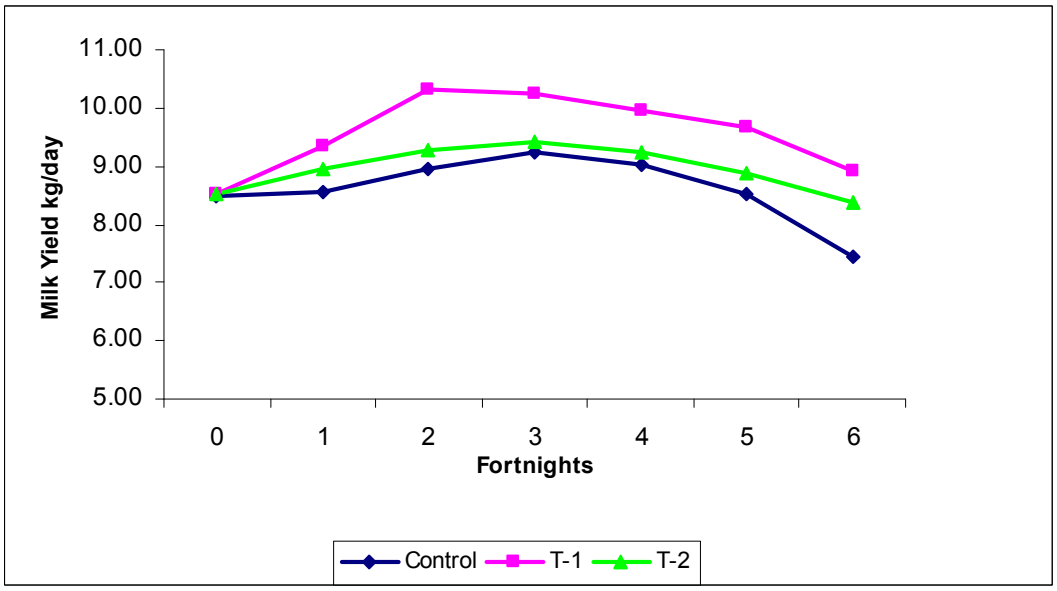

Fig. 4.3 (b): Fortnightly milk yield kg/day

Table 4.16: Fortnightly fat corrected milk yield (FCM, 4% fat) of Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
0	12.85±0.89	13.06±0.76	13.10±1.18
1	12.97±0.80	14.40±0.69	14.07±1.15
2	13.73±0.94	16.21±0.69	14.74±1.07
3	14.21±1.08	15.97±0.37	15.07±1.14
4	13.98±1.26	15.59±0.49	14.74±0.89
5	13.32±1.72	15.65±0.58	14.39±1.04
6	11.69±1.52	14.23±0.38	13.55±1.01
Average*	**13.32^{a} ± 1.13**	**15.34^{b} ± 0.46**	**14.43^{ab} ± 1.03**

* Figures with different superscripts in a row differ significantly ($P<0.05$)

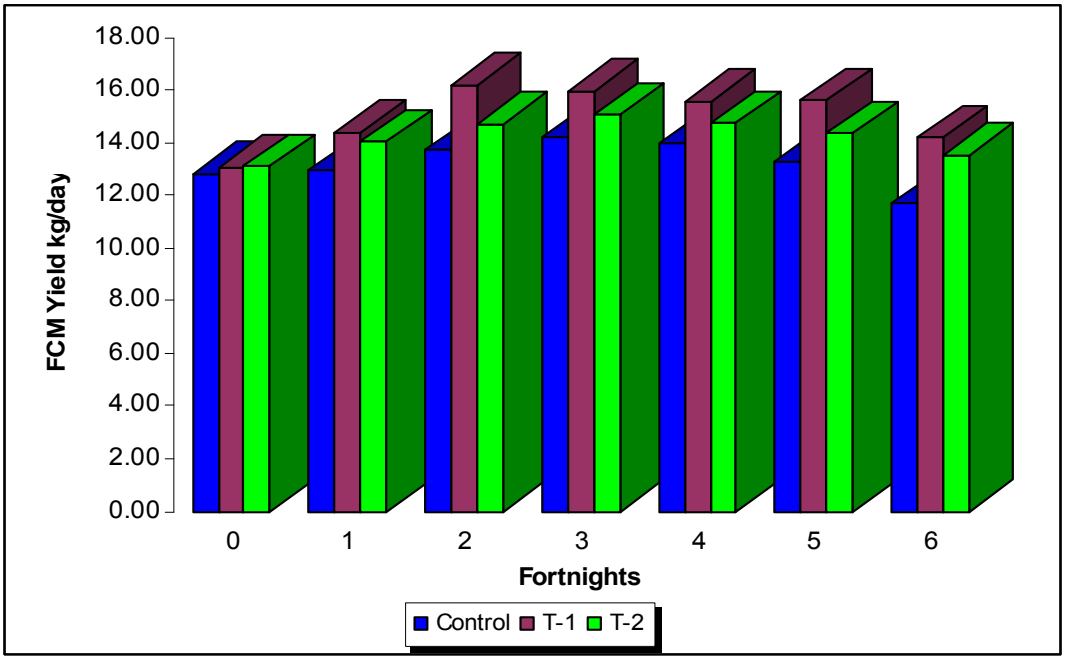

Fig. 4.4 (a): Fortnightly fat corrected milk yield (FCM, 4% fat)

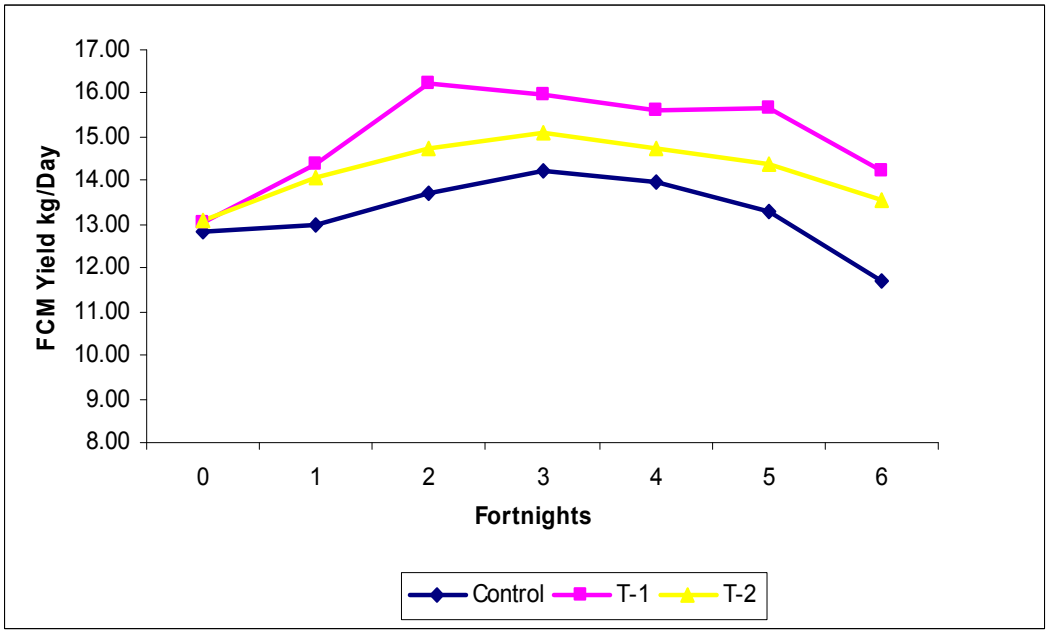

Fig. 4.4 (b): Fortnightly fat corrected milk yield (FCM, 4% fat)

enzymes in the ration of cows (Zheng *et al.*, 2000; Titi, 2003; Shojaeian and Thakur (2007), Miachieo and Thakur (2007).

