Nutritional evaluation of Cumbu Napier (COBN-5) Hybrid Fodder in buffalo bulls

By

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B.V.Sc., & A.H.

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Certificate

M.MADESH has satisfactorily prosecuted the course of research and that the thesis entitled **"Nutritional evaluation of Cumbu Napier (COBN-5) Hybrid Fodder in buffalo bulls"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

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This is to certify that the thesis entitled **"Nutritional evaluation of Cumbu Napier (COBN-5) Hybrid Fodder in buffalo bulls"** submitted in partial fulfillment of the requirements for the degree of MASTER OF VETERINARY SCIENCE of the Sri Venkateswara Veterinary University, Tirupati, is a record of the bonafide research work carried out by M.MADESH, under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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M.MADESH…

DECLARATION

I, **Mr. M.MADESH** hereby declare that the thesis entitled **"Nutritional evaluation of Cumbu Napier (COBN-5) Hybrid Fodder in buffalo bulls"** submitted to Sri Venkateswara Veterinary University, Tirupati for the degree of **MASTER OF VETERINARY SCIENCE** is the result of original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

Date: (M.MADESH)

LIST OF ABBREVATIONS

%	: Percent
±	: Plus or Minus
g	: Grams
g/d	: Grams/day
Kg	: Kilogram
ADF	: Acid detergent fibre
ADL	: Acid detergent lignin
ADICP	: Acid detergent insoluble crude protein
ADIN	: Acid detergent insoluble nitrogen
AFRC	: Agricultural Food and Research Council
AOAC	: Association of Official Analytical Chemists
BIN	: Buffer insoluble nitrogen
BIP	: Buffer insoluble protein
Ca	: Calcium
CF	: Crude fibre
CHO	: Carbohydrates
CNCPS	: Cornell Net Carbohydrate and protein system
CP	: Crude protein
DCP	: Digestible crude protein
DE	: Digestible energy
DM	: Dry matter
DMB	: Dry matter basis
DMI	: Dry matter intake
EE	: Ether extract
ECM	: Early cut Mott grass
ED	: Effective degradability
EDADF	: Effective degradable acid detergent fibre
EDCP	: Effective degradable crude protein
EDDM	: Effective degradable dry matter
EDNDF	: Effective degradable neutral detergent fibre
EFS	: Evolved feeding strategy
FAO	: Food and Agricultural Organization
FFS	: Farmers feeding strategy

ICAR	: Indian Council of Agricultural Research
IVADFD	: *In vitro* acid detergent fibre digestibility
IVCPD	: *In vitro* crude protein digestibility
IVDMD	: *In vitro* dry matter digestibility
IVNDFD	: *In vitro* neutral detergent fibre digestibility
Insoluble N	: Insoluble nitrogen
IP	: Insoluble protein
LCM	: Late cut Mott grass
ME	: Metabolisable energy
MEq	: Milli-equivalent
N	: Nitrogen
NDF	: Neutral detergent fibre
NDICP	: Neutral detergent insoluble crude protein
NDIN	: Neutral detergent insoluble nitrogen
NFE	: Nitrogen free extract
NH_3-N	: Ammonia nitrogen
NPN	: Non protein nitrogen
NSC	: Non-structural carbohydrate
OM	: Organic matter
P	: Phosphorus
RSD	: Residual standard deviation
SP	: Soluble protein
SRL	: Strained rumen liquor
TA	: Total ash
TCA	: Trichloroacetic acid
TDN	: Total digestible nutrients
Total-N	: Total nitrogen
TVFA	: Total volatile fatty acids
W $kg^{0.75}$	: Metabolic body weight

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ABSTRACT

In the present study, six Murrah buffalo bulls (7 yrs; 357.97 ± 43.36 kg) were randomly selected and fed Cumbu Napier (COBN-5) Hybrid Fodder *ad libitum*. The COBN-5 fodder was evaluated for its chemical composition, CNCPS fractionation, *in vitro* digestibility of nutrients at different stages (30, 35, 40, 45 and 50 days) of harvest, *in sacco* degradability at two different stages of harvest (40 and 45 days), rumen fermentation pattern, nutrient utilization and balance in buffalo bulls. The chemical composition showed highest CP (14.00 %) at 30 days of harvest and lowest (12.10 %) at 50 days of harvest but the fiber fractions were inversely proportional to the crude protein content at different stages of harvest. The CNCPS fractionation of COBN-5 fodder revealed higher CB_1 and CB_2 fractions and lower CC fraction harvested at 30 days compared to the fodder harvested at 50 days. Similarly, the protein fractions PA and PB_3 were higher and PC was lower at 30 days of harvest indicating higher degradable portion of carbohydrate and protein in COBN-5 fodder. The oxalate content (%) of COBN-5 fodder was 3.32, 2.89, 2.31, 1.86 and 1.64 at 30, 35, 40, 45 and 50 days of harvest, respectively, and it decreased linearly with the increase in days of harvest.

The *in vitro* digestibility (%) of DM, CP, NDF and ADF in COBN-5 fodder decreased ($P<0.05$) linearly from 30 to 50 days of harvest. Higher DM, CP, NDF and ADF (%) digestibility was observed at 30 days (70.40, 81.01, 64.88 and 56.96) and lowest at 50 days of harvest (60.69, 69.64, 55.82 and 49.30), respectively. Significant ($P<0.05$) differences were observed in *in vitro* digestibility between all stages of harvest. The results of *in sacco* studies in COBN-5 fodder harvested at two different stages (40 and 45 days) of harvest in the rumen of fistulated buffalo bulls revealed that as the period of incubation extended from 0 to 72 h in the rumen, the *in sacco* DM, CP, NDF and ADF disappearance (%) values increased progressively. Significant differences ($p<0.05$) were observed in CP, NDF and ADF disappearance and degradation kinetics of COBN-5 fodder between 40 and 45 days of harvest, while the differences in DM disappearance is non-significant.

Rumen fermentation studies conducted in fistulated buffalo bulls revealed that rumen pH values were highest at 0 h and declined to minimum by 6 h post feeding, while TVFA reached peak at 6 h post feeding and NH_3-N reached peak at 2 h post feeding and later followed a decreasing trend. The digestibility coefficients (%) of gross nutrients and fibre fractions showed better digestibilities. All the buffalo bulls were in positive N, Ca and P balance. The average DMI (kg/d) of buffalo bulls (6.01) met the requirements. The DCP (%) in fodder consumed (8.74) is more than the requirement for maintenance of the buffalo bulls. Furthermore, the DM, DCP, TDN and ME intakes per $Wkg^{0.75}$ in buffalo bulls fed *ad libitum* of COBN-5 fodder (73.03, 6.38, 40.93 and 0.15), respectively, were higher than the values recommended by ICAR (2013) and Kearl (1982) standards.

Based on results of the present study, it is concluded that feeding of COBN-5 fodder *ad libitum* showed higher digestibility of nutrients *in vitro* and *in sacco*. Further, *in vivo* studies revealed better DM intake and digestibility of gross nutrients and fibre fractions. The carbohydrate and protein fractions of COBN-5 fodder showed higher degradable fractions. The oxalate content is higher in early stages compared to mature fodder. Hence, it is recommended that Cumbu Napier (COBN-5) hybrid fodder can be used for feeding to ruminants and can be harvested at 40 days for animal feeding without any adverse effects.

Chapter - I

Introduction

CHAPTER – I

INTRODUCTION

Livestock play an important role in the rural economy of India by providing employment and supplementary family income, which contributes about 21% of the total agricultural income (Velayudham *et al.*, 2011). India is the world's largest single milk producer, with a total of 117 million tonnes of liquid milk produced in 2010 (www.nddb.coop). Dairy production is one of the most important agricultural activities in the country, contributing about 5.3 per cent to the agricultural gross domestic product. Forages usually constitute the major portion of the ruminant feeds and high quality nutritious green fodder is required to exploit the productive potential of dairy animals of our country (Datta, 2013). The low productivity of dairy animals in our country is attributed to inadequate supplies of quality feeds and fodder, gradual genetic deterioration and general neglect of animals over centuries. Due to ever-increasing human population in India, arable land is diverted to production of food and cash crops with little chance for fodder production. The critical constraint in profitable animal production in developing countries is the inadequacy of quality forage. There may be many alternatives to overcome the shortage of forage and one among them is the introduction of high yielding forage varieties (Mahr-un-nisa and Sarwar, 1998). Hence, cultivation of perennial fodders with higher biomass per unit area is the immediate solution to meet the current livestock production.

Napier grass is a tall stout and deep rooted perennial bunch grass well known for its high yielding capability and usage as forage for livestock. It is one of the highest yielding perennial tropical fodder grasses and considered as cut and carry forage (Woodard and Prine, 1991). It can withstand considerable periods of drought and rapidly recovers with the onset of rain (Butt *et al.,* 1993). Several workers reported the potential for improvement in yield and quality of Bajra × Napier hybrids over Napier. The parental materials of both Bajra and Napier have been improved making it possible to develop better hybrids in terms of quality and yield. Forage quality of most of these hybrids was also superior to their ancestors (Gupta and Mhere, 1997). Consequently, high yielding forages including a number of varieties of Napier hybrids have been introduced in many parts of the world, including India.

Napier bajra hybrid, an inter-specific cross between Napier (*Pennisetum purpureum Schumach*) and pearl millet (*Pennisetum americanum*) is an important perennial forage grass with high yield potential. It is popular owing to high yield, palatability and adaptability to varying soil and climatic conditions (Faruqui *et al.,* 2009). It has gained considerable importance in dairy industry because of its quick sprouting and rejuvenating capacity. In recent years, several new cultivars of hybrid Napier were released and are becoming popular among farmers. Along with high yielding potential and climate adaptability, nutritional quality is also considered a criterion for selection by farmers. Due to various reasons, growing forage crops is a new concept for most of farmers, unlike growing food and other cash crops.

With these objectives, breeding work was commenced in Department of Forage Crops, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural

University (TNAU), Coimbatore resulting in a high yielding nutritious forage variety *viz*., Cumbu Napier Hybrid Fodder (COBN-5).

Cumbu Napier (COBN-5) hybrid fodder was developed by the scientists of TNAU at Coimbatore and released for commercial cultivation in 2013. It is an inter-specific hybrid between Fodder Cumbu IP 20594 (*Pennisetum glaucoma*) and Napier grass FD 437 (*Pennisetum purpureum Schumach*). The characteristic features of COBN-5 include robust tillering perennial grass, high biomass potential round the year, high palatability, high leaf to stem ratio and succulent stem with a mean green fodder yield of about 1080 q/ha/yr (Babu *et al.*, 2014). Green forages generally meet the requirements of maintenance, however nutritive evaluation of a particular fodder is the need of the hour for their optimum utilization in ruminant feeding.

Hence, the present work has been proposed with the following objectives:

1. To analyze the chemical composition, carbohydrate and protein fractions of Cumbu Napier (COBN-5) hybrid fodder.

2. To study the effect of feeding COBN-5 fodder on rumen fermentation in cannulated buffalo bulls.

3. To evaluate the nutrient digestibility of COBN-5 fodder through *in vitro* and *in sacco* studies in buffalo bulls.

4. To determine the voluntary feed intake and nutritive value of COBN-5 fodder in buffalo bulls.

Chapter - II

Review of Literature

CHAPTER - II

REVIEW OF LITERATURE

In developing countries, feeding of dairy cattle is often based on crop residues and low quality native hay or pasture. Quality feed and fodder are the key factors for enhancing farm animal milk production and yield level. Consequently, a number of cultivated perennial fodders *viz*. Napier varieties were developed for sustained milk production.

Forage quality of these fodders were further improved by developing Bajra × Napier hybrids which were proved to be superior over Napier varieties. The Cumbu Napier hybrid fodder (COBN-5) is one such variety with high biomass potential and superior nutritive value. The performance of ruminant animals fed various Napier varieties and Napier hybrid fodders is reviewed under this chapter.

2.1 AVAILABILITY OF FODDERS

The increasing number of livestock and changing dynamics of animal husbandry practices, require corresponding increase in the type of quality fodder to meet the requirements of new situations in India. Various studies have been carried out to assess the demand and supply of fodder resources, especially with respect to green and dry fodder (Forestry Statistics India, 2000).

One such project estimate pegs the demand of green fodder and dry fodder in 2006 at 817.25 and 614.93 million tonnes, respectively. Whereas, the project requirement has increased by more than 50%, the fodder availability is estimated to have increased by only 14.5% in the five years between 2001 and 2006. It has resulted in a projected shortage of more than 60% in green fodder and nearly 23% in dry fodder by 2020. Converted into absolute terms, this deficit works out to 728 million tons in respect of green fodder and 157 million tons in respect of dry fodder.

2.2 FODDER YIELD

In recent years, the availability of land is very low for the fodder production due to increased urbanization. So, the high yielding varieties are required to produce more milk yield and to exploit the production potential of the animal. In the high category, Napier fodder is well known for its high yielding capability and usage as fodder for livestock (Woodard and Prine. 1991). It can withstand considerable periods of drought (Butt *et al.*, 1993) and rapidly recovers with onset of rain.

Napier grass (*Pennisetum purpureum* (L) Schumach) was studied using 5 x 3 factorial experiment arranged in a randomised complete block design with three replications to know the dry matter yield at different heights (0.5, 1.0 and 1.5 m). The dry matter (DM) yield at different heights (0.5, 1.0 and 1.5 m) was 5.90, 9.18 and 9.53 t/ha, respectively (Zewdu *et al.*, 2002).

Zewdu (2005) reported varied dry matter yield with the different Napier grass accessions (14983, 14984, 15743, 16834, 16835, 16786, 16971, 16798 and 16836) and it ranged from the 4.6 to 20.5 tonnes of DM /ha, respectively.

A study was conducted to know the yield of CO-3 perennial fodder grass developed by the Tamil Nadu Agricultural University, Coimbatore. This grass is placed in a higher category, especially on tillering capacity, green forage yield, regeneration capacity and leaf to stem ratio. The green fodder yield recorded for CO-3 was 250-350 t/ha/yr under local conditions (Premaratne and Premalal, 2006).

Perez *et al*. (2010) conducted a research on the yield and nutritive value of the Napier grass. The dry matter yield of Napier grass ranged from 11 to 15 t/ha/yr. Because of this dynamic yield, the producers prefer the Napier grass varieties in Cuba.

Bora *et al.* (2011) explored the effect of variety and stage of harvest of CO-3 on the yield and its chemical composition. The dry matter yield was 23.5, 37.5 and 38.1 t/ha at 60, 90 and 120 days of harvesting and the corresponding values for CO-2 were 21.8, 35.5 and 36.2 t/ha, respectively.

Chellamuthu *et al.* (2011) conducted a study on green fodder yield of different Napier varieties (IGFRI-3, IGFRI-7, IGFRI-10, PBN-233, CO-3, CO-4 and KKM-1) and reported a highest yield of 29.77 t/ha in Co-3 and the lowest yield of 24 t/ha in PBN-233.

A study was conducted to know the yield and the chemical composition of Napier grass at different cutting intervals and height. The results showed that the yield is more between 45- 60 days of cutting interval. The dry matter yield at 45 days of cutting interval is 3.56 kg/ha and at 60 days of cutting interval is 4.68 t/ha, respectively (Lounglawan *et al.*, 2014).

Field experiment was carried out to know the growth and fodder yield of Bajra Napier hybrid grass (COCN)-4 as influenced by crop geometry, irrigation regimes and nitrogen fertilization. The study reported a green fodder yield of 368.17 t/ha and dry matter yield of 75.62 t/ha, respectively (Alagudurai and Muthukrishnan, 2014).

Babu *et al.* (2014) conducted a study to know the yield of different Napier varieties (CO-3 and COBN-5 fodder) and recorded higher green fodder yield (326.0 t/ha/yr) in COBN-5 than the CO-3 (296.0 t/ha/yr).

2.3 CHEMICAL COMPOSITION OF FODDERS

The chemical composition of the hybrid Napier varies with the strain, stage of maturity, soil conditions and climate. Young plants are characterized by their high protein content, low cellulose and lignin content and a high digestibility.

Balaraman (1995) nutritionally evaluated the hybrid Napier grass in goats and reported a DM, OM, CF, CP, NFE, EE and Ash content (%) of 13.5, 88.9, 27, 13.5, 42, 6.4 and 11.1, respectively.

Kariuki *et al.* (1998) conducted an experiment to know the effect of feeding the Napier grass, Lucerne and sweet potato vines as a sole diet to the dairy heifers on nutrient intake, weight gain, and rumen degradation. Results showed that Napier grass contained OM, CP, Ash, Ca, P, NDF, ADF, ADL and Silica (g/kg DM) of 796, 118, 204, 3.4, 3.4, 587, 301, 47 and 53, respectively.

Kaitho and Kariuki (1998) studied the effect of feeding Napier grass as basal diet in dairy heifers along with the Desmodium, Sesbania and Calliandra.

The chemical composition and cell wall constituents of the young Napier grass (7 weeks) or old Napier grass (16 weeks) of regrowth showed DM, Ash and CP content (%) as 16.6, 19.8 and 8.5; 23.7, 16.5 and 6.4; NDF, ADF and ADL were 62.7, 29.0 and 2.6 ; 67.5, 34.4 and 3.1 respectively.

Mahr-un-nisa *et al.* (2000) conducted a research on the chemical composition, nutrient digestibility and ruminal characteristics of the Mott grass in buffalo bulls. The Early Cut Mott grass (ECM) and Late Cut Mott grass (LCM) were harvested after 40 days and 60 days of first cutting from an already established stand of Mott grass. The fibre constituents of the ECM and LCM were 74.5, 42.8, 8.6, 34.2 and 31.7; 79.4, 51.3, 13.1, 38.2 and 28.1 for NDF, ADF, ADL, cellulose and hemi-cellulose, respectively.

A study was conducted on rumen degradation and estimation of microbial protein yield and intestinal digestion of Napier grass. In this study, results showed that the chemical composition and fibre constituents of the Napier grass contained (g/kg DM) OM, CP and EE *viz*. 784, 83 and 37; NDF, ADF and ADL *viz*. 541, 303 and 36, respectively (Muia *et al.*, 2001).

Gwayumba *et al.* (2002) compared two varieties of Napier grass (Bana Napier grass vs. French Cameroon Napier grass) and determined the chemical composition, feed intake, digestibility, average daily gain (ADG) and milk yield of lactating Friesian cows. The study reported an average DM, CP, ash, NDF, ADF and lignin content (%DM) were 20.75, 6.65, 9.3, 75.0, 47.9 and 6.25, respectively.

Napier grass (*Pennisetum purpureum* (L) *Schumach*) was studied using 5 x 3 factorial experiments arranged in a randomised complete block design with three replications to know the chemical composition at different heights. Results depicted the DM, CP, ash, NDF, ADF, ADL, cellulose and hemi-cellulose content (%) of Napier grass at 1m height as 18.2, 16.6, 11.5, 61.5, 35.5, 3.6, 23.8 and 26.0, respectively (Zewdu *et al.,* 2002).

Islam *et al.* (2003) evaluated the effect of variety on proportion of botanical fractions and nutritive value of different Napier grasses (Arusha, hybrid Napier, Pusha, 17 and Bazra) and relationship between botanical fractions and nutritive value. Results depicted that the average CP, CF, EE, NFE and ash content (% DM) were 8.4, 31.3, 1.8, 46.9 and 11.8, respectively.

An experiment was conducted to study the feed intake and lactation performance of dairy cows offered Napier grass supplemented with legume hay. The proximate constituents of Napier grass reported to contain DM, CP, Phosphorus and Calcium (g/kg) as 176, 68.4, 1.5, and 3.2, respectively (Nyambati *et al.,* 2003).

Tessema and Baars (2004) conducted an experiment to assess the effect of Napier grass and Sesbania leaves on chemical composition, *in vitro* dry matter digestibility (IVDMD), *in sacco* rumen DM degradability (DMD), organic matter degradability (OMD) and crude protein degradability (CPD). Results showed that the cell wall contents *viz*. NDF, ADF, ADL, cellulose and hemicellulose were 616, 326, 36, 198 and 289 (g/kg DM), respectively.

Kabi *et al.* (2005) conducted an experiment on the beef bulls regarding the evaluation of protein degradation characteristics and metabolisable protein of elephant grass and locally available protein supplements. Results showed that the chemical composition and cell wall constituents of Napier grass as 901, 889, 115.4, 111, 746.9 and 66.6 (g/kg DM) for DM, OM, CP, Ash, NDF and ADL, respectively.

A study was carried out to evaluate intake, digestibility, ruminal fermentation, nitrogen retention and ruminal microbial protein synthesis in lambs fed dwarf elephant grass (*Pennisetum purpureum Schum*. cv. Mott) hay. Results depicted that OM, EE, NSC, NDF, ADF and Lignin content (g/kg DM) of hay as 874, 28, 69, 723, 401 and 39, respectively (Kozloski *et al.*, 2006).

Osuga *et al*. (2006) evaluated the chemical composition, rumen degradation and *in vitro* gas production parameters in some browse forages, grasses and maize stover from Kenya. Results revealed that the OM, CP, NDF, ADF and ADL content of Napier grass (g/kg DM) were 837.3, 173.2, 590.3, 360.5 and 28.7, respectively.

Raja Kishore *et al.* (2008) studied the quality indices of non-leguminous tropical forages (Para grass, CO-1, APBN-1, Jowar, Guinea grass, Guinea grass, Maize, NB-21 and Anjan grass) based on the cell wall contents. Results revealed that the chemical composition and cell wall contents of cultivated APBN -1 contains 6.0, 2.1, 10.2, 56.1, 35.1 and 3.1 (% DM) of CP, EE, Ash, NDF, ADF and ADL, respectively.

Reddy *et al.* (2009) evaluated the hybrid Bajra Napier fodder along with the tree fodder in goats and reported an OM, CP, EE, CF and Ash content (% DM) of 88.0, 10.3, 2.0, 30.2 and 11.9, respectively. The cell wall contents *viz*. NDF, ADF and ADL were 64.3, 39.5 and 4.2 (% DM), respectively.

Ajaib Singh *et al.* (2009) compared the nutritive value of new variety of (PHBF-1) hybrid bajra with the standard variety (FBC-16) in adult male buffaloes and reported DM, CP, CF, EE and NFE (%) content of 15.6, 10.70, 23.50, 2.70 and 52.60; 14.90, 9.60, 25.00, 2.50 and 52.20, respectively. The cell wall constituents *viz*. NDF, ADF, cellulose, hemi cellulose and lignin (% DM) were 64.4, 40.1, 24.8, 24.3 and 5.0; 65.6, 41.7, 26.8, 23.9 and 4.8, respectively, in PHBF-1 and FBC-16.

In another study, yield and nutritive value of Napier grass was studied. Results showed that the dry matter and crude fibre (%) content increased progressively with age from low of 29.95 at 18^{th} day to 35.89 at 81^{st} day but the crude protein (CP %) content decreased with the age from 14.71 at 18^{th} day to 6.11 at 81^{st} day, respectively (Perez *et al.*, 2010).

A study was conducted to estimate the *in situ* and *in vitro* degradations of the forages (Alfalfa hay, Corn silage, Elephant grass, Guinea grass, Corn stover and Oat straw) in ruminants. The proximate composition and cell wall constituents of elephant grass *viz*. DM, CP, ash, NDF and ADF content (g/kg) were 920, 85, 91, 62.4 and 37.3, respectively (Gallardo *et al.*, 2010).

Srinivas Kumar *et al.* (2011) conducted an experiment on 6 graded Murrah buffalo bulls by dividing into two groups of three animals each maintained on 1.5

kg of concentrate mixture and *ad libitum* hybrid Napier (CO-1). Results indicated DM, OM, CP, EE, CF, NFE and Total ash (%) of CO-1 were 24.39, 89.29, 7.88, 2.24, 32.44, 46.73 and 10.71. The cell wall constituents *viz*. NDF, ADF, ADL, cellulose and hemi-cellulose (%) were 73.8, 47.2, 5.9, 39.3 and 26.6, respectively.

Budiman *et al.* (2012) conducted a study to determine the productivity and quality of the Napier grass cultivars (Taiwan, King and Mott) harvested at 8 and 13 weeks after planting. Results showed that the average crude protein (%) content of the Napier grass at 3, 6, 9 and 12 weeks were 15.9, 14.7, 14.4 and 9.3, respectively. The average fibre fractions *viz*. NDF, ADF and ADL at 8 weeks and 12 weeks were 67.1, 43.32 and 6.01; 73.43, 48.73 and 8.25, respectively.

Ferreira *et al.* (2012) evaluated some feedstuffs nutritionally for capybaras and reported an OM, CP, NDF and ADF content (g/kg DM) of 870, 62, 740 and 459, respectively.

The chemical composition of twelve commonly used Indian forages (*Pennisetum purpureum*, *Panicum maxicum* and *Saccharum officinarum*) was studied. The proximate composition of the *Pennisetum purpureum* was reported as 55.6, 863,17.5, 736, 406, 331 and 30.6 for CP, OM, EE, NDF, ADF, cellulose and lignin (g/kg DM), respectively (Sultan Singh *et al.,* 2012).

Morenz *et al.* (2012) evaluated the chemical composition and dry matter intake of elephant grass in eight lactating cows. The CP, EE and ash content (% DM) of elephant grass at two different months (*i.e.* March and April) were 12.8, 1.2 and 9.4; 12.3, 1.3 and 9.2, respectively. The NDF, ADF and lignin content of elephant grass at two different months (*i.e.* March and April) were 72.6, 37.9 and 7.8; 72.7, 38.4 and 7.3, respectively.

The NB-21 hybrid Napier grass was nutritionally evaluated in goats. Results showed that the OM, CP, EE, CF, NFE, total ash, calcium, phosphorus, NDF and ADF (% DM) contents were 87.53, 11.62, 2.45, 30.12, 36.89, 8.56, 0.49, 0.17, 64.67 and 41.51, respectively (Chandra *et al.,* 2012).

Garg *et al*. (2012) conducted a study to assess the nutritional characteristics of green grasses and concentrates. Among the green grasses, eight different hybrid Napier varieties (CO-3, CO-1, PBN-233, PBN-83, PBN-2, PBN-231, RC-2 and CO-4) were evaluated for chemical composition. Results showed that the average DM, CP, EE, CF and total ash contents (%) were 22.35, 12.48, 2.66, 23.10 and 11.88, respectively. And the average cell wall constituents *viz.* NDF and ADF (% DM) ranged from 66.37 (CO-1) to 70.05 (PBN-2) and 36.80 (PBN-233) to 40.13 (PBN-2), respectively.

An experiment was carried out to study the effect of milk dhara on milk yield in graded Murrah buffaloes along with the basal diet of hybrid Napier (APBN-1) fodder to meet the requirements. Results depicted that the OM, CP, EE, CF, NFE, Total ash, NDF, ADF and ADL contents (%DM) were 91.0, 8.5, 2.19, 36.8, 43.5, 8.98, 75.1, 47.8 and 7.4 %, respectively (Srinivas Kumar *et al.*, 2013).

Lounglawan *et al*. (2014) conducted a study to know the yield and chemical composition of the Napier grass. Results showed a DM, CP, EE, CF and Ash content of (%) 17.16, 10.13, 1.95, 36.32 and 11.99, respectively at 45 days of harvest. The protein content (%) is more at 30th day (12.93) and low at 60th day of harvest (8.64).

A study was conducted on 11 popular hybrid Napier varieties (CO-2, CO-3, CO-4, KKM-1, Suguna, Supriya, IGFRI-3, IGFRI-7, DHN-6, PTH and PBN-16) for one year during 2011–2012 to evaluate the nutritional quality. Results showed that mean CP, CF, EE, NFE and total ash content (% DM) were 10.3, 31.09, 1.98, 50.54 and 8.12, respectively (Antony and Thomas, 2014).

Sahoo *et al.* (2014) evaluated the chemical composition of 13 marginal land grass samples (Clover, Kikyu, Guchhi, Rye, Bhimlsa, Brome, Guinea, Sita, Neelima, Neelam, Napier, Kumeria and Kans) and reported an OM, CP, EE, NDF and ADF content (% DM) as 88.5, 6.5, 1.3, 38.7 and 39.3, respectively.

Bueno *et al.* (2015) evaluated four roughages (alfalfa, Tifton, maize silage and acacia) for their chemical composition, cell wall constituents and *in vitro* methane production. The OM, EE, CP, NDF, ADF and ADL contents (g/kg DM) of elephant grass were 898, 47, 60, 770, 520 and 122, respectively.

The chemical composition and fibre fractions of the Napier grass harvested at 35-40 days of age contained an OM, CP, EE, NFC, NDF, ADF and ADL content (% DM) of 82.4, 10.8, 2.74, 5.6, 63.2, 39.2 and 3.92, respectively (Kondo *et al.,* 2015).

Kebede *et al.* (2016) conducted an experiment to study chemical composition and *in vitro* Organic Matter Digestibility of Napier Grass (*Pennisetum purpureum* (L) Schumach) accessions in the mid and Highland Areas of Ethiopia. Results showed that ash and CP content (g/kg DM) were 144.6 and 57.3, respectively. The fibre fractions *viz*. NDF, ADF, ADL, cellulose and hemi cellulose contents (g/kg DM) were 770, 474.6, 70.4, 404.2 and 295.4, respectively.

2.4 CNCPS FRACTIONATION OF FORAGES

2.4.1 Carbohydrate and protein components and fractions

Carbohydrates are composed of structural and non-structural carbohydrates or soluble and insoluble fractions. The proportions of these fractions are imported in determining the animal response to forage (Van Soest, 1994). In nitrogen fractionation of ruminant feeds, non-protein nitrogen is denoted as ‘A’ fraction while true protein is broken down into B_1, B_2, and B_3 fractions based on decreasing solubility. The nitrogen that is insoluble in acid detergent is denoted as the ‘C’ fraction, and it is assumed to be indigestible (Licitra *et al.,* 1996).

Bhadauria *et al.* (2002) evaluated the shrubs and tree leaves for carbohydrate and nitrogen fractions. In tree leaves, lignin (% NDF) was lowest in *Harawickia binate* (10.20) and highest in *Albizia lebbeck* (42.79) whereas, non-structural carbohydrate (% NSC) was maximum in *Leucaena leucocephala* (27.34). Starch (% NSC) varied from 42.79 to 100. Lignin (% NDF) widely varied among different shrubs, from 11.43 (*Secuinegia virosa*) to 57.09 (*Carissa spinarum*). The quantity of NSC (% starch) in different shrubs also varied widely from 21.53 to 100. In tree leaves, the carbohydrate fraction C (% CHO) was lowest (21.39) in *Hardwickia binate* and highest (25.91) in *Dalbergia sissoo*. Similarly, low content of fraction C (% CHO) was reported in *Securinega virosa* (13.46) in shrubs compared to the other species. Further, the results showed that the soluble protein (% CP) among different shrubs varied from 61.97 (corn silage) to 90.30 (*Acacia leucophloea*). The corresponding values for ADIP and NDIP ranged from 2.37 to 18.03 and 3.70 to 20.12, respectively.

The chemical composition, carbohydrate and protein fractions of timothy and alfalfa fodder were analyzed. The results showed that alfalfa contains higher CA (36.0 vs. 14.7 % CHO), and CC (35.1 vs.16.4 % CHO), but lower CB_2 (27.4 vs. 67.6 % CHO) compared to timothy. The NSC (% DM) and lignin (% NDF) content of alfalfa ranged from 22.4 to 28.9 and 17.1 to 23.1, respectively. In case of protein fractions (% CP), PA (41.5 vs.16.5), PB_3 (27.2 vs. 21.3) and PC (8.4 vs.5.2) were higher, but PB_1 (8.4 vs. 23.6) and PB_2 (10.7 vs. 33.5) were lower in alfalfa fodder compared to timothy hay, indicating large and potential differences in rumen CP degradation characteristics (Yu *et al.*, 2003).

Twelve grasses (guinea grass, Napier, Napier thin, Napier-3, Napier-7, Nandi grass, marvel grass, vetiver grass, *Bracharia sp*, lemon grass, *Stylosanthus hamata*and *scabra*) were analysed for proximate and fiber constituents, nitrogen solubility, fibre bound nitrogen and nitrogen fractions. The protein fractions (% CP), PA ranged from 23.8 (*Pennisetum polystachyon*) to 36.6 (Napier-3), PB_1 ranged from 1.4 (*Pennisetum polystachyon)* to 9.6 (Napier-3), PB_2 ranged from 18.7 (Napier-3) to 27.8 (*Pennisetum purpureum*), PB_3 ranged from 9.1 (Napier-7) to 32.8 (*Pennisetum polystachyon*) and PC ranged from 14.0 (*Pennisetum purpureum*) to 23.4 (Napier-7), respectively (Sharma *et al.*, 2004).