B) Milk fat content (%):

Average fortnightly milk fat contents (Table 4.18) ranged between 7.52 to 7.93, 7.63 to 8.15 and 7.59 to 8.25 in control, T-1 and T-2 groups, respectively. The average milk fat content was 7.69, 7.82 and 7.99 % in control, T-1 and T-2 groups respectively, which are similar in the three groups. The results of the present study are in line with the results of other workers (Beauchemin *et al.*, 1999; Schingoethe *et al.*, 1999; Vicini *et al.*, 2003); Shojaeian and Thakur (2007) who also reported that milk fat (%) was not affected by feeding enzyme supplemented diets to dairy cows. In contrast, Bowman *et al.* (2002) reported significantly higher values for milk fat (%) in cows fed enzyme supplemented diets.

C) Milk protein content (%):

Average milk protein contents (Table 4.19) were similar in control (3.77%), T-1 (3.82%) and T-2 (3.78%) groups. The treatment differences were found to be statistically non significant. Various research workers have also reported no effect of enzyme supplementation to the diet on protein percentage of milk in lactating cows (Schingoethe *et al.,* 1999; Kung *et al.,* 2002; Knowlton *et al.* 2002; Shojaeian and Thakur 2007; and Miachieo and Thakur 2007).

D) Solid not fat (SNF) content (%):

Average SNF content (Table 4.20) was numerically similar in control (10.41%), T-1 (10.48%) and T-2 (10.44%). SNF content ranged between 10.32 to 10.53, 10.40 to 10.54 and 10.37 to 10.50 % in control, T-1 and T-2 groups, respectively during the experimental period of 12 weeks. The treatment differences were found to be non significant. Similar findings for solids not fat content in milk were also reported by Knowlton *et al.* (2002); Titi (2003); Shojaeian and Thakur, (2007); Miachieo and Thakur (2007) in cows fed enzyme supplemented diets.

E) Milk lactose content (%):

Average milk lactose contents (Table 4.21) were similar in control (5.75%), T-1 (5.78%) and T-2 (5.75%) groups. The treatment differences were found to be statistically non significant (P<0.05).

The Summarized results of milk yield, FCM yield and milk composition have been presented in Table 4.22

4.2.6 Effect on Milk Production Efficiency

DM intake per kg milk yield (Table 4.23, Fig.4.5) was 1.62, 1.41 and 1.47 kg in control, T-1 and T-2 respectively, which was statistically similar in all groups. It differed with the advancement of lactation and ranged between 1.41 to 2.06, 1.37 to 1.55 and 1.38 to 1.60 in control T-1, and T-2, respectively, over different fortnights. Similarly, DMI per kg FCM yield (Table 4.24, Fig. 4.6) was similar (P> 0.05) in both groups and the mean values were 1.03, 0.89 and 0.91 kg in control, T-1 and T-2, respectively. It also did not differ in different fortnights and mean values ranged between 0.91 to 1.27, 0.86 to 0.97 and 0.86 to 0.98 in control, T-1 and T-2, respectively.

CP intake per kg milk yield (Table 4.25, Fig.4.7) was 183.88, 160.50 and 166.79 g in control,T-1 and T-2, respectively, which did not differ (P> 0.05) among different groups. However, CP intake g per kg milk yield varied with the advancement of lactation and the mean values ranged between 160.51 to 234.87, 151.59 to 173.37 and 157.16 to 181.86 in control, T-1 and T-2, respectively in different fortnights. Similar trend was observed in case of CP intake per kg FCM yield (Table 4.26, Fig. 4.8) and overall mean values were 116.84, 101.74 and 103.84 g in control, T-1 and T-2 groups. These ranged between 103.47 to 145.04, 97.52 to 109.94 and 97.86 to 111.99 g, respectively in different fortnights.

Mean TDN intake g per kg milk yield (Table 4.27, Fig.4.9) was 918.41, 846.63, and 853.91 in control, T-1 and T-2 groups, respectively, which was statistically similar in different groups. TDN intake per kg milk yield showed

Table 4.17: Fortnightly milk total solid (TS) content (%) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
0	17.97±0.32	18.03±0.15	17.99±0.47
1	18.05±0.37	18.13±0.13	18.28±0.44
2	18.14±0.33	18.39±0.33	18.50±0.32
3	18.13±0.36	18.27±0.26	18.53±0.34
4	18.14±0.35	18.27±0.15	18.49±0.39
5	18.25±0.39	18.57±0.29	18.67±0.34
6	18.25±0.37	18.41±0.27	18.53±0.21
Average	**18.16 ± 0.34**	**18.34 ± 0.21**	**18.50 ± 0.31**

Table 4.18: Fortnightly Fat (%) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
0	7.52±0.31	7.57±0.16	7.59±0.37
1	7.52±0.37	7.63±0.15	7.83±0.37
2	7.61±0.31	7.85±0.33	7.99±0.23
3	7.66±0.33	7.77±0.24	8.05±0.27
4	7.74±0.32	7.79±0.17	8.05±0.31
5	7.89±0.29	8.15±0.25	8.25±0.28
6	7.93±0.25	8.02±0.19	8.16±0.23
Average	**7.69 ± 0.31**	**7.82 ± 0.21**	**7.99 ± 0.29**

Table 4.19: Fortnightly milk protein content (%) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
0	3.75±0.05	3.74±0.04	3.70±0.06
1	3.72±0.06	3.77±0.04	3.73±0.06
2	3.75±0.05	3.78±0.04	3.77±0.06
3	3.72±0.05	3.79±0.03	3.75±0.05
4	3.80±0.04	3.85±0.02	3.73±0.08
5	3.84±0.05	3.85±0.03	3.82±0.05
6	3.78±0.08	3.89±0.06	3.88±0.06
Average	**3.77 ± 0.04**	**3.82 ± 0.03**	**3.78 ± 0.05**