Five species each of tree (*Albizia lebbek, Leucaena leucocephala, Hardwickia binate*, *Grewia optiva, Anogeissus pendula*), shrubs (*Acacia catechu, Securengia virosa*, *Zizyphus xylophyrus*, *Dichrostachys cinera*, *Helictris isora*) and grasses (*Heteropogan contortus, Sehima nervosum, Chrysopogon fulvus, Cenchrus ciliarus, Dichianthium annulatum*) were nutritionally evaluated for their chemical composition, carbohydrates, proteins and nitrogen fractions. Grasses proved to be richest in total carbohydrates compared to the shrubs and tree leaves. The carbohydrate fractions (% CHO) *viz.* CA (20.57) and CB_1 (23.81) were higher and CC (12.15) was lower is grasses indicating the superiority of carbohydrate quality. The mean (% CP) values for fraction A, B_2, and C were 25.07, 29.57 and 19.15 in tree leaves; 10.95, 31.12 and 26.84 in shrubs and 12.93, 15.02 and 46.02 in grasses, respectively (Chaurasia *et al.*, 2006).

Morenz *et al.* (2012) predicted dry matter intake and milk production of grazing crossbred cows using CNCPS model. The results showed that the carbohydrate fractions of the elephant grasses *viz*. total carbohydrate, non-fibrous carbohydrate ($A+B_1$), B_2 and C were 76.7, 9.0, 48.7 and 18.8, respectively. The mean values for carbohydrate fractions (% CHO) were 27.1, 27.1 and 3.5 for A, B_1 and B_2, while protein fractions (% CP) were 14.2, 45.5, 32.9 and 7.3 for A, $B_1 + B_2$, B_3 and C, respectively.

Sultan Singh *et al.* (2012) evaluated 12 commonly used Indian green forages (*Hordeum Vulgare, Avena sativa, Trifolium alaxendrium, Medicago* *sativa, Pennisetum purpureum, Panicum maximum, Saccharum officinarum, Arachis hypogea, Grewia optiva Leucaena laeucocephala* and *Sorghum bicolor*) for *in vitro* ruminal fermentation, protein and carbohydrate fractionation and methane prediction. The carbohydrate components *of Pennisetum purpureum viz*. TCHO, NSC, SC and starch (g/kg DM) were 790, 79.2, 710 and 21.7 and the carbohydrate fractions (% CHO) *viz*. CA, CB_1, CB_2, CC were 57.5, 21.7, 637 and 73.5, respectively. The protein fractions of *Pennisetum purpureum viz*. NPN, BSP, NDSP, ADSP, ADIP (g/kg DM) were 9.43, 6.85, 13.6, 19.6 and 6.10, respectively.

An experiment was conducted to study dry matter and NDF degradation kinetics of roughages (Maize, Pearl millet, Sorghum, Oats Fenugreek, Cowpea, Berseem, Guar, Paddy Straw, Wheat Straw, Sugarcane Tops, Sorghum Stover, Guinea Grass, Sehima Grass, Crysopogon Grass, Heteropogan Grass, Buffel Grass, Bermuda Grass and Babool Grass) in relation to carbohydrate and protein fractions. Results showed that the total CHO (% DM) in forages ranged from 26.51 (berseem) to 86.61 (wheat straw), whereas the NSC (% DM) ranged from 1.9 (buffel grass) to 46.07(gaur), respectively. The CA fraction (% CHO) ranged from 0.81 (wheat straw) to 60.73 (fenugreek) and CB_1 fraction (% CHO) ranged from 4.91 (Sehima grass) to 21.55 (maize). Fraction CB_2 (% CHO) averaged 47.58 with highest in Sehima (66.45) and lowest in oats fodder (2.28). Further the PA (% CP) fraction was highest in Bermuda grass (37.88) and lowest in wheat straw (6.25) whereas, PB_1 (% CP) fraction was highest in heteropogon (30.77) and lowest in wheat straw (2.5). The PB_2 (% CP) fraction ranged from 22.51 (Heteropogon) to 77.68 (cowpea). The PB_3 (% CP) fraction was lowest in guar fodder (1.61) and highest in Cryosopogon (37.93). PC (% CP) fraction was the lowest (1.25) in cowpea and highest (49.31) in paddy straw (Prusty *et al.*, 2013).

Tropical grasses (*Cenchrus ciliarus, Leucaena leucocephala, G.optiva, M.alba, P.purpureum, P.maximum, C.ciliaris, S.nervosum*) were evaluated for carbohydrate and protein fractionation. The carbohydrate components of *Pennisetum purpureum* (g/kg DM) *viz*. TCHO, NSC, SC were 739, 225, 514, respectively, and the carbohydrate fractions (g/kg DM) *viz*. CA, CB_1, CB_2 and CC were 0.26, 0.05, 0.66 and 0.03, respectively. The protein fractions of *Pennisetum purpureum* were 37, 373, 259, 312 and 17 (g/kg CP) for NPCP, BSP, NDSP, ADSP and ADIP, respectively (Sultan Singh *et al.*, 2014).

Das *et al.* (2015) fractionated some forages (Para grass, Guinea grass, Hedge Lucerne, Seteria grass and Hybrid Napier) by CNCPS model. Results showed that the carbohydrate components *viz*. CHO (%DM), NSC (% DM), SC (% DM), NSC (% DM), SC (% CHO), starch (% DM), starch (% NSC) and ADL (% NDF) were 76.4, 8.0, 68.4, 10.4, 89.6, 5.1, 64.4 and 5.6, respectively, and carbohydrate fractions (% CHO) *viz*. CA, CB_1, CB_2 and CC were 10.6, 19.2, 59.3 and 10.9, respectively, in hybrid Napier. The protein components of hybrid Napier *viz*. TP, NPN, SP, IP, SP (% CP) and NPN (% SP) were 7.6, 3.3, 3.9, 7.0, 35.8 and 84.6, respectively, while protein fractions PA, PB_1, PB_2, PB_3 and PC (% CP) were 30.3, 5.5, 4.6, 43.2 and 16.4, respectively.

2.5 *IN VITRO* DIGESTIBILITY OF FORAGES

Napier grass (*Pennisetum purpureum* (L) Schumach) was studied using a 5 x 3 factorial experiment arranged in a randomised complete block design with three replications to know the IVDMD at different heights. The IVDMD (%) of Napier grass variety at different heights (0.5, 1.0 and 1.5 m) was 71.74, 65.50 and 61.03, respectively (Zewdu *et al.*, 2002).

Nyambati *et al.* (2003) evaluated Napier grass alone and with the supplements like mucuna hay, lablab hay and commercial dairy meal for *in vitro* digestibility. The results showed that the IVDMD (g/kg) of the Napier grass alone is 602.

Shaikh *et al.* (2004) conducted an *in vitro* experiment on *Stylosanthes* varieties (RSS-2000-95 (V_1), RSS-2000-14(V_2), RSS-2000-10(V_3), RSS-2000-70A (V_4), RSS-2000-46(V_5), RSS-2000-70(V_6), RSS-2000-13(V_7),RSS-2000-72(V_8), *S. hamata* (V_9), *S. scabra* (V_{10}) at different stages of growth. The *in vitro* DM digestibility (IVDMD) was significantly affected by varieties and stages of harvest. Irrespective of varieties the IVDMD (%) was more (61.9) at 3rd stage and lowest at 2nd stage (47.7). The interaction effect between varieties and stages of growth in *Stylosanthes* had significant effect on IVDMD.

An experiment was conducted to assess the effect of Sesbania leaves on IVDMD, *in sacco* rumen DM degradability (DMD), organic matter degradability (OMD) and crude protein degradability (CPD) of Napier grass and Sesbania mixtures was conducted. The IVDMD (g/kg DM) and ME (MJ/kg DM) of Napier grass were 668 and 8.61, respectively (Tessema and Baars, 2004)

Ten Napier grass accessions (14983, 14984, 15743, 16834, 16835, 16786, 16971, 16798and 16836) were evaluated for the IVDMD at the Adet Agricultural research centre. The IVDMD (%) of the Napier accessions ranged from 62.6 to 70.7. The metabolizable energy (MJ/kg DM) of Napier accessions ranged from 8.61 to 9.77, respectively (Zewdu, 2005)

A study was conducted to determine the *in vitro* dry matter digestibility of Napier grass at different stages of growth and reported a decreasing trend in IVDMD as the maturity of the grass increased. The experimental outcome of IVDMD (%) of the Napier grass at different heights (50 cm, 75 cm, 1 m, 1.25 m, 1.5 m) were 64, 40, 47, 42 and 35, respectively (Aganga *et al.*, 2005).

Zewdu (2006) conducted an *in vitro* digestibility experiment on mixture of Napier grass and Sesbania leaves. The IVDMD (%) of Napier alone is 66.78 but on proportion of Sesbania in the mixture it increased to 70.95, respectively.

An experiment was conducted to know the *in vitro* true digestibility (IVTD %) of Napier grass at different plant heights (48 to 360 cm). The IVTD % of Napier grass at different plant heights ranged from 58 to 89. The digestibility of Napier grass decreased as the height increases (Chen *et al.,* 2006).

Two varieties of hybrid Bajra fodder *viz*. PHBF-1 and FBC-16 were compared nutritionally in six buffaloes and reported an IVDMD (%) of 64.12 and 62.80, respectively (Ajaib Singh *et al*., 2009).

Wadhwa *et al*. (2010) evaluated *in vitro* true organic matter digestibility in non-leguminous fodders. The results explored that the digestibility (%) is lower (48.76) in bajra compared to sorghum (52.26) and maize (58.16), respectively.

Bora *et al.* (2011) compared two varieties of Hybrid Napier (CO-2 and CO-3) at three stages of harvest on *in vitro* dry matter and organic matter digestibility. The IVDMD (%) of CO-3 and CO-2 variety decreased from 54.0 to 49.0 and 47.61 to 44.12, respectively on DM basis from 60 to 120 days. The IVOMD (%) decreased from 61.12 to 58.72 (CO-3) and 56.39 to 53.23 (CO-2) from 60 to 120 days of harvesting, respectively. In two varieties, CO-3 is highly digestible than the CO-2 in all different stages of harvest.

An experiment was conducted to determine the *in vitro* dry matter digestibility of Napier grass cultivers (Taiwan, king and Mott) harvested at 8 and 13 weeks after planting. The results concluded that the Mott cultivers has an average *in vitro* dry matter digestibility (%) of 63.58 compared to Taiwan (53.02) and King (53.66). The IVDMD (%) was higher (66.63) at 8 weeks after planting compared to 13 weeks (47.78) in Mott grass (Budiman *et al.*, 2012).

Morenz *et al.* (2012) evaluated Napier grass for CNCPS model on prediction of dry matter intake and digestibility. The IVDMD (%) of Napier grass was 64.4 and 67.3 in March and April, respectively.

Sahoo *et al.* (2014) evaluated thirteen marginal land grasses samples (*Trifolium repens*, *Pennisetum clandestinum, Dactylis glomerata*, *Lolium perenne, Pennisetum orientale*, *Bromus inermis, Panicum maximum*, *Setaria sphacelata, Poa pratensis, Panicum antidotale, Pennisetum purpureum, Heteropogon* *contortus and Saccharum spontaneum*) for *in vitro* nutritional evaluation. The IVOMD (%) of the thirteen marginal land grasses ranged from 50.9 to 79.5 and reported an IVOMD (%) of 63.8 in Napier grass.

The *in vitro* ruminal fermentation of tropical grasses was studied in wet season in the Philippines. The NDF degradability (%) of the Napier grass is 58.6 and degradable NDF content (% DM) was 37 (Kondo *et al.,* 2015).

Kebede *et al.* (2016) conducted an experiment on the chemical composition and *in vitro* Organic Matter Digestibility of Napier Grass (*Pennisetum purpureum* (L) Schumach) accessions (15743, 16783, 16791, 16792, 16794, 16813, 16815, 16817 and 16819) in the mid and Highland Areas of Ethiopia. The IVOMD of Napier grass accessions (g/kg DM) ranged from 488 and 534.7, respectively.

2.6 *IN SITU* DEGRADABILITY OF FORAGES

Singh *et al.* (1992) evaluated the rate and extent of *in sacco* degradation of dry matter and cell wall constituents of various grass species (Oats, Para grass, Rhode grass, Guinea grass, Kikuyu grass, Setaria anceps cv. Kazungula, Setaria anceps cv. Nandi , Setaria anceps cv.navok and Maize). The average amount of potentially digestible (%) DM, NDF, and ADF were 63.71, 55.74 and 53.08 (24 h), 77.57, 73.22 and 71.94 (36 h) and 86.01, 83.39 and 83.04 (48 h), respectively in various grass species.

Five crossbred (*Bos taurus* X *Bos indicus*) steers each fitted with a flexible rubber cannula, were used to estimate changes in rumen ammonia nitrogen (NH3-N) and *in sacco* degradation characteristics of forages. The DM degradation (%) of Napier grass such as a, b, a + b and c were 20.5, 59.1, 79.6 and 0.032, respectively (Abdulrazak *et al.*, 1996).

Four fistulated Friesian steers were offered Napier grass to assess the effect of dry matter intake, degradation and rumen fermentation. The DM degradation constants of Napier grass *viz.* a, b, c, PD and ED of Napier grass were 197, 492, 0.044, 667 and 468 (g kg/DM), respectively (Kariuki *et al.*, 2001).

Tessema and Baars (2004) conducted an experiment to assess the effect of Napier grass and Sesbania leaves on *in sacco* rumen DM degradability (DMD), organic matter degradability (OMD) and crude protein (CP) degradability (CPD). The 'a', and 'b' fractions of Napier grass for DM, OM and CP (g/kg) were 191 and 559; 149 and 660; 175 and 678, respectively.

Kabi *et al.* (2005) evaluated the protein degradation characteristics and metabolisable protein of elephant grass (*Pennisetum purpureum*) along with locally available protein supplements. The effective CP degradable fractions of Napier grass *viz*. a, b, a + b and c (g/kg CP) were 354.9, 351.9, 706.8 and 0.03, respectively.

Osuga *et al*. (2006) evaluated the chemical composition along with the rumen degradation and *in vitro* gas production parameters in some browse forages, grasses and maize stover from Kenya. The *in situ* DM disappearance (%) of *Pennisetum purpureum* at 24h and 48h of incubation were 66 and 77.9, respectively. The results showed that DM degradation kinetics (%) of a and b in Napier grass were 30.8 and 59.9, respectively.

A study was conducted to know the *in situ* degradability of the forages (Alfalfa hay, Corn silage, Elephant grass, Guinea grass, Corn stover and Oat straw) in ruminants. The *in vitro* study of elephant grass revealed that the DM degradation fractions (%) *viz*. a, b, and a + b (% DM) were 16.6, 46.5 and 63.6. The study reported that degradable fractions *viz*. b and c of NDF and ADF (%) were 61.8, 3.8 and 44.6, 2.6, respectively (Gallardo *et al.*, 2010).

Eight different forages (Sugarcane, Corn silage, Elephant grass (50 days), Elephant grass (250 days), Corn straw, Signal grass hay and Coast cross hay) were evaluated for DM and NDF degradability by nylon (50 μm) bag technique. The elephant grass (50 days old and 250 days old) revealed that the fractions *viz*. 'a', 'b' and 'c' of DM were 16.37, 56.95 and 0.0520; 13.33, 35.54 and 0.0296, respectively. Degradation parameters of elephant grass for NDF (50 days old and 250 days old) revealed that the fractions *viz*. 'a', 'b' and 'c' were 70.09, 26.58 and 0.0258; 41.34, 52.94 and 0.0285, respectively (Valente *et al.,* 2011).

Nineteen forages (Maize, Pearl millet, Sorghum, Oats Fenugreek, Cowpea, Berseem, Guar, Paddy Straw, Wheat Straw, Sugarcane Tops, Sorghum Stover, Guinea Grass, Sehima Grass, Crysopogon Grass, Heteropogan Grass, Buffel Grass, Bermuda Grass and Babool Grass) were evaluated for their *in sacco* degradation and their carbohydrate and nitrogen fractions as per CNCPS system. The DM degradation (%) at 96 h was highest in gaur fodder (87.23) followed by oats green (87.15) and fenugreek (86.24). Sorghum Stover (62.95) had highest indigestible DM (%) followed by buffel grass (60.11) and sugarcane tops (47.58). The instantly soluble (%) DM (0 h) was highest in fenugreek (40.45) and lowest in Heteropogon (8.46). The 48h degradability (%) was also highest in fenugreek (85.57) and lowest in sorghum Stover (31.38), respectively (Prusty *et al.*, 2013).

2.7 RUMEN FERMENTATION PARAMETERS

Five crossbred (*Bos taurus X Bos indicus*) steers each fitted with a flexible rubber cannula, were used to estimate changes in rumen ammonia nitrogen (NH3-N) and *in sacco* degradation characteristics of Napier fodder alone and along with the gliricidia / leucaena forages. Results showed that the rumen pH and ammonia nitrogen of Napier grass were 6.62 and 130 mg/L, respectively (Abdulrazak *et al.*, 1996).

Four ruminally cannulated buffalo calves (2yrs, 250 kg BW) were used in a 4×4 Latin Square design to know the ruminal characteristics of Mott grass with or without berseem hay. The mean rumen ammonia concentration of 17 mg/100 ml was observed in buffalo calves fed *ad libitum* Mott grass, while the value is 12 mg/100 ml in calves fed restricted Mott grass (Mahr-un-nisa and Sarwar, 1998).

Mahr-un-nisa *et al.* (2000) evaluated the ruminal characteristics of the Mott grass diets using 4×4 Latin square design in four ruminally cannulated buffalo bulls. The ruminal pH tend to be higher in first 6 h post-feeding in animals fed nitrogen fertilized Mott grass. The ammonia concentration of the early cut with nitrogen fertilized Mott grass had 25 mg/dl at 3 h and least concentration is seen in late cut and non-fertilized Mott grass (10-15 mg/dl) at 3 h. But the concentration was decreased as the time progressed and lowest concentration of ammonia was noticed at 12 h of post feeding.

Four fistulated Friesian steers were offered Napier grass to assess the effect of dry matter intake, degradation and rumen fermentation. The rumen fermentation characteristics *viz*. rumen pH, NH3-N (mg/L) and total VFA (mmol/L) in steers fed Napier diets were 6.6, 130 and 87.3, respectively (Kariuki *et al.,* 2001).

Muia *et al.* (2001) conducted an experiment to determine the feed intake and rumen fermentation parameters of Napier grass using 4×4 Latin square design in four rumen fistulated Friesian steers. The rumen pH, ammonia nitrogen (mg/L) and volatile fatty acid (mmol/L) were 6.68, 101.01 and 123.75; 7.10, 51.16 and 101.79, respectively, in steers fed medium (10 wks) and old mature Napier grass (15 wks).

The study was carried out to evaluate intake, digestibility, ruminal fermentation, nitrogen (N) retention and ruminal microbial protein synthesis in lambs fed dwarf elephant grass (*Pennisetum purpureum* Schum. cv. Mott) hay. The results showed that the pH and ammonia nitrogen (mg/dL) of strained rumen liquor was 7.27 and 8.34, respectively (Kozloski *et al.,* 2006).

Mondal *et al.* (2010) assessed the total ammonia nitrogen concentration in some ruminant feeds (Mustard Cake (MC), FA Treated MC, Groundnut Cake, FA Treated GNC, Maize Grain, Wheat Grain, Wheat Bran, Barley, Maize Fodder, Wheat Straw) through *in vitro* method after 48hrs of incubation. The ammonia nitrogen concentration (mg/dL) was 11.65 (Maize fodder) and 1.34 (Wheat straw).

Bandeswaran *et al.* (2013) conducted an experiment on rumen parameters of Napier grass based diets with different feeding strategies. They compared the FFS (farmers feeding strategy) and EFS (evolved feeding strategy) and reported the pH and ammonia nitrogen (mg %) as 6.94 and 10.19 (FFS); 6.91 and 9.42 (EFS), respectively.

2.8 NUTRIENT DIGESTIBILITY AND NUTRITIVE VALUE OF FORAGES

Balaraman (1995) evaluated the nutritive value of hybrid Napier grass in goats. The digestibility (%) of DM, OM, CP, CF and NFE were 39.11, 41.97, 48.53, 50.11 and 36.65, respectively. The nutritive value of hybrid Napier was 6.54 % DCP and 39.38 % TDN, respectively.

Abdulrazak *et al.* (1996) evaluated the effects of supplementation with *Gliricidia sepium* or *Leucaena leucocephala* forage on intake, digestion and live weight gains of *Bos taurus X Bos indicus* steers offered Napier grass. The DMI of Napier grass (g/kg) was 5.2 and the DMD and OMD (g/kg DM) were 608 and 638, respectively.

Kariuki *et al.* (1998) conducted an experiment to study the nutrient intake and average daily gain on 33 Friesian heifers fed Napier grass for 104 days. The nutritive intake (kg/day) of CP, NDF, Ca and P were 0.59, 2.9, 0.017 and 0.017, respectively. The DMI and ADG (kg/d) were 5 and 0.50, respectively.

Kaitho and Kariuki (1998) studied the effect of feeding young Napier (7 weeks) and old Napier (16 weeks) on heifers. The outcome showed differences ($P<0.05$) on intake of DM, OM, CP and NDF. Animals fed young Napier grass had higher ($P<0.05$) dry matter intake and live weight gain than those fed old Napier grass. The total dry matter intake (% live weight) of 3.1 was observed on the heifers fed young Napier grass.

Mahr-un-nisa *et al.* (2000) evaluated the digestibilities of nutrients of the Mott grass diets in four buffalo bulls. The DMD, OMD, NDFD, ADFD, cellulose digestibility and hemi-cellulose digestibility (%) were 62.8, 64.7, 59.8, 52.7, 57.9 and 70.0, respectively.

Gwayumba *et al.* (2002) compared two varieties of Napier grass (Bana Napier grass vs. French Cameroon Napier grass) to determine the digestibility and ADG. The DM and NDF intake (% BW) of Bana and French Cameroon grass were 2.25 and 1.7; 2.26 and 1.8, respectively. The nutrient digestibility (%) of DM, CP, ADF and NDF for Bana grass and French Cameroon grass were 57.5, 59.9, 52.5 and 54.5; 57.6, 52.6, 58.0 and 59.4, respectively.

Eight multiparous Friesian cows maintained on Napier grass were used in two 4×4 Latin squares to evaluate the apparent digestibility and average weight gain. The mean intake of DM, CP and apparent dry matter digestibility of Napier grass (g/kg) were 108, 695 and 558, respectively (Nyambati *et al.*, 2003).

A study was carried out to evaluate the intake, digestibility, ruminal fermentation, nitrogen (N) retention and ruminal microbial protein synthesis in lambs fed dwarf elephant grass (*Pennisetum purpureum* Schum. cv. Mott) hay.

Apparent DM, OM, NDF, energy digestibility and OMTD (%) were 60, 62, 69, 59 and 75, respectively. Apparent and true N digestibility (%), urinary excretion and retention of N (g/day) were 52.0, 89.0, 6.1 and 0.5, respectively in lambs fed elephant grass (Kozloski *et al.*, 2006).

Ajaib Singh *et al.* (2009) nutritionally evaluated the newly developed variety of Hybrid bajra fodder (PHBF-1) and compared with standard variety (FBC-16) in six adult male buffaloes. Apparent digestibility coefficients of DM, CP, CF and EE (%) for PHBF-1 and FBC-16 were 65.18, 69.16, 68.56 and 60.97; 63.53, 66.90, 67.75 and 59.73, respectively. Nitrogen retained (%) for PHBF-1 and FBC-16 in steers was 36.99 and 26.57. The TDN and DCP (%) values were 65.0, 7.40; 63.4, 6.42, respectively, in buffaloes fed PHBF-1 fodder.

Six metabolism trials were conducted using four male goats in a cross over design to study the different supplementary feeding value along with goats fed with *ad libitum* quantity of Napier bajra green fodder as a basal feed. The total dry matter intake of the Napier grass alone was 344 g/day. The digestible coefficients (%) of DM, OM, CP, EE, CF, NFE, NDF, ADF, hemi cellulose and cellulose were 52.03, 54.72, 64.07, 56.47, 52.92, 53.56, 52.85, 43.63, 67.10 and 46.17, respectively. The TDN and DCP (%) content of Napier grass was 49.55 and 6.65. Daily balances of nitrogen, calcium and phosphorus (g/day) were 2.17, 0.81 and 0.34 whereas, DCP and TDN intake (g/day) are 22.86 and 170.71, respectively (Reddy *et al.,* 2009).

Wadhwa *et al.* (2010) evaluated the non-leguminous forages such as Bajra, sorghum and Maize for dry matter intake, nutrient digestibility and nitrogen retention in animals. The results revealed that the DMI of Bajra was 9.85 (kg/day). The digestibilities (%) of DM, OM, CP, NDF, ADF, Cellulose and hemicellulose were 59.78, 64.26, 67.10, 56.06, 47.85, 65.04 and 67.83, respectively. Nitrogen retained (% nitrogen intake) was highest in animals fed Bajra (56.22) but comparable with those fed maize fodder (55.14).

A study was conducted to know the *in vivo* digestibility of nutrients of the ten re-growth ages (18, 25, 32, 39, 46, 53, 60, 74 and 81 d) of Napier grass. The results concluded that DMD (%) was higher in the 18th day (73.84) of growth and then it decreased as the days of growth increased to 46th day (59.04). Highest CPD (89.48 %) and CFD (78.57 %) were reported at 18th day of growth and lowest was noted at 81st day (Perez *et al.*, 2010).

Chandra *et al.* (2012) nutritionally evaluated NB-21 hybrid Napier in six female Sikkim local goats with an average body weight of 11.20 kg. The digestibility (%) of DM, OM, CP, EE, NDF, and ADF were 55.03, 57.12, 65.22, 56.14, 54.55 and 41.26, respectively. The dry matter intake of the goats was 2.42 % of the body weight and 45 g/kg metabolic body size. Nutritive value (%) in terms of DCP and TDN was 7.10 and 52.25, respectively. Feeding of green hybrid Napier (NB-21) provided sufficient nitrogen, calcium and phosphorus leading to positive balances of nitrogen (2.17 g), calcium (0.90 g) and phosphorus (0.35 g).

A study was conducted to assess the nutritional characteristics of some feedstuffs. The ME intake (MJ/kg DM) and TDN (%) of CO-3, CO-1, PBN-223, PBN-83, PBN-231, RVC-2 and CO-4 were 7.13, 7.46, 7.30, 8.15, 7.55, 7.41,7.53

and 8.40; 48.36, 50.13, 49.30, 53.83, 50.61, 49.90, 50.53 and 55.20, respectively (Garg et al., 2012)

2.9 OXALATE CONTENT OF NAPIER VARIETIES

Ahuja *et al.* (1998) conducted an experiment to know the effect of seasonal variation in oxalate content of hybrid Napier Bajra and its amelioration through ensiling. A promising cultivar of hybrid Napier bajra (PBN-231) was compared with the standard variety PBN-83 for its seasonal variation in oxalate content and its possible reduction through ensiling. The oxalate content (%) varied from 2.10 to 3.92 in both the cultivars of hybrid Napier. Total oxalate content increased linearly with the successive cut and highest levels were recorded during June and July in both the cultivars.

Ajaib Singh *et al.* (2009) reported average oxalate content (%) of 2.30 in two bajra varieties (PHBF-1 and FBC-16).

Rahman *et al.* (2009) conducted an experiment to know the effect of clipping interval (2, 4 and 8 weeks) and nitrogen fertilization (urea) on oxalate content in pot grown Napier grass (*Pennisetum purpureum*). The soluble oxalate concentrations in harvested material was about 15 g/kg DM, while insoluble and total oxalate concentrations were about 11.5 and 26.6 g/kg DM, respectively. The insoluble oxalate levels showed a declining trend as clipping interval increased at both N fertilizer rates.

Rahman *et al.* (2011) conducted an experiment to know the involvement of calcium in elevation of oxalate content in some plant species during fertilization at different seasons. The soluble and total oxalate content (g/kg) of the Napier grass ranged from 11.9 to 23.4 and 16.5 to 27.6, respectively.

Rahman and Kawamura (2011) conducted an experiment to know the involvement of some Agronomic, Climatic and Genetic Aspects in elevation of oxalate content in some plant species. The results showed that the soluble, insoluble and total oxalate content (%) of the Napier grass were 1.15, 0.31 and 1.47, respectively.

A study was conducted to know the oxalate content in different fodders [Group I (Seteria), Group II (Buffel Grass, Pangla Grass and Napier Grass), and Group III (Creeping Signal Grass, Para Grass, Bracharia Marandu, Rhodes Grass, Giant Star Grass, Leucaena Leaves, Guinea Grass, Sugarcane Tops and Korean Lawn-Grass)] in ruminants. The fodder plants with oxalate content more than 20 g/kg is considered as toxic, 10-20 g/kg is probably safe and less than 10 g/kg is safe. The results showed that the soluble fraction and total oxalate content in Napier grass ranged from 9.85 to 19.41 and 14.92 to 20.26, respectively. It was concluded that the Napier grass will come in probably safer zone, still it varies with different regions (Rahman *et al.*, 2013).

Anthony and Thomas. (2014) conducted an experiment with 11 popular cultivers of hybrid Napier *viz*. CO-2, CO-3, CO-4, KKM-1, Suguna, Supriya, IGFRI-3, IGFRI-7, DHN-6, PBN-16 and PTH for nutrient composition and oxalate contents of leaves and stems. The results indicated that leaves of 6 cultivers showed more oxalate content than stem, but in 5 cultivers stem showed more oxalate content than leaves. Results showed that the average oxalate content in Napier varieties ranged from 2.65 (CO-4) to 3.88 (Suguna), respectively.

Chapter - III

Materials and Methods

CHAPTER – III

MATERIALS AND METHODS

The present work has been undertaken to evaluate the nutritional quality of COBN-5 fodder. The fodder was analyzed for proximate principles, forage fibre constituents, CNCPS fractionation and evaluated *in vitro* for nutrient digestibility. The fodder was evaluated *in situ* for DM, CP, NDF and ADF degradability using four rumen fistulated Murrah buffalo bulls. Further, the COBN-5 fodder was evaluated *in vivo* in Murrah buffalo bulls fed as a sole feed to study the effect on rumen fermentation pattern, nutrient digestibility, balance of nutrients and nutritive value by conducting metabolism trial. The study was conducted at Department of Animal Nutrition, NTR College of Veterinary Science, Gannavaram.

3.1 PROCUREMENT OF GREEN FODDER

The green fodder of COBN-5 variety was procured from the cultivated fields of Instructional Live-stock Farm Complex (ILFC), NTR College of Veterinary Science, Gannavaram. First cut was done on 75th day of plantation. Subsequently, the fodder was harvested on 30th, 35th, 40th, 45th and 50th day in the second cut and evaluated for its chemical composition and nutritive value.

3.2 *IN VITRO* STUDIES

The chopped, dried and ground COBN-5 fodder was evaluated for *in vitro* DM, CP, NDF and ADF digestibility (Tilley and Terry, 1963) with strained rumen liquor (SRL) collected from rumen cannulated adult male Murrah buffalo bulls maintained on the same fodder diet for a period of 60 days.

3.3 *IN SACCO* STUDIES

The COBN-5 fodder was evaluated for rumen degradable DM, CP, NDF and ADF by *in situ* nylon bag technique (AFRC, 1993) using three adult Murrah buffalo bulls fitted with permanent rumen cannula. The animals were fed *ad libitum* COBN-5 fodder daily to meet the maintenance (ICAR, 2013). All the animals were offered fresh clean drinking water at free of choice.

3.4 RUMEN FERMENTATION STUDIES

During the metabolism trial, at the end of collection period, rumen liquor was collected from four rumen fistulated buffalo bulls just prior to feeding (0 h) and at 2, 4, 6 and 8 h post feeding. On the days of rumen liquor collection, feed was offered at 8 AM (before 0 h collection) and at 4 PM (after 8 h collection) to avoid the effect of continuous feeding of COBN-5 fodder on rumen metabolites. The animals were offered water one hour before start of collection and after last collection to eliminate influence of water on nitrogen concentration. The collected rumen liquor samples were strained through four layers of muslin cloth and resultant liquid was designated as strained rumen liquor (SRL). About 100 ml of the SRL was drawn at each collection into a clean sterile polythene bottle. The pH of rumen liquor was determined immediately by using digital pH meter.

The ammonia nitrogen in SRL was determined immediately after collection. The remaining SRL was preserved after adding 1 ml of saturated mercuric chloride to stop the microbial activity. All the samples were stored in polyethylene bottles and preserved at sub-zero temperature for further analysis.

3.5 *IN VIVO* STUDIES

3.5.1 Selection of animals

Six graded Murrah buffalo bulls (7yrs; 357.97 ± 43.36 kg), four buffalo bulls fitted with permanent rumen cannula were used for the rumen fermentation study. All the animals were vaccinated against HS and FMD and were dewormed one week prior to the start of the experiment.

3.5.2 Housing and management

The animals were housed in well ventilated conventional sheds maintained in good hygienic condition and are stall fed throughout the experimental period. Fresh, clean drinking water was provided to animals at about 9.00AM and 3.00PM daily. Two days prior to the collection period, the animals were shifted to the metabolism stalls for adaptation.

3.5.3 Feeding regimen:

The chopped COBN –5 fodder was fed *ad libitum* to six adult Murrah buffalo bulls. The refusals were collected every day and the average intake of feed was assessed after a period of 60 days.