Table 4.20: Fortnightly solid not fat (SNF) content (%) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
0	10.45±0.06	10.46±0.06	10.40±0.14
1	10.53±0.07	10.50±0.07	10.44±0.13
2	10.53±0.05	10.54±0.08	10.50±0.13
3	10.47±0.05	10.50±0.09	10.49±0.11
4	10.41±0.06	10.48±0.07	10.44±0.12
5	10.36±0.26	10.42±0.05	10.42±0.09
6	10.32±0.14	10.40±0.08	10.37±0.05
Average	**10.43 ± 0.10**	**10.48 ± 0.07**	**10.44 ± 0.10**

Table 4.21: Fortnightly milk lactose content (%) in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
0	5.75±0.05	5.79±0.07	5.65±0.06
1	5.73±0.06	5.73±0.05	5.69±0.09
2	5.80±0.07	5.77±0.06	5.74±0.09
3	5.81±0.07	5.75±0.04	5.71±0.08
4	5.82±0.04	5.77±0.04	5.74±0.08
5	5.77±0.10	5.85±0.04	5.93±0.09
6	5.62±0.11	5.81±0.07	5.66±0.06
Average	**5.75 ± 0.07**	**5.78 ± 0.05**	**5.75 ± 0.08**

Table 4.22: Overall Milk yield and composition in Murrah buffaloes fed control and enzyme supplemented diets

Particulars	Groups		
	Control	T-1	T-2
Milk Yield (kg/day)	8.62^{a}±0.34	9.74^{b}±0.18	9.01^{a}±0.27
4% FCM Yield (kg/day)	13.32^{a}±0.44	15.34^{b}±0.26	14.43^{ab}±0.39
Milk Composition (%)			
TS	18.16±0.34	18.34±0.21	18.50±0.31
Fat	7.69±0.31	7.82±0.21	7.99±0.29
Protein	3.77±0.04	3.82±0.03	3.78±0.05
SNF	10.43±0.10	10.48±0.07	10.44±0.10
Lactose	5.75±0.07	5.78±0.05	5.75±0.08

* Figures with different superscripts in a row differ significantly (P<0.05)