3.5.4 Weighing of animals

The animals were weighed at the beginning and ending of the metabolism trial. Every time, weighing was done on two consecutive days before offering feed and water and the average body weight was calculated.

3.5.5 Metabolism trial

The experiment consists of 60 days preliminary period and 6 days collection period. The buffalo bulls were fed chopped COBN-5 fodder at 9.00AM, 12.00 PM, 2.00 PM and 4.00 PM all through the experimental period. On 58th day of the preliminary period, the buffaloes were shifted to the metabolism stalls for adaptation so as to reach their normal feed consumption. Daily feed intake, feed refusals if any as well as faces and urine voided were recorded daily at 9.00 AM.

3.6 COLLECTION OF SAMPLES

3.6.1. Feed and feed residue

Representative samples of feed offered and feed residue if any during collection period were collected and pooled for 6 days, dried and ground in a laboratory Wiley mill through 2 mm screen and preserved in air tight bottles for subsequent analysis.

3.6.2 Faeces

Twenty four hour collection of faeces was recorded on every day morning at 9.00 AM for six days. The faeces were weighed, mixed thoroughly and representative sample (2%) was taken in polythene bag separately for each animal and stored at -10ºC in a deep freeze. For dry matter, a representative sample of daily faeces voided by each animal was taken in previously weighed petri dishes and dried overnight in hot air oven at 100 ± 5^{0}C. After completion of each trial, pooled faecal samples were thawed to room temperature mixed properly and fresh samples were taken for nitrogen (8-10 g) and DM (50-60 g) analysis. For further analysis, faeces was dried at 60ºC and ground to pass through 1mm screen and preserved in air tight bottles.

3.6.3 Urine

Twenty four hour collection of urine was recorded on every day morning at 9.00 AM for six days. The urine voided by each animal was measured, mixed thoroughly and representative sample (2%) was taken in glass bottle for each animal and stored at 4ºC in a refrigerator after addition of few drops of concentrated H_2SO_4.

3.7 ANALYTICAL METHODS

3.7.1 Proximate Analysis

Samples of fodder and faeces were analyzed for proximate constituents and urine for N according to AOAC (2007) methods. The crude protein (N x 6.25) was estimated with fresh faeces samples. Nitrogen analysis was done by using Turbotherm and Vapodest (Gerhardt, Germany) analyzer. The crude protein (N x 6.25) of feeds and leftover were also estimated with fresh material.

3.7.2 Analysis of Cell-wall constituents

Cell-wall constituent's *viz.*, Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Cellulose, Acid Detergent Lignin (ADL), and silica were determined for fodder and faeces by using the methods described by Van Soest *et al.* (1991). Hemi-cellulose was calculated as NDF - ADF.

3.7.3 Carbohydrate and protein fractionation

3.7.3.1 CNCPS fractionation of carbohydrates

The carbohydrate fraction viz., A, B_1, B_2 and C of COBN-5 fodder were estimated using procedures of Sniffen *et al.* (1992). This fractionation is based on degradation rates of feed stuffs. Fraction 'A' is fast and is sugars, fraction B_1 is intermediate and is starch, fraction B_2 is slow and is available cell wall and fraction C is unavailable cell wall. Starch of the feed samples was determined by the procedures of Clegg (1956). Procedure to calculate carbohydrate fractions using the chemical analysis data of feed is listed below.

Carbohydrate fractions:

CHO (%DM) = 100- {CP (%DM) +FAT (%DM) + ASH (%DM)}

CC (%CHO) = 100 [NDF (%DM) × 0.01 LIGNIN (%NDF) × 2.4] / CHO (%DM)

CB_2 (%CHO) = 100 {NDF (%DM)-[NDIP (%CP) ×0.01×CP (%DM)]-[NDF (%DM) ×0.01×LIGNIN (%NDF) ×2.4]}/CHO (%DM)

CNSC (%CHO) = 100-B_2 (%CHO)-C (%CHO)

CB_1 (%CHO) = STARCH (%NSC) [100-B_2 (%CHO) – C (%CHO)]/100

CA (%CHO) ={100-STARCH (%NSC)} * [100 – B_2 (%CHO)–C_j (%CHO)] /100

Where,

CP (%DM) = percentage of crude protein of the jth feedstuff

CHO (%DM) = percentage of carbohydrate of the jth feedstuff

FAT (%DM) = percentage of fat of the jth feedstuff

ASH (%DM) = percentage of ash of the jth feedstuff

NDF (%DM) = percentage of neutral detergent fibre of the jth feedstuff

NDIP (%DM) = percentage of neutral detergent insoluble protein of the jth feedstuff

LIGNIN (%NDF) = percentage of lignin of the jth feedstuff NDF

STARCH (%NSC) = percentage of starch in the non-structural carbohydrate of jth feedstuff

SUGAR (%NSC) = percentage of sugar in the non-structural carbohydrate of jth feedstuff

CA (%CHO) = percentage of the carbohydrate of j^{th} feedstuff, that is sugar

CB_1 (%CHO) = percentage of the carbohydrate of j^{th} feedstuff, that is starch +NSP

CB_2 (%CHO) = percentage of the carbohydrate of j^{th} feedstuff, that is available fibre

CC (%CHO) = percentage of the carbohydrate of j^{th} feedstuff, that is unavailable fibre

3.7.3.2 CNCPS fraction of proteins

In this method, the feed CP was divided in to five fractions – A, B_1, B_2, B_3 and C, which sum to unity. The five fractions have different rates of ruminal degradation (k_d). Fraction A (NPN) is the percentage of CP that is instantaneously solubilized at time zero, which is assumed to have a degradation rate (k_d) of infinity. It is determined chemically as that proportion of CP that is soluble in Borate-phosphate buffer but not precipitated with the protein denaturant, tri-choroaceticacid (TCA). Fraction C is determined chemically as the percentage of total CP recovered with ADF (i.e.ADIN) and is considered to be un-degradable. Fraction C contains proteins associated with lignin and tannins and heat damaged proteins such as the Maillard reaction products. The remaining B fractions represent potentially degradable true protein. The amounts of each of these 3 fractions that are degraded in the rumen are determined by their fractional rates of degradation (k_d) and passage (kp). A single kp value was used for all fractions. Fraction $B_1$is the percent of CP that is soluble in Borate - phosphate buffer and precipitated with 10 %TCA. Fraction B_3 is calculated as the difference between the portions of total CP recovered with NDF (i.e.NDIN) and ADF (i.e. ADIN or fraction). Fraction B_2 is the remaining CP and is calculated as total CP minus the sum of fractions A, B_1, B_3 and C. The non-protein nitrogen (NPN), soluble protein (SP), neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were estimated by the methods (Fig.1) of Licitra *et al.* (1996).

PA (%CP) = NPN (%SOLP) × 0.01 SOLP (%CP)

PB_1 (%CP) = SOLP (%CP) – A (%CP)

PC (%) = ADIP (%CP)

PB_3 (%CP) = 100-A (%CP) – B_1 (%CP) – B_3 (%CP) – C (%CP)

Where,

CP (%DM) = percentage of the crude protein of the feed stuff

NPN (%CP) = percentage of the crude protein of the feed stuff that is non-protein nitrogen × 6.25

SOLP (%CP) = percentage of the crude protein of the feed stuff that is soluble protein

NDIP (%DM) = percentage of the feed stuff that is neutral detergent insoluble protein

ADIP (%CP) = percentage of the feed stuff that is acid detergent insoluble protein

PA (%CP) = percentage of the crude protein of the feed stuff that is non-protein nitrogen

PB_1 (%CP) = percentage of the crude protein of the feed stuff that is rapidly degraded protein

PB_2 (%CP) = percentage of the crude protein of the feed stuff that is intermediately degraded protein

PB_3 (%CP) =percentage of the crude protein of the feed stuff that is slowly degraded protein

PC (%CP) = percentage of the crude protein of the feed stuff that is bound protein

3.7.4 Analysis of minerals

Calcium and Phosphorus in fodder and faeces were determined according to methods described by Talapatra *et al.* (1940). Calcium and Phosphorus in urine samples were determined by Ferro and Ham (1957) and Fiske and Subba Row (1925), respectively.

3.7.5 *In vitro* studies

The *in vitro* technique was used to measure the digestibility of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) for COBN-5 fodder in the rumen as per the procedure of

Tilley and Terry, (1963). In the first stage, around 0.5 g of finely ground (particle size < 1mm) sample is taken and incubated for 48 h with the buffered rumen liquor in a tube under anaerobic conditions. In the second stage, the microbial activities are stopped by acidifying with hydrochloric acid to pH 2.0 and then digested by incubating with pepsin for another 24 h. In the last stage, the insoluble residue is filtered off, dried at 100℃ overnight and weighed.

3.7.6 *In sacco* nylon bag technique

The *in sacco* nylon bag technique was used to measure the dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) degradability of COBN-5 fodder in the rumen. Pre-weighed nylon bags (13.5cm × 7.5cm) containing 5g of air dried (ground through 2mm screen) were incubated in the ventral sac of rumen each Murrah buffalo bull. The COBN-5 were incubated for 0, 3, 6, 12, 24, 48 and 72 h using 3 rumen cannulated Murrah buffalo bulls. The bags containing samples of 0 h were washed without incubation in the rumen.

The *in sacco* degradability of DM, CP, NDF, and ADF of COBN-5 fodder were determined as per the procedure of Orskov and McDonald (1979). The bags with feed samples incubated in the rumen were drawn in a sequential removal method (Osuji *et al.*, 1993). After removal of bags from rumen they were placed in a bucket of cold water to stop ongoing microbial activity. Then bags were washed under slow running tap water by rubbing between fingers and the thumb finger for ten minutes, oven dried at 70℃ for 48h to a constant weight and DM loss during the incubation was calculated. The CP, NDF, and ADF content in the residue was analyzed to determine their respective degradabilities. From the degradability data obtained at different intervals, the constant a, b and c from the expression

$$P=a + b\ (a-e^{-ct})$$

where,

P = percentage of degradability for response variables at t

t = time relative to incubation (h)

a = highly soluble or readily degradable fraction (%),

b = insoluble and slowly degradable fraction (%)

c = rate constant for degradation (/h)

e = 2.7182 (Natural logarithm base)

The effective nutrient degradability (%) of COBN-5 were calculated by time measurements and fitted values in NEWAY program (Model based on Mc Donald, 1981) using a computer, assuming an outflow rate (K) of 0.05/hr.

3.7.7 Rumen metabolic profile studies

Rumen metabolic profile studies in terms of rumen pH, ammonia nitrogen (NH_3-N) and total volatile fatty acids (TVFA) concentration were determined in strained rumen liquor (SRL). The pH of the rumen liquor was measured immediately after collection of rumen liquor using digital pH meter and ammonia nitrogen was determined by micro-diffusion method of Conway (1957) using mixed indicator (Livingston *et al.,* 1964). The TVFA concentration of SRL was determined by using the procedure of Barnett and Reid. (1957).

3.8 Oxalate Estimation

The COBN-5 fodder samples were dried and milled to pass through a 1mm screen using Wiley mill. The samples were analyzed for oxalates at different stages of harvest following the method suggested by Abaza *et al.* (1968).

3.9 STATISTICAL ANALYSIS

The data was analysed statistically (Snedecor and Cochran, 1994) using SPSS 17.0 version.

Fig 1. FLOW DIAGRAM FOR ESTIMATION OF PROTEIN FRACTIONS IN FEEDS AND FORAGES

Step - 1: Estimation of Crude Protein (CP %)

0.5 gm dried feed sample

↓

Digestion

↓

Distillation

↓

Titration

(Digestion, Distillation, Titration: Kjeldahl Method)

↓

C.P% of feed sample

Step - 2: Estimation of Non Protein Nitrogen (NPN)

0.5 gm dried feed sample

↓

Add 50 ml distilled water

↓

Add 10 ml solution of TCA (10%)

↓ Allow to stand for 30 minutes

Filtration through No. 54 filter paper

↓ → Filtrate / Soluble Protein (SP)

Transfer filter paper with residue into Kjeldahl flask

↓

Kjeldhal method

↓

Insoluble Protein / True Protein (TP)

NPN (FRACTION 'A' ESTIMATED BY DIFFERENCE)
= CP – TP

Step - 3: Estimation of Buffer Insoluble Protein (BIP)

0.5 gm dried feed sample

↓

Add 50 ml borate phosphate buffer + 1ml sodium azide (10%)

↓ ← Allow to stand for 3 h

Filtration through No. 54 filter paper → Filtrate / Buffer Soluble Protein (BSP)

↓

Transfer filter paper with residue into Kjeldahl flask

↓ ← Estimate CP by Kjeldhal Method

Buffer Insoluble Protein (IP)

'B_1' FRACTION = True Protein (TP) - Insoluble Protein (IP)

Step - 4: Estimation of Neutral Detergent Insoluble Protein (NDIP)

0.5 gm dried feed sample

↓ ← Estimate NDF

NDF residue

↓ ← Estimate CP by Kjeldahl Method

NDIP

'B_2' FRACTION =
Insoluble Protein (IP) - Neutral Detergent Insoluble Protein (NDIP)

Step - 5: Estimation of Acid Detergent Insoluble Protein (ADIP)

0.5 gm dried feed sample

↓ ← Estimate ADF

ADF residue

↓ ← Estimate CP by Kjeldahl Method

ADIP

'B_3' FRACTION =
Neutral Detergent Insoluble Protein (NDIP) -
Acid Detergent Insoluble Protein (ADIP)

'C' FRACTION = ADIP

COMPUTATION OF PROTEIN FRACTIONS

FRACTION	CALCULATION
A	CP - TP
B_1	TP - IP
B_2	IP - NDIP
B_3	NDIP - ADIP
C	ADIP

Chapter - IV

Results

CHAPTER - IV

RESULTS

The COBN-5 fodder is procured from the cultivated fields of ILFC, NTR College of veterinary science, Gannavaram. The fodder is evaluated for chemical composition, nutrient digestibility, balance of minerals, nutritive value using six Murrah buffalo bulls and rumen fermentation pattern using four rumen cannulated Murrah buffalo bulls. Further, the COBN-5 fodder is evaluated for their CNCPS fractionation, *in situ* and *in vitro* degradability of DM, CP, NDF and ADF.

4.1 EVALUATION OF COBN-5 FODDER

4.1.1 Chemical composition of COBN-5 fodder at different stages of harvest

The chemical composition of COBN-5 fodder used in the current study is presented in Table 1. The per cent DM, OM, TA, CP, EE, CF and NFE at different stages of harvest (30, 35, 40, 45 and 50 days) of COBN-5 fodder were 16.68, 82.49, 17.51, 14.00, 3.27, 19.24 and 45.98; 17.02, 84.27, 15.73, 13.41, 2.90, 23.07 and 44.89; 17.42, 86.05, 13.95, 13.02, 2.82, 27.96 and 42.25; 18.03, 87.20, 12.80, 12.70, 2.72, 30.50 and 41.28 and, 20.54, 88.17, 11.83, 12.10, 2.41, 34.69 and 38.97, respectively. The % DM in COBN-5 fodder was higher ($P<0.05$) at 50th day of harvest as compared to other stages of harvest. However, non-significant differences were observed between the other stages of harvest.