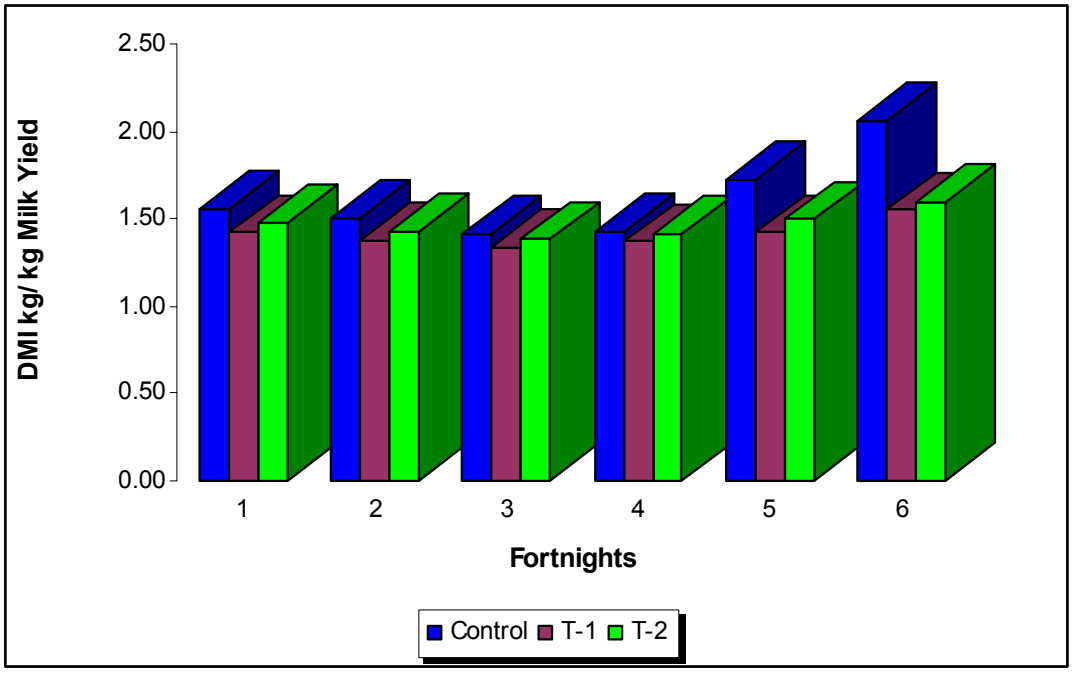

Fig. 4.5: Fortnightly DMI kg/kg milk yield

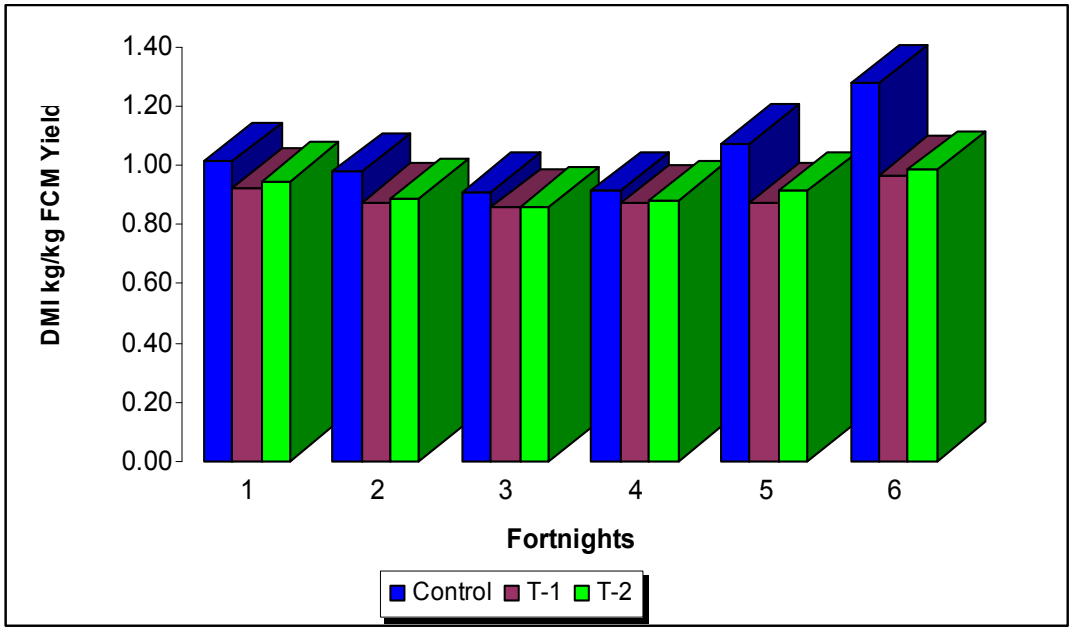

Fig. 4.6: Fortnightly DMI kg/kg FCM yield

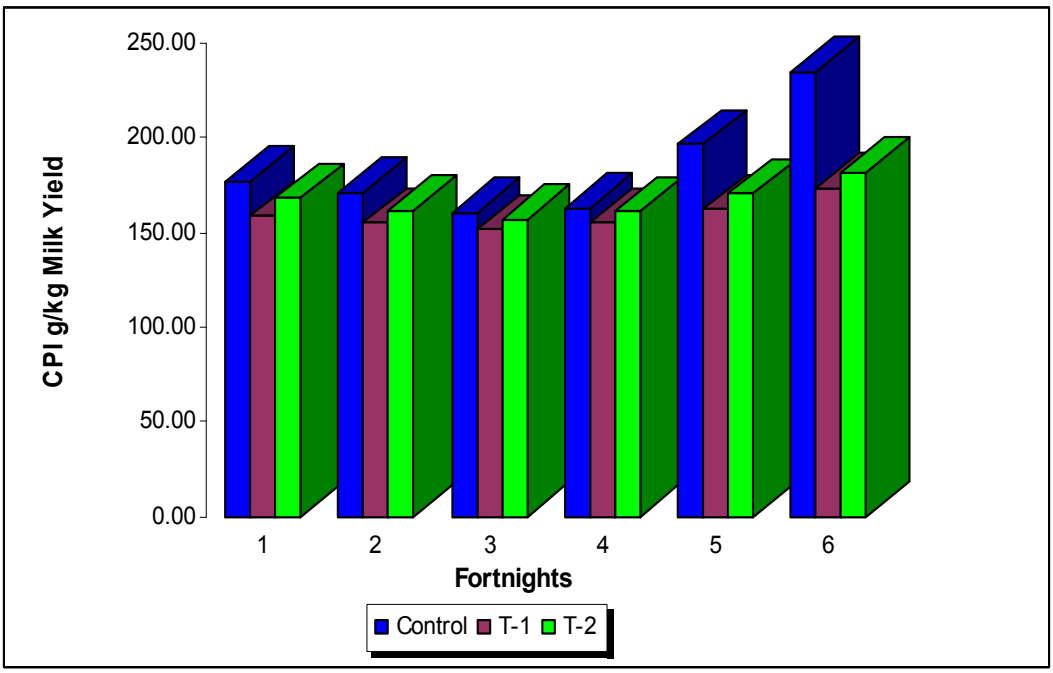

Fig. 4.7: Fortnightly CPI (g) per kg Milk yield

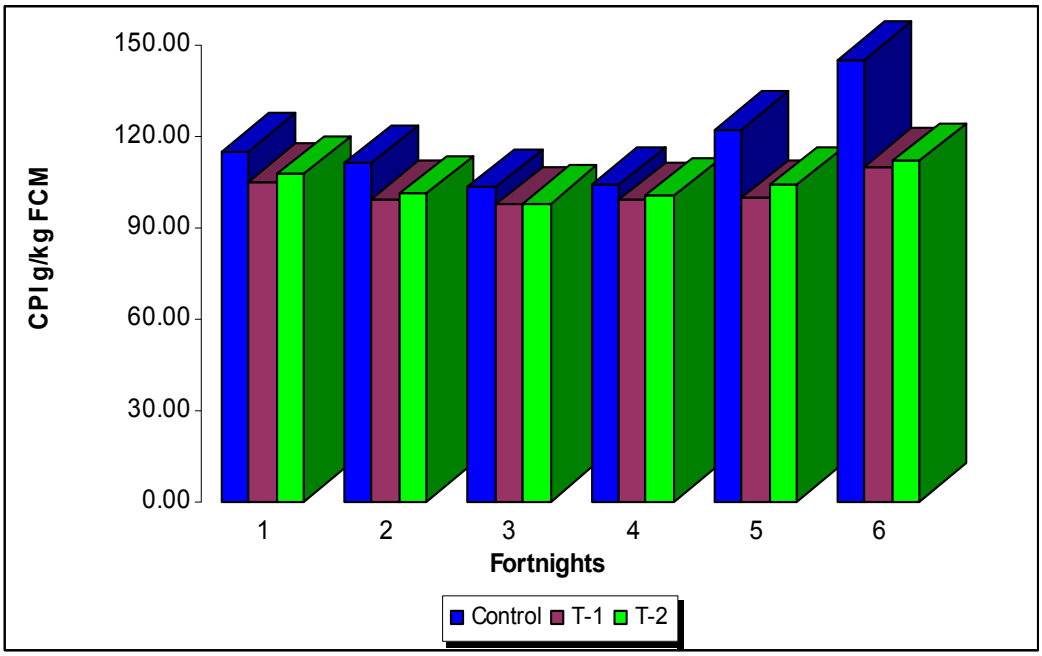

Fig. 4.8: Fortnightly CPI (g) per kg FCM yield

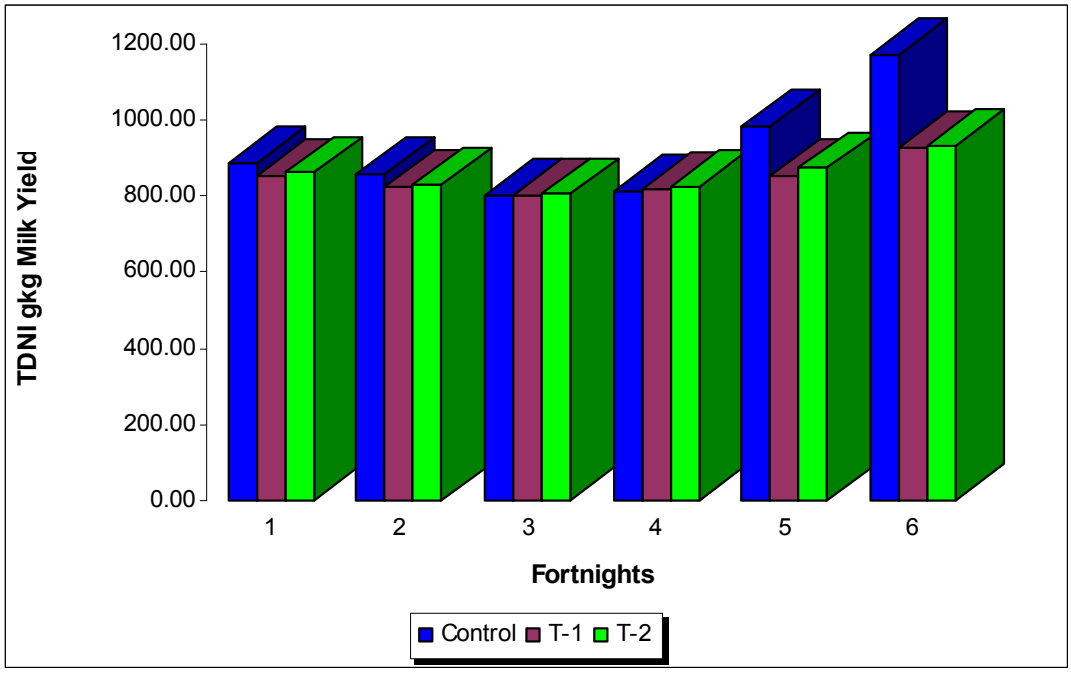

Fig. 4.9: Fortnightly TDN intakes (g) per kg milk yield

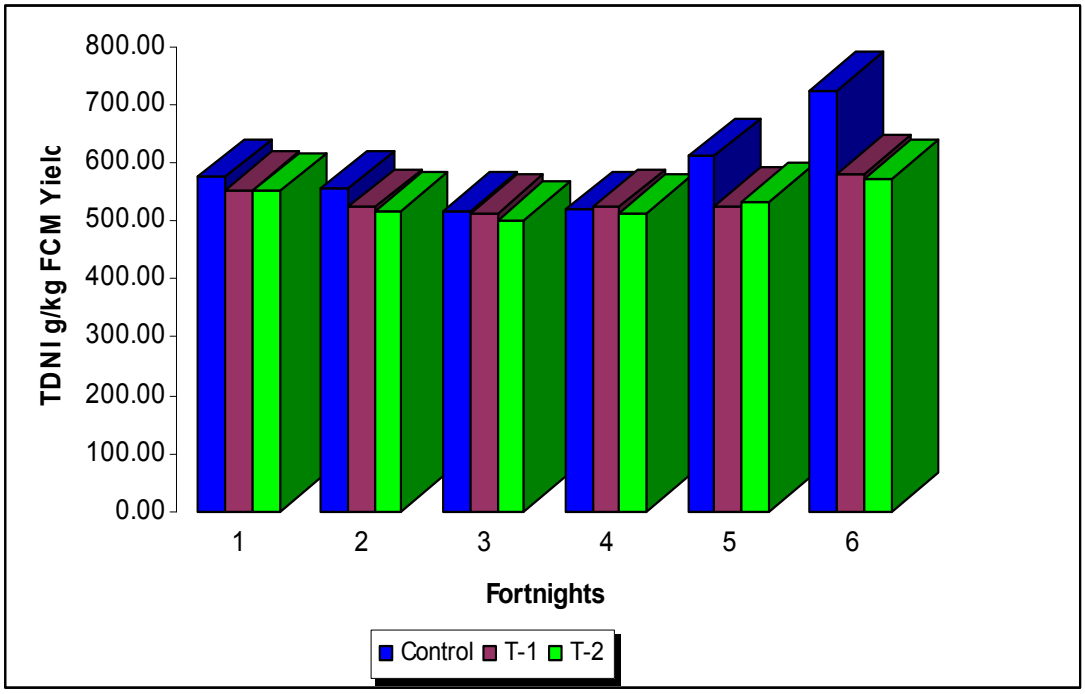

Fig. 4.10: Fortnightly TDN intakes (g) per kg FCM yield

increasing trend with advancement of lactation and ranged between 801.69 to 1173.14, 803.76 to 929.02 and 804.56 to 931.02 g in control, T-1 and T-2, respectively, over the entire experimental period. Neither dietary treatments nor experimental period affected TDN intake per kg FCM yield (Table 4.28, Fig.4.10) and the mean values were 563.89, 534.94 and 530.08 g in control, T-1 and T-2, respectively, and the same ranged between 516.81 to 724.41, 514.42 to 579.94 and 501.01 to 573.33 in control, T-1 and T-2 respectively, in different fortnights.

The effect of dietary enzyme supplementation on nutrient intake per kg milk yield or FCM production is presented in condensed Table 4.29. In the present study, it was observed that the DM intake per kg milk yield or per kg FCM yield was numerically better in the treatment group as compared to the control group but these differences were statistically non-significant. Shojaeian and Thakur (2007) also recorded similar findings.

4.2.7 Effect on Digestibility of Nutrients

4.2.7.1. Chemical composition of feedstuffs

The chemical composition of feedstuffs (Concentrate, Green maize, Wheat straw and TMR) during digestible trial is presented in Table 4.30.

4.2.7.2 Effect on Body weights and nutrient intake

Average body weights and nutrients intake during digestibility trial is presented in Table 4.31. Average body weights were 566.61, 569.36 and 563.58 in control, T-1 and T-2 groups, respectively which did not differ significantly among different groups. Similar trend was observed in case of metabolic body weight ($W^{0.75}$) which was 116.01, 116.55, and 115.62, in control T-1 and T-2 respectively.

The Total DMI as kg/d, kg/100 kg weight and g/kg $w^{0.75}$ were 12.80, 2.26 and 109.97 in control group, 13.64, 2.40 and 117.07 on T-1 group and 12.65, 2.27 and 110.17 in T-2 group. Average CP intakes expressed as kg/d,

g/100 kg body weight, and g/ kg $W^{0.75}$ were 1.51, 265.25 and 12.93 in control, 1.60, 280.83 and 13.71 T-1 and 1.49, 266.99 and 12.97 in T-2 groups, respectively. There was no significant difference in the CP intake between the different groups.

Mean TDN intakes expressed as kg/d, kg/100 kg body weight and g/kg $W^{0.75}$ were 7.28, 1.28 and 62.50 in control group, 819, 1.44 and 70.28 in T-1group and 7.37, 1.32 and 64.19 in T-2 group. There was no significant difference in TDN intake between the different groups.

4.2.7.3 Effect on Digestibility coefficients of TMRS

The digestibility coefficients of the different nutrients with or without exogenous fibrolytic enzyme mixture supplementation are presented in Table 4.32. The digestibility coefficients of DM, OM, CP, EE, and NFE were 55.60, 55.98, 58.21, 70.06 and 65.57% in control, 57.83, 58.47, 60.69, 75.22,and 67.54% inT-1 and 56.49, 57.05, 59.28, 71.29 and 66.28% in T-2 groups, respectively, and these were similar in the three groups.

The digestibility coefficients of CF, NDF, ADF, cellulose and Hemicellulose were 49.84, 50.75, 43.53, 50.64, and 62.31 (control) 55.15, 54.65, 48.26, 55.15, and 66.50 (T-1) and 52.43, 52.17, 46.88, 52.40 and 63.69 (T-2) groups. However, there was improvement ($P<0.05$) in the digestibility of CF, NDF, ADF, Cellulose and Hemicellulose by 10.65, 7.68, 10.86, 8.90, and 6.72 percent in buffaloes of T-1 group over those of control. Though there was increase in CF, NDF, ADF, Cellulose and Hemicellulose digestibility in T-2 group by 5.19, 2.79, 7.69, 3.47, and 2.21% over that of control, but the same was statistically similar. The increased digestion of CF, NDF, ADF, cellulose and hemicellulose ($P<0.05$) suggests that the fibrolytic activity of the rumen microbes may have been increased due to synergistic effect of exogenous fibrolytic enzymes with ruminal enzymes produced by rumen microbes.