Table 1. Chemical composition (% DM basis) of COBN-5 fodder at different stages of harvest

Nutrient	Days of harvest					
	30	35	40	45	50	Mean± SE
Dry matter	16.68[a]	17.02[a]	17.42[ab]	18.03[b]	20.54[c]	17.94 ± 1.09
Organic matter	82.49[a]	84.27[b]	86.05[c]	87.20[cd]	88.17[d]	85.64 ± 1.61
Total ash	17.51[e]	15.73[d]	13.95[c]	12.80[b]	11.83[a]	14.36 ± 1.61
Crude protein	14.00[e]	13.41[d]	13.02[c]	12.70[b]	12.10[a]	13.05 ± 0.51
Ether extract	3.27[d]	2.90[c]	2.82[c]	2.72[b]	2.41[a]	2.82 ± 0.22
Crude fibre	19.24[a]	23.07[b]	27.96[c]	30.50[d]	34.69[e]	27.09 ± 4.30
Nitrogen free extract	45.98[e]	44.89[d]	42.25[c]	41.28[b]	38.97[a]	42.67 ± 1.99
Neutral detergent fibre	62.00[a]	65.00[b]	67.06[c]	69.10[d]	73.38[e]	67.31 ± 3.03
Acid detergent fibre	38.29[a]	39.26[b]	40.20[c]	41.64[d]	44.80[e]	40.84± 2.41
Hemi-cellulose	23.71[a]	25.74[b]	26.86[c]	27.46[d]	28.58[e]	26.47± 0.44
Cellulose	30.94[a]	32.61[b]	34.25[c]	36.70[d]	40.87[e]	35.07± 2.97
Acid detergent lignin	4.97 [a]	5.31 [b]	5.68 [c]	6.13 [d]	6.54[e]	5.73± 2.74
Silica	2.95 [a]	3.17 [b]	3.42 [c]	3.69 [d]	3.82[e]	3.41 ± 0.25
Calcium (%)	0.74[b]	0.81[c]	1.15[d]	0.48[a]	0.47[a]	0.73 ± 0.20
Phosphorus (%)	0.68[c]	0.51[b]	0.75[d]	0.45[a]	0.44[a]	0.57 ± 0.10

[abcde]Values in the rows bearing different superscripts differ significantly $P<0.05$
N= Mean of 3 observations for each set

The mean CP % in COBN-5 fodder was 13.05. The CP % is highest at 30th day (14.00) and lowest at 50th day (12.10) of harvest. Further, significant differences ($p<0.05$) were observed between all stages of harvest. The mean EE % in COBN-5 fodder was 2.82. The EE % is highest at 30th day (3.27) and lowest at 50th days (2.41) of harvest. Further, significant differences ($p<0.05$) were observed between all stages of harvest, except between 30 and 35 days of harvest. The mean CF % in COBN-5 fodder was 27.09. The CF % is highest at 50th day (34.69) and lowest at 30th days (19.24) of harvest and significant differences ($p<0.05$) were observed between all stages of harvest.

4.1.2 Cell-wall constituents of COBN-5 fodder at different stages of harvest

The cell wall constituents of COBN-5 fodder used is presented in Table 1. The per cent NDF, ADF, hemi-cellulose, cellulose, ADL and silica content of COBN-5 fodder at different stages (30, 35, 40, 45 and 50th day) of harvest were 62.00, 38.29, 23.71, 30.94, 4.97 and 2.95; 65.00, 39.26, 25.74, 32.61, 5.31 and 3.17; 67.06, 40.20, 26.86, 34.25, 5.68 and 3.42; 69.10, 41.64, 27.46, 36.70, 6.13 and 3.69 and, 73.38, 44.80, 28.58, 40.87, 6.54 and 3.82, respectively. The mean NDF % in COBN-5 fodder was 67.31. The NDF % is highest at 50th day (73.38) and lowest at 30th days (62.00) of harvest with significant differences ($p<0.05$) between all stages of harvest. The mean ADF % in COBN-5 fodder was 40.84 and is highest at 50th day (44.80) and lowest at 30th days (38.29) of harvest. Further, significant differences ($p<0.05$) were observed between all stages of harvest. The mean hemi-cellulose % in COBN-5 fodder was 26.47. The hemi-cellulose % is highest at 50th day (28.58) and lowest at 30th days (23.71) of harvest with significant differences ($p<0.05$) between all stages of harvest. The mean cellulose % in COBN-5 fodder was 35.07 with highest value at 50^{th} day (40.87) and lowest at 30^{th} day (30.94) of harvest. Further, significant differences ($p<0.05$) were observed between all stages of harvest.

The per cent calcium and phosphorous (Table 1) content at different stages of harvest (30, 35, 40, 45 and 50 days) of COBN-5 were 0.74 and 0.68; 0.81 and 0.51; 1.15 and 0.75; 0.48 and 0.45; 0.47 and 0.44, respectively. The mean calcium % in COBN-5 fodder was 0.73 with highest value at 40^{th} day (1.15) and lowest at 50^{th} day (0.47) of harvest. Further, similar values were observed between 30^{th} and 35^{th} and, 40^{th} and 45^{th} days of harvest. The mean phosphorus % in COBN-5 fodder was 0.57 with highest value at 40^{th} day (0.75) and lowest at 50^{th} day (0.44) of harvest. Similar values were observed between 30^{th}, 35^{th} and 40^{th} days of harvest.

4.1.3 Oxalate content of COBN-5 fodder at different stages of harvest

The oxalate (%) content of COBN-5 fodder was 3.32, 2.89, 2.31, 1.86 and 1.64 at 30, 35, 40, 45 and 50 days of harvest, respectively (Table 2). The oxalate content of COBN-5 decreased ($p<0.01$) linearly with increase in time of harvest. The mean oxalate content (%) of COBN-5 fodder was 2.40 with higher ($p<0.05$) value at 30^{th} day (3.32) and lower ($p<0.05$) at 50^{th} day (1.64) of harvest.

Table 2. Oxalate Content (%) of COBN-5 fodder at different stages of harvest

Days	**Oxalate (%)**
30	$3.32^{a} \pm 0.24$
35	$2.89^{b} \pm 0.21$
40	$2.31^{c} \pm 0.36$
45	$1.86^{d} \pm 0.27$
50	$1.64^{e} \pm 0.31$
Mean ± SE	**2.40± 0.50**

[abcde]Values in the column bearing different superscripts differ significantly ($P<0.01$)
N= Mean of 3 observations for each set

4.1.4 Carbohydrate and protein components of COBN-5 fodder at different stages of harvest

The carbohydrate and protein components of COBN-5 fodder at different stages of harvest are presented in Table 3. The carbohydrate components *viz*. lignin (% NDF), NSC (% DM) and starch (% NSC) of COBN-5 fodder at different stages (30, 35, 40, 45 and 50 days) of harvest were 4.50, 10.82 and 71.36; 5.00, 10.46 and 67.00; 5.82, 11.05 and 63.92; 6.41, 10.48 and 60.20; 7.27, 8.38 and 57.50, respectively. Higher ($p<0.05$) values for lignin (% NDF), NSC (% DM) and starch (% NSC) were reported at 50^{th} day compared to other stages of harvest.

The protein components *viz*. SP (% CP), NPN (% SP), BIP(% CP), NDICP (% CP) and ADICP (% CP) of COBN-5 fodder at different stages (30, 35, 40, 45 and 50 days) of harvest were 41.35, 92.66, 62.15, 54.52 and 8.71; 37.22, 91.37, 67.39, 56.06 and 11.43; 33.61, 90.10, 70.51, 60.46 and 16.42; 30.71, 89.74, 72.44, 61.43 and 18.12 and, 25.47, 86.41, 74.32, 66.63 and 25.34, respectively. The SP (% CP) and NPN (% SP) were higher ($p<0.05$) at 30^{th} day compared to other stages of harvest, while BIP, NDICP and ADICP (% CP) were higher ($p<0.05$) at 50^{th} day compared to other stages of harvest.

Table 3. Carbohydrate and protein components of COBN-5 fodder at different stages of harvest

Parameter	Days of harvest					
	30	**35**	**40**	**45**	**50**	**Mean ± SE**
Carbohydrate Components						
Lignin (% NDF)	4.50[a] ± 0.14	5.00[b] ± 0.21	5.82[c] ± 0.14	6.41[d] ± 0.18	7.27[e] ± 0.11	5.8 ±0.78
NSC (% DM)	10.82[d] ± 1.08	10.46[b] ± 1.02	11.05[e] ± 1.01	10.48[c] ± 1.08	8.38[a] ± 1.10	10.24 ±0.75
Starch (% NSC)	71.36[e] ± 1.30	67.00[de] ± 1.33	63.92[cd] ± 1.29	60.20[b] ± 1.18	57.50[a] ± 1.20	64.00 ±3.87
Protein Components						
SP (% CP)	41.35 [e] ± 1.45	37.22 [d] ± 1.50	33.61 [c] ± 1.35	30.71 [b] ± 1.41	25.47 [a] ± 1.62	33.67 ±4.30
NPN (% SP)	92.66 [e] ± 0.56	91.37 [d] ± 0.58	90.10 [c] ± 0.51	89.74 [b] ± 0.48	86.41 [a] ± 0.54	90.06 ±1.66
BIP (% CP)	62.15 [a] ± 1.14	67.39 [b] ± 1.52	70.51 [c] ± 1.22	72.44 [d] ± 1.05	74.32 [e] ± 1.33	69.36 ±3.38
NDICP (% CP)	54.52 [a] ± 1.15	56.06[b] ± 1.20	60.46 [c] ± 1.15	61.43 [d] ± 1.24	66.63 [e] ± 1.87	59.82 ±3.38
ADICP (% CP)	8.71 [a] ± 1.66	11.43 [b] ± 1.45	16.42 [c] ± 1.89	18.12 [d] ± 1.65	25.34 [e] ± 1.72	16.00 ±4.56

[abcde]values in the rows bearing different superscripts differ significantly. $P<0.05$ N= Mean of 3 observations for each set

Table 4. Carbohydrate and protein fractions of COBN-5 fodder at different stages of harvest

Parameter	Days of harvest					
	30	35	40	45	50	Mean ± SE
Carbohydrate fraction (% CHO)						
CA	2.94^{a} ± 0.23	5.09^{b} ± 0.21	5.66^{c} ± 0.28	5.81^{d} ± 0.25	4.81^{e} ± 0.23	4.86 ± 0.81
CB1	11.77^{a} ± 0.70	10.33^{ab} ± 0.69	10.03^{ab} ±0.66	8.79^{ab} ±0.75	6.51^{b} ± 0.72	9.49 ± 1.40
CB2	73.25^{a} ± 1.15	73.10^{b} ± 1.10	70.96^{c} ±1.32	70.59^{d} ±0.98	71.30^{e} ± 0.90	71.84 ± 0.88
CC	10.26^{a} ± 1.65	11.48^{b} ± 1.21	13.34^{c} ±0.89	14.81^{d} ± 1.84	17.38^{e} ± 1.59	13.45 ± 1.98
Protein fractions (% CP)						
PA	38.31^{a} ± 1.03	34.01^{b} ± 1.20	30.28^{c} ±1.25	27.56^{d} ±1.10	22.01^{e} ±1.41	30.43 ± 4.39
PB1	3.46^{a} ± 0.04	3.33^{b} ± 0.01	3.24^{c} ±0.03	3.15^{d} ±0.04	3.02^{e} ±0.01	3.24 ± 0.12
PB2	3.71^{a} ± 0.03	6.6^{b} ± 0.03	6.02^{c} ±0.01	7.86^{d} ±0.08	8.34^{e} ±0.05	6.51 ± 1.29
PB3	45.81^{a} ± 0.42	44.63^{b} ± 0.40	44.04^{c} ±0.55	43.31^{d} ±0.84	41.29^{e} ±0.36	43.82 ± 1.19
PC	8.71^{a} ± 1.66	11.43^{b} ± 1.45	16.42^{c} ±1.89	18.12^{d} ±1.65	25.34^{e} ±1.72	16.00 ± 4.56

^{abcde}Values in the rows bearing different superscripts differ significantly
$P<0.05$ N= Mean of 3 observations for each set

4.1.5 Carbohydrate and protein fractions of COBN-5 fodder at different stages of harvest

The carbohydrate and protein fractions of COBN-5 fodder at different stages of harvest were presented in Table 4. The carbohydrate fractions *viz*. CA, CB_1, CB_2, and CC (% CHO) of COBN-5 at different stages (30, 35, 40, 45 and 50 days) of harvest were 2.94, 11.77, 73.25 and 10.26; 5.09, 10.33, 73.10 and 11.48; 5.66, 10.03, 70.96 and 13.34; 5.81, 8.79, 70.59 and 14.81 and, 4.81, 6.51, 71.30 and 17.38, respectively. Higher ($p<0.05$) proportion of CA was observed at 45^{th} day, while that of CC at 50^{th} day of harvest.

The protein fractions *viz*. PA, PB_1, PB_2, PB_3 and PC (% CP) of COBN-5 at different stages (30, 35, 40, 45 and 50 days) of harvest were 38.31, 3.46, 3.71, 45.81 and 8.71; 34.01, 3.33, 6.60, 44.63 and 11.43; 30.28, 3.24, 6.02, 44.04 and 16.42; 27.56, 3.15, 7.86, 43.31 and 18.12 and, 22.01, 3.02, 8.34, 41.29 and 25.34, respectively. Higher ($p<0.05$) proportions of PA was reported at 30^{th} day, while that of PC was observed at 50^{th} day of harvest.

4.2 *IN VITRO* STUDIES

The *in vitro* digestibility of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) at different stages of harvest (30, 35, 40, 45 and 50 days) of COBN-5 fodder studied using rumen liquor collected from buffalo bulls fed *ad libitum* COBN-5 fodder is presented in Table 5.

4.2.1 *In vitro* digestibility of DM in COBN-5 fodder at different stages (30, 35, 40, 45 and 50 days) of harvest

The *in vitro* DM digestibility (%) was 70.40 ± 0.36, 67.50 ± 0.30, 64.60 ± 0.41, 63.24 ± 0.34 and 60.69 ± 0.39 in COBN-5 fodder at 30, 35, 40, 45 and 50 days of harvest, respectively (Table 5). The IVDMD (%) of COBN-5 fodder decreased ($p<0.05$) linearly with increase in the time interval of harvest. Further, the study revealed that IVDMD was higher ($p<0.05$) at 30^{th} day (70.40) and lower at 50^{th} day (65.29) of harvest as compared to the other stages of harvest.

4.2.2 *In vitro* digestibility of CP in COBN-5 fodder at different stages (30, 35, 40, 45 and 50 days) of harvest

The *in vitro* CP digestibility (%) was 81.01 ± 0.65, 79.07 ± 0.61, 76.79 ± 0.71, 71.97 ± 1.62 and 69.64 ± 0.70 in COBN-5 fodder at 30, 35, 40, 45 and 50 days of harvest, respectively (Table 5). The IVCPD (%) of COBN-5 fodder decreased ($p<0.05$) linearly with increase in the time interval of harvest. Further, the study revealed that IVCPD was higher ($p<0.05$) at 30^{th} day (81.01) and lower at 50^{th} day (69.64) of harvest as compared to the other stages of harvest.

4.2.3 *In vitro* digestibility of NDF in COBN-5 fodder at different stages (30, 35, 40, 45 and 50 days) of harvest

The *in vitro* NDF digestibility (%) was 64.88 ± 0.125, 62.53 ± 1.29, 60.08 ± 1.17, 59.38 ± 1.05 and 55.82 ± 0.83 in COBN-5 fodder at 30, 35, 40, 45 and 50 days of harvest, respectively (Table 5). The IVNDFD (%) of COBN-5 fodder decreased ($p<0.05$) linearly with increase in the time interval of harvest. Further, the study revealed that IVNDFD was higher ($p<0.05$) at 30th day (64.88) and lower at 50th day (55.82) of harvest as compared to the other stages of harvest.

4.2.4 *In vitro* digestibility of ADF in COBN-5 fodder at different stages (30, 35, 40, 45 and 50 days) of harvest

The *in vitro* ADF digestibility (%) was 56.96 ± 0.13, 54.43 ± 0.27, 53.26 ± 0.17, 52.68 ± 0.25 and 49.30 ± 0.16 in COBN-5 fodder at 30, 35, 40, 45 and 50 days of harvest, respectively (Table 5). The IVADFD of COBN-5 fodder decreased ($p<0.05$) linearly with increase in the time interval of harvest. Further, the study revealed that IVNDFD was higher ($p<0.05$) at 30th day (56.96) and lower at 50th day (49.30) of harvest as compared to the other stages of harvest.

4.3 *IN SACCO* STUDIES

The *in sacco* degradability of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF) of COBN-5 fodder at 40 and 45 days of harvest was studied using three rumen cannulated buffalo bulls and the results were presented in Tables 6 to 9.

4.3.1. *In sacco* DM disappearance (%) and degradation kinetics of COBN-5 fodder at 40 and 45 days of harvest

In sacco disappearance (%) and degradation kinetics of dry matter in rumen cannulated buffalo bulls fed with *ad libitum* COBN-5 fodder is presented in Table 6. The dry matter disappearance (%) after 72h of incubation in the

Table 5. *In vitro* digestibility (%) of COBN-5 fodder at different stages of harvest

Nutrient digestibility (%)	Days of Harvest					
	30	35	40	45	50	Mean ± SE
IVDMD	$70.40^{e} \pm 0.36$	$67.50^{d} \pm 0.30$	$64.60^{c} \pm 0.41$	$63.24^{b} \pm 0.34$	$60.69^{a} \pm 0.39$	65.29 ± 2.67
IVCPD	$81.01^{e} \pm 0.65$	$79.07^{d} \pm 0.61$	$76.79^{c} \pm 0.71$	$71.97^{b} \pm 1.62$	$69.64^{a} \pm 0.70$	75.70 ± 3.38
IVNDFD	$64.88^{e} \pm 1.25$	$62.53^{d} \pm 1.29$	$60.08^{c} \pm 1.17$	$59.38^{b} \pm 1.05$	$55.82^{a} \pm 0.83$	60.54 ± 2.41
IVADFD	$56.96^{e} \pm 0.13$	$54.43^{d} \pm 0.27$	$53.26^{c} \pm 0.17$	$52.68^{b} \pm 0.25$	$49.30^{a} \pm 0.16$	53.33 ± 1.97

^{abcde}Values in the rows bearing different superscripts differ significantly
$P<0.05$
N= Mean of 3 observations for each set

ventral sac of the rumen was higher ($p<0.05$) at 40th day (82.54) harvest as compared to the fodder harvested at 45th day (78.44). The rapidly soluble fraction (a) was higher ($p<0.05$) in bulls fed 40th day harvested fodder (38.37) but the insoluble but degradable fraction (b) was higher ($p<0.05$) in 45th day harvested fodder (50.27). The potentially degradable fraction (a+b) was higher ($p<0.05$) in 45th day fodder. The effective degradability of dry matter (EDDM %) was 58.50 and 54.20 in 40th and 45th day harvested fodder, respectively.