The result of the present study are similar to those of Yang *et al.,* (1999, 2000), Bowman *et al.* (2002) and Shojaeian and Thakur (2007), who also reported increased digestibility of DM and organic matter when fibrolytic

Table 4.23: Fortnightly DMI kg/kg milk yield in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	1.56±0.16	1.42±0.07	1.48±0.07
2	1.51±0.15	1.37±0.06	1.42±0.08
3	1.41±0.13	1.34±0.05	1.38±0.07
4	1.43±0.15	1.37±0.06	1.42±0.05
5	1.73±0.18	1.42±0.07	1.50±0.11
6	2.06±0.12	1.55±0.08	1.60±0.07
Average	**1.62 ± 0.13**	**1.41 ± 0.06**	**1.47 ± 0.07**

Table 4.24: Fortnightly DMI kg/kg FCM yield in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	1.01±0.08	0.92±0.05	0.95±0.06
2	0.98±0.09	0.87±0.04	0.89±0.04
3	0.91±0.07	0.86±0.03	0.86±0.04
4	0.91±0.08	0.87±0.04	0.88±0.03
5	1.07±0.09	0.88±0.04	0.91±0.05
6	1.27±0.09	0.97±0.05	0.98±0.04
Average	**1.03 ± 0.10**	**0.89 ± 0.05**	**0.91 ± 0.04**

Table 4.25: Fortnightly CPI (g) per kg Milk yield in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	177.21±10.71	158.74±7.81	168.28±8.01
2	171.42±11.67	155.12±6.88	161.91±8.58
3	160.51±11.86	151.59±5.52	157.16±7.74
4	162.76±12.68	154.92±6.49	161.04±6.07
5	196.49±15.53	162.10±8.50	170.53±9.02
6	234.87±17.70	173.37±8.72	181.86±7.94
Average	**183.88 ± 14.03**	**160.50 ± 6.91**	**166.79 ± 7.87**

Table 4.26: Fortnightly CPI (g) per kg FCM yield in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	115.13±8.85	105.00±6.07	107.67±6.77
2	111.21±9.84	99.23±4.78	101.27±4.94
3	103.47±8.26	97.52±3.58	97.86±4.50
4	103.95±8.75	99.14±3.88	100.35±3.56
5	122.23±9.60	99.62±4.86	103.90±5.84
6	145.04±10.53	109.94±5.20	111.99±4.09
Average	**116.84 ± 9.63**	**101.74 ± 4.50**	**103.84 ± 4.60**

Table 4.27: Fortnightly TDN intakes (g) per kg milk yield in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	885.10±88.45	852.43±41.19	861.52±41.03
2	856.17±83.25	822.69±36.28	828.88±43.94
3	801.69±74.22	803.76±29.11	804.56±39.63
4	812.93±83.32	820.14±34.22	824.43±31.07
5	981.42±88.41	851.76±44.85	873.02±61.56
6	1173.14±92.17	929.02±46.02	931.02±40.65
Average	**918.41 ± 84.45**	**846.63 ± 36.46**	**853.91 ± 40.27**

Table 4.28: Fortnightly TDN intakes (g) per kg FCM yield in Murrah buffaloes fed control and enzyme supplemented diets

Fortnights	Control	T-1	T-2
1	575.03±44.20	553.89±32.04	551.20±34.64
2	555.49±49.17	523.45±25.20	518.45±25.29
3	516.81±41.24	514.42±18.88	501.01±23.03
4	519.19±43.69	522.98±20.46	513.72±18.24
5	610.51±47.89	525.51±25.62	531.92±29.90
6	724.41±52.50	579.94±27.41	573.33±20.95
Average	**563.98 ± 46.45**	**534.94 ± 23.44**	**530.08 ± 23.38**

Table: 4.29: Overall Milk production efficiency in Murrah buffaloes fed control and enzyme supplemented diets

Particulars	Groups		
	Control	T-1	T-2
DMI (kg)/kg milk yield	1.62±0.13	1.41±0.06	1.47±0.07
DMI (kg)/kg FCM	1.03±0.10	0.89±0.05	0.91±0.04
CPI (g)/kg milk yield	183.88±14.03	160.50±6.91	166.79±7.87
CPI (g)/kg FCM yield	116.84±9.63	101.74±4.50	103.84±4.60
TDNI (g)/kg milk yield	918.41±84.45	846.63±36.46	853.91±40.27
TDNI (g)/kg FCM yield	563.98±46.45	534.94±23.44	530.08±23.38

Table: 4.30: Chemical composition of feed ingredient during digestibility trial

Particulars	Concentrate mixture	Green Maize	Wheat Straw	TMR
Proximate principles (% DM basis)				
DM	89.97	12.28	89.88	41.40
OM	92.15	88.75	91.79	91.40
CP	20.18	9.76	4.38	11.32
EE	3.80	1.71	1.22	2.34
CF	9.21	27.74	41.96	26.68
NFE	58.96	49.54	44.23	51.06
Total ash	7.85	11.25	8.21	8.60
Cell wall constituents % DM basis				
NDF	31.03	52.61	78.08	56.46
ADF	13.68	32.43	50.75	33.32
Hemi cellulose	17.35	20.18	27.33	22.14
Cellulose	8.55	24.70	39.07	25.11
ADL	4.10	5.33	8.36	6.43
Cell Contents	59.21	47.39	21.92	43.54
Silica	1.18	2.31	3.40	2.62

Table 4.31: Nutrient intake in the experimental Murrah buffaloes during digestibility trial.

Particulars	Groups		
	Control	T-1	T-2
Av. Body Weight (kg)	566.61±26.71	569.36±8.91	563.58±17.25
Metabolic body weight ($w^{0.75}$)	116.01±4.09	116.55±1.35	115.62±2.65
DMI (kg/d)	12.80±0.94	13.64±0.42	12.65±0.51
DMI, kg/100 kg BW	2.26±0.11	2.40±0.08	2.27±0.16
DMI (g/kg $w^{0.75}$)	109.97±5.70	117.07±3.67	110.17±6.85
CP Intake (kg/d)	1.51±0.11	1.60±0.05	1.49±0.06
CP Intake (g/kg BW)	265.25±12.52	280.83±9.09	266.99±18.58
CP Intake kg $w^{0.75}$ (g/d)	12.93±0.66	13.71±0.43	12.97±0.80
TDN Intake (kg/d)	7.28±0.54	8.19±0.25	7.37±30
TDN Intake (kg/100 kg BW)	1.28±0.06	1.44±0.05	1.32±0.09
TDN Intake per kg $w^{0.75}$ (g/d)	62.50±3.24	70.28±2.20	64.19±3.99

Table 4.32: Nutrient digestibility and nutrient value of TMR fed to the experimental Murrah buffaloes.

Digestibility Coefficients (%)			
Parameters	Control	T-1	T-2
DM	55.60±0.58	57.83±0.63	56.49±1.55
OM	55.98±0.47	58.47±0.88	57.05±1.67
CP	58.21±1.71	60.69±2.44	59.28±1.48
EE	70.06±1.65	75.22±1.02	71.29±2.93
CF	49.84^{a}±0.62	55.15^{b}±1.61	52.43^{ab}±1.06
NFE	65.57±1.21	67.54±1.11	66.28±1.78
NDF	50.75^{a}±1.41	54.65^{b}±1.01	52.17^{ab}±0.99
ADF	44.53^{a}±1.03	48.26^{b}±0.47	46.88^{ab}±0.64
Cellulose	50.64^{a}±0.92	55.15^{b}±0.60	52.40^{ab}±0.57
Hemi cellulose	62.31^{a}±0.98	66.50^{b}±0.76	63.69^{ab}±0.83
Nutritive values of ration (%DM basis)			
DCP	6.59±0.16	6.87±0.23	6.71±0.14
TDN	57.06±1.37	60.03±1.60	58.26±1.86

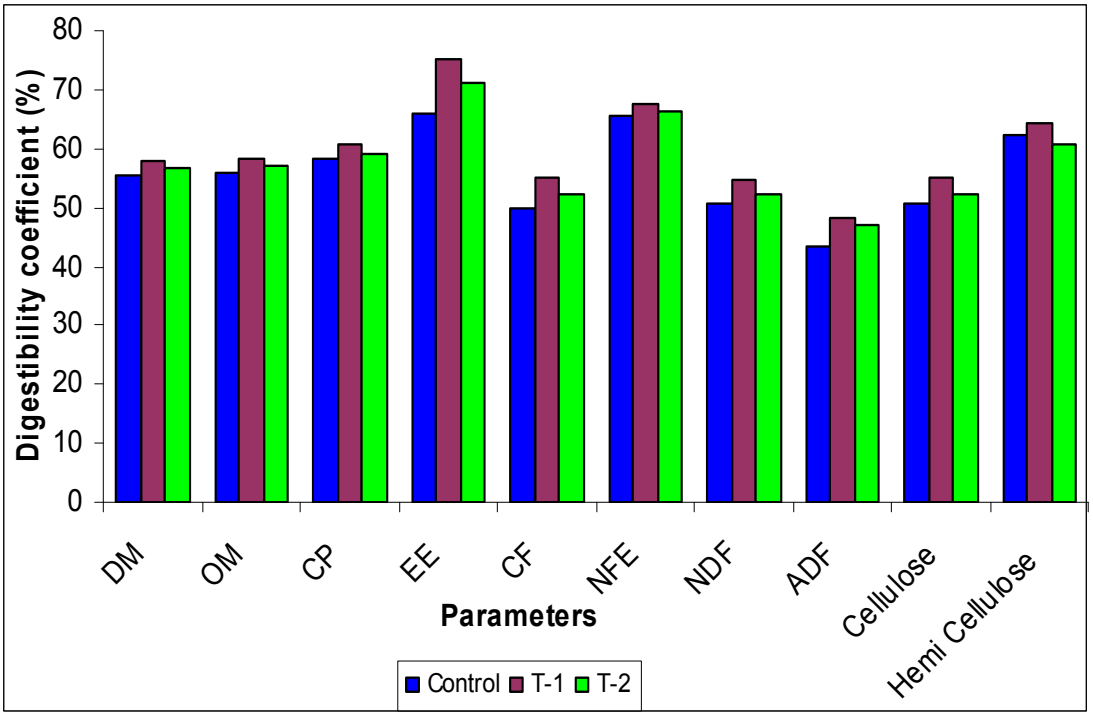

Fig. 4.11: Digestibility coefficient of different nutrients

enzymes were supplemented through the concentrate portion of the ration. Shojaeian and Thakur, (2007) reported increased NDF and ADF digestibility on fortification of wheat straw based ration with cellulase and xylanase. Morgavi *et al.* (2000a) reported that the total hydrolytic capacity of the rumen increases due to synergistic effect of the exogenous enzymes with the hydrolases of the ruminal micro organisms. Exogenous fibrolytic enzymes have also been reported to enhance the attachment of rumen micro organisms to the feed particles thereby increasing the hydrolytic activity of the rumen (Morgavi *et al.*, 2000a; Wang *et al.*, 2001).

4.2.8 Effect of EFE supplementation on blood parameters in experimental Murrah buffaloes.

Some of the blood biochemical constituents such as blood glucose and blood urea nitrogen recorded in the present experiment have been depicted in Table 4.33. The blood glucose concentration at 0 day was 50.11, 52.92 and 51.95 mg/dl in control, T-1 and T-2 groups respectively, and at the end of experiment, the concentration was 51.61, 53.18, and 52.53 mg/dl in control, T-1, and T-2 groups, respectively. Blood glucose concentration remained within the normal range and no significant difference was observed among the three groups. Similarly, the blood urea nitrogen concentration in control and treatments group T-1 and T-2 at 0 day were 22.70, 23.67, and 23.88 mg/dl respectively, and at the end of experiment, the concentration were 24.72, 25.95 and 24.76 mg/dl in control, T-1 and T-2 respectively, which were statistically similar. The results obtained for blood glucose and urea level are in agreement with the levels reported by Javid *et al.* (2008) and Turkar *et al* (2008) in buffaloes.

Table 4.33: Effect of EFE supplementation on blood parameters in experimental Murrah buffaloes

Parameters	Control		T-1		T-2	
	0 Day	Final Day	0 Day	Final Day	0 Day	Final Day
Blood Glucose (mg/dl)	50.11 ±2.14	51.61 ±1.76	52.92 ±2.30	53.18 ±2.32	51.95 ±1.55	52.53 ±1.50
Blood urea nitrogen (mg/dl)	22.70 ±2.62	24.72 ±2.00	23.67 ±.31	25.95 ±1.35	23.88 ±2.31	24.76 ±2.21

CHAPTER – 5

Summary and Conclusions

5. SUMMARY AND CONCLUSION

5.1 SUMMARY

The present investigations were carried out to ascertain the effect of the exogenous fibrolytic enzymes supplementation (cellulase 4000 µM glucose/g/h and xylanase 7990 µM xylose/g/min) mixed in 50:50, w/w) at 3 levels viz. 0.0g, 1.5g, and 3.0g per kg DM, on nutrient digestion, milk yield, its composition and certain blood metabolites in buffaloes.

5.1.1 Effect of Supplementing Exogenous Fibrolytic Enzymes (EFE) on Nutrient Intake and Digestibility, Milk Yield and Composition in Lactating Murrah buffaloes

- To study the effect of supplementing fibrolytic enzyme mixture at 1.5 g and 3.0g (cellulase 4000 µM glucose/g/h and xylanases 7990 µM xylose/g/min) /kg DM of TMR to the concentrate portion of the diet on milk production, eighteen Murrah buffaloes were allotted to two dietary treatments on the basis of milk yield (8.48, 8.52 and 8.53 kg/d) and stage of lactation (68.5, 80.33 and 82 days).
- The control group was fed a TMR containing 45% wheat straw, 15% green maize and 40% concentrate (on DM basis), whereas, the treatment group was fed the same diet plus exogenous fibrolytic enzymes mixture (cellulase: xylanase mixture, 50:50 w/w) at 1.5g (T-1 group) and 3.0g/kg DM of TMR (T-2 group) by mixing the same in powder form to the concentrate portion of the diet.
- The animals were fed TMR so as to meet their requirements as per Kearl (1982).
- No significant difference in fortnightly body weights was recorded between the control, T-1 and T-2 groups.
- There was no significant difference in DM intake (kg/d, kg/100kg body weight and g/kg $W^{0.75}$) between the control and two treatment groups throughout the experimental period.