4.3.2 *In sacco* CP disappearance (%) and degradation kinetics of COBN-5 fodder at 40 and 45 days of harvest

In sacco disappearance (%) and degradation kinetics of crude protein in cannulated buffalo bulls fed *ad libitum* COBN-5 fodder is presented in Table 7. The protein disappearance (%) at 72 h of incubation in the ventral sac of rumen was higher ($p<0.05$) in the fodder harvested at 40th day as compared to the 45th days of harvest. The rapidly soluble fraction 'a' was higher ($p<0.05$) in the fodder harvested at 40 days (55.18) as compared to the 45th (53.47) day of harvest. The insoluble but degradable fraction (b) was lower ($p<0.05$) in 40th day (39.33) when compared to the 45th day (44.56) harvested fodder. The rate constant/h (c) was higher ($p<0.05$) in 40th day, while the potentially degradable fraction (a+b) was lower ($p<0.05$) in 40th day (94.51) as compared to that on 45th day (98.03) harvested fodder. The effective degradability of crude protein (EDCP %) was 76.10 and 74.80 in fodder harvested at 40 and 45 days, respectively.

Table 6. Average *in sacco* DM disappearance (%) and degradation kinetics of COBN-5 fodder at 40 and 45 days of harvest

Incubation period(h)	Days of Harvest		
	40	**45**	**Mean ± SE**
0	38.37 [b] ± 0.23	37.04 [a] ± 0.16	37.70 ± 0.67
3	40.47 [b] ± 0.42	39.20 [a] ± 0.15	39.83 ± 0.64
6	48.01[b] ± 0.74	46.97[a] ± 0.23	47.49 ± 0.52
12	57.23[b] ± 0.51	51.87[a] ± 0.14	54.55 ± 2.68
24	70.05[b] ± 0.67	67.49[a] ± 0.15	68.77 ± 1.28
48	77.46[b] ± 0.44	72.55[a] ± 0.15	75.00 ± 2.45
72	82.54[b] ± 0.15	78.44[a]± 0.24	80.49 ± 2.05
Degradation kinetics			
a	38.37[b] ± 0.42	37.04[a] ± 0.38	37.705 ± 0.67
b	43.98[a] ± 0.27	50.27[b] ± 0.21	47.125 ± 3.15
c	0.056[b] ± 0.25	0.0240[a] ± 0.23	0.04 ± 0.02
PD (a+b)	82.35[a] ± 0.31	87.31[b] ± 0.35	84.83 ± 2.48
RSD	1.22 ± 0.16	2.45 ± 0.19	1.835 ± 0.62
EDDM (0.05%)	58.50[b] ± 0.76	54.20[a] ± 0.84	56.35 ± 2.15

[ab]Values in the rows bearing different superscripts differ significantly
$P<0.05$
N= Mean of 3 observations for each set

4.3.3 *In sacco* NDF disappearance (%) and degradation kinetics of COBN-5 fodder at 40 and 45 days of harvest

In sacco disappearance (%) and degradation kinetics of NDF in cannulated buffalo bulls fed *ad libitum* COBN-5 fodder is presented in Table 8. The % NDF disappearance at 72 h of incubation in the ventral sac of rumen was higher ($p<0.05$) at 40^{th} day (76.54) harvest as compared to that on 45^{th} day (71.68). The study indicated that the rapidly soluble fraction (a) is higher ($p<0.05$) on 40^{th} day (25.34) when compared to that harvested on 45^{th} day (18.98). The insoluble but degradable fraction (b) is similar between 40 and 45 days of harvest. The rate constant/h (c) and potentially degradation fraction (a+b) were lower ($p<0.05$) in fodder harvested on 45^{th} day as compared to 40^{th} day. The effective degradability of NDF (EDNDF %) was 48.9 and 43.1 for fodder harvested on 40^{th} day and 45^{th} day.

4.3.4 *In sacco* ADF disappearance (%) and degradation kinetics of COBN-5 fodder at 40 and 45 days of harvest

In sacco disappearance (%) and degradation kinetics of ADF in cannulated buffalo bulls fed *ad libitum* COBN-5 fodder is presented Table 9. The % ADF disappearance at 72 h of incubation in the ventral sac of rumen was higher ($p<0.05$) in fodder harvested on 40^{th} (76.52) day as compared to 45^{th} day (71.06). The rapidly soluble fraction (a) was higher ($p<0.05$) in 40^{th} day (19.11) when compared to that harvested on 45^{th} day (14.46). The rate constant/h (c) and potentially degradable fraction (a+b) were lower ($p<0.05$) in 45^{th} day harvested fodder when compared to the 40^{th} day. The effective degradability of ADF

Table 7. Average *in sacco* CP disappearance (%) and degradation kinetics of COBN-5 fodder at 40 and 45 days of harvest

Incubation period(h)	**Days of Harvest**		
	40	**45**	**Mean ± SE**
0	55.18 [b] ± 0.53	53.47 [a] ± 0.72	54.33 ± 0.86
3	62.99 [b] ± 0.22	60.49 [a] ± 0.43	61.74 ± 1.25
6	69.74 [b] ± 0.43	67.84 [a] ± 0.36	68.79 ± 0.95
12	73.85 [b] ± 0.38	71.73 [a] ± 0.29	72.79 ± 1.06
24	79.05 [b] ± 0.23	75.41 [a] ± 0.87	77.23 ± 1.82
48	88.73 [b] ± 0.15	86.12 [a] ± 0.20	87.43 ± 1.31
72	92.10 [b] ± 0.41	91.84 [a] ± 1.08	91.97 ± 0.13
Degradation kinetics			
a	55.18 [b] ± 0.32	53.47 [a] ± 0.29	54.33 ± 0.86
b	39.33 [a] ± 0.65	44.56 [b] ± 0.62	41.95 ± 2.62
c	0.0356 [b] ± 0.02	0.0243 [a] ± 0.01	0.030 ± 0.01
PD (a+b)	94.51 [a] ± 0.78	98.03 [b] ± 0.65	96.27 ± 1.76
RSD	1.81 ± 0.25	2.45 ± 0.23	2.13 ± 0.32
EDCP (0.05%)	76.10 [b] ± 0.69	74.80 [a] ± 0.58	75.45 ± 0.65

[abcde]Values in the rows bearing different superscripts differ significantly $P<0.05$

N=Mean of 3 observations for each set

Table 8. Average *in sacco* NDF disappearance (%) and degradation kinetics of COBN-5 fodder at 40 and 45 days of harvest

Incubation period(h)	**Days of Harvest**		
	40	**45**	**Mean ± SE**
0	25.34 [b] ± 0.18	18.98 [a] ± 0.24	22.16 ± 3.18
3	30.16 [b] ± 0.23	24.36 [a] ± 0.24	27.26 ± 2.90
6	36.20 [b] ± 0.12	32.05 [a] ± 0.05	34.13 ± 2.08
12	44.98 [b] ± 0.15	42.64 [a] ± 0.32	43.81 ± 1.17
24	63.13 [b] ± 0.38	53.78 [a] ± 0.07	58.46 ± 4.68
48	71.54 [b] ± 0.29	62.08 [a] ± 0.19	66.81 ± 4.73
72	76.54 [b] ± 0.41	71.68 [a] ± 0.27	74.11 ± 2.43
Degradation kinetics			
a	25.34 [b] ± 0.25	18.98 [a] ± 0.22	22.16 ± 3.18
b	52.41 [a] ± 0.47	52.48 [a] ± 0.45	52.45 ± 0.04
c	0.0501 [b] ± 0.03	0.0449 [a] ± 0.01	0.048±0.001
PD (a+b)	77.75 [b] ± 0.72	71.46 [a] ± 0.75	74.61 ± 3.15
RSD	1.91 ± 0.31	2.67 ± 0.34	2.29 ± 0.38
EDNDF (0.05%)	48.90 [b] ± 0.58	43.10 [a] ± 0.55	46.00 ± 2.90

[abcde]Values in the rows bearing different superscripts differ significantly $P<0.05$

N=Mean of 3 observations for each set

(EDADF %) was higher ($p<0.05$) in 40th day (46.74) harvested fodder when compared to that on 45th day (39.99), respectively.

4.4 RUMEN FERMENTATION STUDIES

The rumen fermentation pattern in respect of pH, ammonia nitrogen and total volatile fatty acids (TVFA) in strained rumen liquor (SRL) of buffalo bulls fed *ad libitum* COBN-5 fodder harvested at 40-45 days is presented in Table 10.

4.4.1 Rumen pH

The mean pH of rumen fluid in buffalo bulls as affected by feeding COBN-5 fodder harvested at 40-45 days with time of sampling are presented in Table 10. The pH values were 6.52, 6.43, 6.33, 6.21 and 6.31 for 0, 2, 4, 6, and 8 h, respectively. The pH of rumen liquor showed a decreasing trend up to 6 h post feeding in all the buffalo bulls and increased thereafter. There was a significant difference ($p<0.01$) in rumen pH 6h post feeding.

4.4.2 Ammonia nitrogen

The ammonia nitrogen concentration (mg/100 ml SRL) of buffalo bulls fed *ad libitum* COBN-5 fodder harvested at 40-45 days as affected by time of sampling is presented in Table 10. The ammonia nitrogen concentration in SRL of buffalo bulls was 12.50, 16.6, 15.54, 14.00 and 12.23 mg/100 ml SRL at 0, 2, 4, 6 and 8 h, respectively. Rumen ammonia nitrogen concentration increased linearly up to 2 h post feeding beyond which there was a decline in its concentration.

Table 9. Average *in sacco* ADF disappearance (%) and degradation kinetics of COBN-5 fodder at 40 and 45 days of harvest

Incubation period(h)	**Days of Harvest**		
	40	**45**	**Mean ± SE**
0	$19.11^{b} \pm 0.92$	$14.46^{a} \pm 0.17$	16.79 ± 2.32
3	$25.39^{b} \pm 0.87$	$19.14^{a} \pm 0.01$	22.27 ± 3.13
6	$33.77^{b} \pm 0.54$	$26.74^{a} \pm 0.29$	30.26 ± 3.52
12	$44.69^{b} \pm 0.49$	$38.46^{a} \pm 0.32$	41.58 ± 3.11
24	$61.01^{b} \pm 0.71$	$52.96^{a} \pm 0.83$	56.99 ± 4.02
48	$71.42^{b} \pm 0.36$	$63.24^{a} \pm 0.23$	67.33 ± 4.09
72	$76.52^{b} \pm 0.37$	$71.06^{a} \pm 0.27$	73.79 ± 2.73
Degradation kinetics			
a	$19.11^{b} \pm 0.21$	$14.46^{a} \pm 0.27$	16.79 ± 2.32
b	$58.11^{b} \pm 0.51$	$57.05^{a} \pm 0.53$	57.58 ± 0.53
c	$0.0530^{b} \pm 0.02$	$0.0478^{a} \pm 0.02$	0.05 ± 0.002
PD (a+b)	$77.22^{b} \pm 0.83$	$71.51^{a} \pm 0.74$	74.37 ± 2.86
RSD	0.94 ± 0.05	1.64 ± 0.03	1.29 ± 0.35
EDADF (0.05%)	$46.74^{b} \pm 0.84$	$39.99^{a} \pm 0.88$	43.37 ± 3.38

^{abcde}Values in the rows bearing different superscripts differ significantly $P<0.05$

N=Mean of 3 observations for each set

Table 10. Rumen fermentation parameters in buffalo bulls fed COBN-5 fodder harvested at 40-45 days

Parameter	Hours					
	0	**2**	**4**	**6**	**8**	**Mean ± SE**
pH	6.52 ± 0.02[d]	6.43 ± 0.01[c]	6.33 ± 0.03[b]	6.21 ± 0.04[a]	6.31 ± 0.03[b]	6.36 ± 0.08
Ammonia Nitrogen (mg/100 ml SRL)	12.50 ± 0.03[b]	16.60 ± 0.03[e]	15.54 ± 0.04[d]	14.00 ± 0.06[c]	12.23 ± 0.05[a]	14.17 ±1.34
TVFA (meq/L of SRL)	65.75 ± 2.46[a]	78.75 ± 3.01[b]	83.00 ± 2.94[c]	92.50 ± 2.90[e]	87.25 ± 2.59[d]	81.45 ±7.17

[abcde]values in the rows bearing different superscripts differ significantly
P<0.01
N=Mean of 3 observations for each set

Further, significant differences (p<0.01) were observed between all different time intervals.

4.4.3 Total volatile fatty acids (TVFA)

The total volatile fatty acid (TVFA) concentration (meq/L of SRL) of buffalo bulls fed *ad libitum* COBN-5 fodder harvested at 40-45 days as affected by time of sampling is presented in Table 10. The TVFA concentration (meq/L of SRL) of buffalo bulls were 65.75, 78.75, 83.00, 92.5and 87.25 at 0, 2, 4, 6, and 8 h, respectively. Time after feeding linearly increased the TVFA concentration up to 6 h post feeding beyond which there was a decline in its concentration. Further, significant differences (p<0.01) were observed between all different time intervals.

4.5 METABOLISM STUDIES IN MURRAH BUFFALO BULLS

4.5.1 Apparent digestibility coefficients of proximate constituents

The apparent digestibility coefficients of proximate constituents in buffalo bulls fed *ad libitum* COBN-5 fodder harvested at 40-45 days are presented in Table 11. The digestibility coefficients (%) of DM, OM, CP, EE, CF and NFE were 60.62, 62.38, 68.74, 62.60, 55.72 and 62.74, respectively in buffalo bulls fed *ad libitum* COBN-5 fodder.

Table 11. Apparent digestibility (%) of nutrients in buffalo bulls fed *ad libitum* COBN-5 fodder harvested at 40-45 days

Nutrient	COBN-5
Dry matter	60.62 ± 2.17
Organic matter	62.38 ± 2.30
Crude protein	68.74 ± 1.58
Ether extract	62.60 ± 1.30
Crude fibre	55.72 ± 2.64
Nitrogen free extract	62.74 ± 3.56
Neutral detergent fibre	55.07 ± 1.21
Acid detergent fibre	46.18 ± 2.64
Hemi cellulose	71.30 ± 2.62
Cellulose	47.00 ± 2.10

N=Mean of 6 observations for each set

4.5.2 Apparent digestibility coefficients of cell wall constituents

The apparent digestibility coefficients of cell wall constituents in buffalo bulls fed *ad libitum* COBN-5 fodder harvested at 40-45 days are presented in Table 11. The digestibility coefficients (%) of NDF, ADF, hemi-cellulose and Cellulose were 55.07, 46.18, 71.30 and 47.00, respectively.

4.5.3 Nitrogen balance

Daily nitrogen intake, outgo and retention in buffalo bulls fed *ad libitum* COBN-5 fodder harvested at 40-45 days are presented in Table 12. All the animals were in positive balance for nitrogen. The average nitrogen intake and outgo as (g/d) were 676.67 and 274.36, respectively. The nitrogen retention expresses as, g/d, % intake and % absorbed are 402.31, 59.45 and 86.44, respectively.

4.5.4 Calcium balance

Calcium intake, outgo and retention in buffalo bulls fed *ad libitum* COBN-5 fodder harvested at 40-45 days are presented in Table 13. All the animals were in positive calcium balance. The average calcium intake and outgo as (g/d) were 160.00 and 138.29, respectively. The calcium retention expresses as, g/d, % intake and % absorbed are 21.71, 13.57 and 77.81, respectively. The average calcium excretion through faeces and urine (g/d) were 132.10 and 6.19, respectively.