- Average CP intakes were 1452.93, 1548.35 and 1475.57g/d, in control, T-1 and T-2 respectively. Overall average CP intake per 100 kg body weight was 254.62, 272.85 and 264.18 g/day in control, T-1 and T-2 groups, respectively. Average CP intake per kg $W^{0.75}$ was 12.45, 13.26 and 12.84 for control, T-1 and T-2 groups, respectively, which did not differ between the control and two treatment groups.
- Average TDN intakes were 7.26, 8.17, and 7.55 kg/day in control, T-1 and T-2 groups respectively, which were significantly higher ($P<0.05$) by 12.53 percent in the T-1 over that of control group. There was no difference between Control and T-2 groups. TDN intake was significantly higher ($P<0.05$) in the first, second and fourth fortnight in T-1 than that of control group.
- Average TDN intake, kg/100 kg BW, was 1.27, 1.43, and 1.36 in control, T-1 and T-2 respectively, which was significantly higher ($P<0.05$) by 12.59 percent in the T-1 over that of control group. Average TDN intakes g / kg $W^{0.75}$ were 62.20, 70.04 and 65.89 in control T-1 and T-2 groups respectively, which were also significantly higher ($P<0.05$) by 12.60 percent in T-1 over that of control. There was no difference between Control and T-2 groups.
- The average milk yield (kg/d) of 90 days lactation trial was 8.62, 9.74, and 9.01 kg in control, T-1 and T-2 groups respectively. Though milk yield (kg/d) was similar at the start of the experiment ; 8.48 in control, 8.52 in T-1 and 8.53 kg in T-2 groups, the average milk yield during the entire experimental period was higher ($P<0.05$) by 12.99 % in group T-1 over that of control and 8.10 % in T-1 over that of T-2, whereas, there was no difference between milk yields of T-2 and control groups.
- Average fat corrected milk (4% FCM) yield over the entire experimental period was 13.32, 15.34 and 14.43 kg/d in control, T-1 and T-2 groups, respectively, showing significantly higher FCM yield ($P<0.05$) by 15.17 in T-1 over that of control. There was no difference between Control and T-2 groups.

- There was no effect of EFE supplementation on the milk composition. Total solids content was 18.16, 18.34 and 18.50%, fat was 7.69, 7.82 and 7.99 %, lactose was 5.75, 5.78 and 5.75%, SNF was 10.43, 10.48 and 10.44% and milk protein was 3.77, 3.82 and 3.78% in control, T-1, and T-2 groups respectively.
- DM intake per kg milk yield was 1.62, 1.41 and 1.47 kg in control, T-1 and T-2 respectively, which was statistically similar in all groups. Similarly, DMI per kg FCM yield was similar (P> 0.05) in control and experimental groups, and the mean values were 1.03, 0.89 and 0.91 kg in control, T-1 and T-2 respectively.
- Average CP intake per kg milk yield was 183.88, 160.50 and 166.79 g in control, T-1 and T-2 respectively which did not differ (P> 0.05) among different groups. Similar trend was observed in case of CP intake per kg FCM yield and overall mean values were 116.84, 101.74 and 103.84 g in control, T-1 and T-2 respectively.
- Average TDN intake per kg milk yield was 918.41, 846.63, and 853.91g in control, T-1 and T-2, which was statistically similar in different groups, similar trend was observed in TDN intake per kg FCM yield which were 563.89, 534.94 and 530.08 g in control, T-1 and T-2 respectively.
- The Total DMI intake(kg/d, kg/100 kg weight and g/kg $w^{0.75}$) during the seven days digestibility trial was were 12.80, 2.26 and 109.97 in control, 13.64, 2.40 and 117.07 in T-1 and 12.65, 2.27 and 110.17 in T-2, which were similar in the three groups.
- Average CP intakes expressed as (kg/d, g/100 kg body weight, and g/ kg $W^{0.75}$) during the digestibility trial were 1.51, 265.25 and 12.93 in (control), 1.60, 280.83 and 13.71 in T-1 and 1.49, 266.99 and 12.97 in T-2. There was no significant difference in the CP intake between the three groups.
- Mean TDN intake during the digestibility trial, kg/d, kg/100 kg body weight and g/kg $W^{0.75}$ were 7.28, 1.28 and 62.50 in control, 8.19, 1.44 and 70.28 in T-1 and 7.37, 1.32 and 64.19 in T-2. There was no significant difference in TDN intake between the different groups.

- The digestibility coefficients(%) of DM, OM, CP, EE, and NFE were 55.60, 55.98, 58.21, 70.06 and 65.57 in control, 57.83, 58.47, 60.69, 75.22,and 67.54 in T-1 and 56.49, 57.05, 59.28, 71.29 and 66.28 in T-2 groups respectively, and these were similar in different groups.
- The digestibility coefficients of CF, NDF, ADF, cellulose and Hemicellulose were 49.84, 50.75, 43.53, 50.64, and 62.31 in control, 55.15, 54.65, 48.26, 55.15, and 66.50 in T-1 and 52.43, 52.17, 46.88, 52.40 and 63.69 in T-2 groups. Improvement ($P<0.05$) in the digestibility of CF, NDF, ADF, Cellulose and Hemicellulose by 10.65, 7.68, 10.86, 8.90, and 6.72 percent in buffaloes of T-1 over that of control was observed.

5.2 CONCLUSIONS:

1. Supplementation of exogenous fibrolytic enzymes (cellulase 4000 µM glucose/g/h and xylanase 7990 µM xylose/g/min) mixed in 50:50 w/w ratio) at 1.5 and 3.0g/kg DM increased the daily milk yield ($P<0.05$) by 12.99 and 8.99 percent in group T-1 over that of control and T-2 groups, respectively in Murrah buffaloes fed TMR containing 45% wheat straw , 15 % green maize and 40% concentrate on DM basis.
2. Average TDN intake, kg/d, kg/100 kg body weight and g/kg $W^{0.75}$ was higher ($P<0.05$) in T-1 by 12.53, 12.59 and 12.60 percent and 8.21, 5.15, 6.30 percent over that of control and T-2.
3. Digestibility coefficients of CF, NDF, ADF, Cellulose and Hemicellulose were improved ($P<0.05$) by 10.65, 7.68, 10.86, 8.90 & 6.72 percent in T-1 over that of control.
4. There was no effect of EFE supplementation on milk production efficiency.
5. There was no effect on blood glucose and blood urea nitrogen level in buffaloes fed ration fortified with fibrolytic enzymes.

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