Table 12. Nitrogen utilization in buffalo bulls fed *ad libitum* COBN-5 fodder harvested at 40-45 days

Parameter	**COBN- 5**
Nitrogen intake, g/d	676.67 ± 71.96
Nitrogen outgo, g/d	
Faeces	211.25 ± 22.09
Urine	63.11 ± 16.78
Total	274.36 ± 36.73
Nitrogen retention	
Retention, g/d	402.31 ± 48.46
% intake	59.45± 1.80
% absorbed	86.44 ± 1.87

N=Mean of 6 observations for each set

Table 13. Calcium utilization in buffalo bulls fed *ad libitum* COBN-5 fodder harvested at 40-45 days

Parameter	COBN-5
Calcium intake, g/d	160.00 ± 17.02
Calcium outgo, g/d	
Faeces	132.10 ± 20.34
Urine	6.19 ± 0.30
Total	138.29 ± 20.48
Calcium retention	
Retention, g/d	21.71 ± 3.96
% intake	13.57 ± 2.86
% absorbed	77.81 ± 1.46

N=Mean of 6 observations for each set

4.5.5 Phosphorus balance

Phosphorus intake, outgo and retention in buffalo bulls fed *ad libitum* COBN-5 fodder harvested at 40-45 days are presented in Table 14. All the animals were in positive phosphorus balance. The average phosphorus intake and outgo (g/d) were 148.00 and 123.84, respectively. The phosphorus retention expressed as, g/d, % intake and % absorbed are 24.16, 16.32 and 55.72, respectively. The average calcium excretion through faeces and urine (g/d) were 104.64 and 19.20, respectively.

4.5.6 Plane of nutrition of buffalo bulls

The data on plane of nutrition of buffalo bulls fed *ad libitum* COBN-5 fodder harvested at 40-45 days is presented in Table 15. The DM intake expressed as (%) of body weight was 1.68. The DCP and TDN content expressed as (%) in the diet consumed or as intake (kg/d) was 8.74 and 56.05; 0.53 and 3.37, respectively. The DE and ME (Mcal) intake in the animals fed COBN-5 fodder were 14.85 and 12.18, respectively. The DM, DCP, TDN (g) and ME (Mcal) intake per unit metabolic body weight were 73.03, 6.38, 40.93 and 0.15, respectively. The ratio of the DCP (g) /ME (Mcal) is 43.12.

The DM intake (kg/d) in buffalo bulls fed COBN-5 fodder was 6.01and is higher than ICAR, 2013 (5.25) and Kearl, 1982 (5.7), recommended intake. Similarly, the intake of DM, DCP, TDN (g/WKg$^{0.75}$) and ME (Mcal) were also higher than the values recommended by ICAR, 2013 and Kearl, 1982 standards.

Table 14. Phosphorus utilization in buffalo bulls fed *ad libitum* COBN-5 fodder harvested at 40-45 days

Parameter	**COBN-5**
Phosphorus intake, g/d	148.00 ± 15.95
Phosphorus outgo, g/d	
Faeces	104.64 ± 8.22
Urine	19.20± 1.39
Total	123.84 ± 8.19
Phosphorus retention	
Retention, g/d	24.16 ± 14.86
% intake	16.32 ± 5.74
% absorbed	55.72 ± 3.81

N=Mean of 6 observations for each set

Table 15. Plane of nutrition of buffalo bulls for maintenance fed COBN-5 fodder harvested at 40-45 days

Treatment	Avg. B .Wt	W kg$^{0.75}$	DMI (kg/d)	DMI as % B.Wt.	DCP		TDN		DE* Intake (Mcal)	ME* Intake (Mcal)	Intake /unit W kg$^{0.75}$				DCP (g) / ME (Mcal)
					% in diet consumed	Intake (kg/d)	% in diet consumed	Intake (kg/d)			DM (g)	DCP (g)	TDN (g)	ME (Mcal)	
COBN-5	357.97	82.30	6.01	1.68	8.74	0.53	56.05	3.37	14.85	12.18	73.03	6.38	40.93	0.15	43.12
ICAR 2013	350.00	80.92	5.25	1.5	-	0.23	-	2.7	11.46	9.4	61.79	2.84	33.87	0.12	24.48
Kearl, 1982	350.00	80.92	5.7	1.6	-	0.23	-	2.6	11.59	9.5	70.44	2.85	32.13	0.12	23.75

*DE intake (Mcal) = TDN intake (Kg) × 4.408

*ME intake (Mcal) = = TDN intake (Kg) × 3.616

Chapter - V

Discussion

CHAPTER – V

DISCUSSION

The effect of feeding COBN-5 fodder on digestibility, rumen fermentation pattern and nutrient utilization in buffalo bulls are discussed here in conjunction with the available literature.

5.1 EVALUATION OF COBN-5 fodder

The COBN-5 fodder was procured from the cultivated fields of ILFC, NTR College of Veterinary Science, Gannavaram at different stages (30, 35, 40, 45 and 50 day) of harvest. The fodder was evaluated for its chemical composition, cell wall constituents, oxalate content and CNCPS fractions.

5.1.1 Chemical composition of COBN-5 fodder

The chemical composition (Table 1) revealed that DM (%) content of COBN-5 fodder fed to buffalo bulls during the experimental period is 18.03. The DM (%) content of harvested fodder at different stages (30, 35, 40, 45 and 50 days) ranged from 16.68 to 20.54. Further, CP, EE and total ash (%) contents were higher in COBN-5 fodder harvested on 30th day and lower on 50th day except for CF (%) which was higher at 50th day (34.69) and lower on 30th day (19.24). The mean CP content of different stages of harvest was 13.05 per cent. Similar values for CP were reported in Napier grass varieties by earlier workers (Balaraman, 1995; Morenz *et al.,* 2012 and Garg *et al.,* 2012). In contrast, lower

(Kariuki *et al.*, 1998; Kabi *et al.*, 2005; Ajaib Singh *et al.*, 2009; Sahoo *et al.*, 2014 and Kebede *et al.*, 2016) and higher (Zewdu, 2002 and Osuga *et al.*, 2006) CP content was reported in Bajra × Napier varieties as compared to the CP content observed in the present study.

The mean EE content at different stages of harvest (30, 35, 40, 45 and 50 days) is 2.82 per cent. These values are in line with the findings of Kozloski *et al.* (2006), Ajaib Singh *et al.* (2009) and Garg *et al.* (2012) who reported similar EE values in Napier grass varieties. On the other hand, lower (Raja Kishore *et al.*, 2008; Reddy *et al.*, 2009; Srinivas Kumar *et al.*, 2011 and Sultan Singh *et al.*, 2012) and higher (Balaraman, 1995; Muia *et al.*, 2006 and Bueno *et al.*, 2015) EE values in bajra × napier varieties were also reported.

The mean CF content of different stages of harvest (30, 35, 40, 45 and 50 days) is 27.09 per cent. The values reported by Balaraman (1995) in Napier grass also showed concomitant values with the present findings. However, lower values were also reported by Ajaib Singh *et al.* (2009) and Garg *et al.* (2012) in Napier grass varieties. Meanwhile, higher values were also reported by earlier workers (Islam *et al.,* 2003; Reddy *et al.,* 2009; Perez *et al.,* 2010; Chandra *et al.,* 2012 and Srinivas Kumar *et al.,* 2013).

The mean total ash content of different stages of harvest (30, 35, 40, 45 and 50 days) is 14.36 per cent. The values reported by Islam *et al.* (2003) in Napier grass in line with the values reported in the present study. The mean calcium and phosphorus contents were 0.73 and 0.57 per cent, respectively. But the present values of calcium and phosphorus were in contrary to the reports of Chandra *et al.* (2012).

5.1.2 Cell-wall constituents of COBN-5 fodder

The cell wall constituents of COBN-5 fodder fed to buffalo bulls during the experimental period is presented in Table 1. The NDF, ADF, hemi-cellulose and cellulose contents were higher in COBN-5 fodder harvested on 50^{th} day and lower at 30^{th} day. The mean NDF content of different stages of harvest is 67.31 per cent. Similar findings were reported in Napier grass varieties by Kaitho and Kariuki (1998) and Garg *et al.* (2012). However, lower NDF values compared to the present study were also reported earlier (Kariuki *et al.*, 1998; Muia *et al.*, 2001; Tessema and Baars, 2004; Osuga *et al.*, 2006; Ajaib Singh *et al.*, 2009; Sahoo *et al.*, 2014 and Kondo *et al.*, 2015). Meanwhile, some researchers also reported higher NDF values in Napier grass varieties compared to the values reported in the present study (Gwayumba *et al.*, 2002; Kabi *et al.*, 2005; Kozloski *et al.*, 2006; Srinivas Kumar *et al.*, 2011; Budiman *et al.*, 2012; Bueno *et al.*, 2015 and Kebede *et al.*, 2016).

The mean ADF content of different stages of harvest (30, 35, 40, 45 and 50 days) is 40.84 per cent. The present findings were in agreement with the values of ADF reported in various Napier varieties by several workers (Kozloski *et al.*, 2006; Reddy *et al.*, 2009; Sultan Singh *et al.*, 2012; Chandra *et al.*, 2012; Garg *et al.*, 2012; Sahoo *et al.*, 2014 and Kondo *et al.*, 2015). On the other hand, lower (Kariuki *et al.*, 1998; Muia *et al.*, 2001; Tessema and Baars. 2004; Osuga *et al.*, 2006 and Raja Kishore *et al.,* 2008) and higher (Gwayumba *et al.,* 2002; Srinivas Kumar *et al.,* 2011; Budiman *et al.,* 2012; Ferreira *et al.,* 2012; Bueno *et al.,* 2015 and Kebede *et al.,* 2016) ADF values in bajra × napier varieties were also reported earlier.

The mean hemi-cellulose content of different stages of harvest (30, 35, 40, 45 and 50 days) is 26.47 per cent. Similar findings were also reported in Napier grass varieties by earlier workers (Mahr-un-nisa *et al.,* 2000; Zewdu *et al.,* 2002; Tessema and Baars, 2004; Ajaib Singh *et al.,* 2009 and Srinivas Kumar *et al.,* 2011). However, Kebede *et al.* (2016) reported concomitant values which are higher than those reported in the present study.

The mean cellulose content of different stages of harvest (30, 35, 40, 45 and 50 days) is 35.07 per cent. The present findings were in agreement with the reports of Mahr-un-nisa *et al.* (2000) and Tessema and Baars. (2004). However, lower values were also reported by some earlier workers (Zewdu *et al.,* 2002; Ajaib Singh *et al.,* 2009 and Sultan Singh *et al.,* 2012). Meanwhile, higher values as compared to the present findings were reported by Srinivas Kumar *et al.* (2011) and Kebede *et al.* (2016).

The mean acid detergent lignin (ADL) (%) of different stages of harvest (30, 35, 40, 45 and 50 days) was 5.73. The present findings were in agreement with the reports of Budiman *et al.* (2012). However, lower (Kariuki *et al.,* 1998; Muia *et al.,* 2001; Zewdu *et al.,* 2002; Tessema and Baars. 2004; Kozloski *et al.* 2006; Osuga e*t al.,* 2006; Raja Kishore *et al.,* 2008; Ajaib Singh *et al.,* 2009 and

Sultan Singh *et al.*, 2012) and higher (Mahr-un-nisa *et al.*, 2000, Gwayumba *et al.*, 2002, Kabi *et al.*, 2005 and Morenz *et al.*, 2012) values were reported by some workers.

These differences in proximate composition and cell wall constituents observed in the present study with COBN-5 variety as compared to other bajra × napier varieties might be attributed to the differences in breeding, variety of cultivar used, stage of harvest, type of soil, season, cultivation practices etc.

5.1.3 Oxalate content

The oxalate content in COBN-5 fodder harvested during early stages is high when compared to that in late stages (Table 2). It is reported that oxalate is the common anti-nutritional factor present in almost all the Napier varieties which leads to poisoning when fed in large quantities. The total oxalate content in Napier grass increased linearly with the successive cut and it also varies with different seasons (Ahuja *et al.*, 1998; Rahman *et al.*, 2008).

Oxalate content reported in different stages of COBN-5 fodder in the present study corroborated with the values reported by Ahuja *et al.* (1998) in PBN-83, Rahman *et al.* (2008) in PB-231 and Ajaib Singh *et al.* (2009) in Napier grass. In contrast, lower oxalate content was reported by Rahman and Kawamura. (2011) as compared to the values observed in the present study. On the other hand, Anthony and Thomas (2014) reported higher oxalate content in 3rd harvest of 11 popular cultivars of hybrid Napier when compared to the values in the present study which might be due to differences in clipping intervals. The oxalate content of the plant varies with many internal and external factors like fertilizer management, forage species, harvesting practices and seasonal variations etc., (Rahman *et al.*, 2011). The safer levels of soluble oxalate content in Napier grass is 10-20 g/kg (Rahman *et al.*, 2013). Feeding of early cut COBN-5 fodder (30th and 35th day) may cause oxalate toxicity, while the late cut fodder revealed lower nutrient digestibility. Hence, the COBN-5 harvested at 40 days with moderate oxalate content (2.31 %) and high nutrient digestibility is the recommended optimum age of harvest for ruminant feeding.

5.1.4 Carbohydrate and protein components of COBN-5 fodder at different stages of harvest

The carbohydrate and protein components of COBN-5 fodder (Table 3) revealed that lignin (% NDF) content was 11.61 per cent. Similar values were reported in *Hardwickia binate* tree leaves and *Securinega virosa* shrubs while higher values were observed in *Azadirachta indica* and *Ficus religiosa* leaves than the values (5.8) in present experiment (Bhadauria *et al.*, 2002). The NSC (% DM) content of the present study was 19.48 per cent. Similar results were observed in *Ficus religiosa* tree leaves and *Bauhinia racemose* shrubs while higher values were reported in *Azadirachta indica* and *Leucaena leucocephala* (Bhadauria *et al.*, 2002). The NSC (%) of the present study (10.24) is lower than in Alfalfa (24.7) and Timothy (13.13) but the lignin (%) was vice-versa (Yu *et al.*, 2003). Total carbohydrates were slightly lower and NSC content was higher in the present study compared to the values reported in hybrid napier grass (Sultan Singh *et al.*, 2012; Das *et al.,* 2015). The results of TCHO and NSC of present study corroborated with the values reported by Sultan Singh *et al*. (2014) in Napier fodder with little differences.

The SP (% CP) content of COBN-5 fodder in the present study corroborated with the mean value reported for grasses (*Heteropogon contortus, Sehima nervosum Cenchrus ciliarus*) by different workers (Chaurasia *et al.,* 2006; Prusty *et al.,* 2013; Das *et al.,* 2015) while, antithetical values were reported in tree leaves and shrubs (Bhadauria *et al.,* 2002). The NPN (% SP) content of the COBN-5 fodder was 89.74 and is in agreement with the value reported for tree and shrubs (Bhadauria *et al.,* 2002; Prusty *et al.,* 2013) with an exception of lower value in *Ziziphus nummularia* shrub. However, higher values of NPN (% SP) and SP (% CP) content were reported in alfalfa than COBN-5 fodder (Yu *et al.,* 2003).

The NDICP and ADICP values (% CP) of the present study were 77.16 and 33.86. Lower values were reported for most trees and shrubs (Bhadauria *et al.,* 2002; Chaurasia *et al.,* 2006; Prusty *et al.,* 2013); for alfalfa (Yu *et al.,* 2003) and hybrid Napier varieties (Das *et al.,* 2015). Further, the NPN (% SP) and ADICP (% CP) values in the present study were higher when compared to the values reported in Napier grass (Sharma *et al.,* 2004). The BSP and NDICP (% CP) values in the present study corroborated with the findings of Sultan Singh *et al.* (2014) in Napier grass.

5.1.5 Carbohydrate and protein fractions of COBN-5 fodder at different stages of harvest

The carbohydrate fractions viz. CA, CB_1, CB_2 values of most of the tree and shrubs were lower as compared to COBN-5 fodder but higher CC values were reported (Bhadauria *et al.,* 2002). Higher CC fraction may be attributed to the presence of high amount of insoluble or non-degradable part of CHO. This indicates presence of higher degradable CHO portion in COBN-5 fodder when compared that in trees and shrubs. The CA, CC fractions were higher in timothy and alfalfa while, CB_1 and CB_2 fractions were lower when compared to respective fractions in COBN-5 fodder. Higher CA and CB_1 content was reported in *Heteropogan contortus* compared to the value observed in the present study. However, Chaurasia *et al.* (2005) reported higher CB_2 and CC values in grasses (*Heteropogan contortus, Sehima nervosum etc.*) than the values in present study. Similar results as compared to the present findings were reported for CB_2 fraction in Napier grass but lower values were observed for C ($A+B_1$) and CC fractions (Morenz *et al.,* 2012). Further, higher CA and CB_2 values were reported in the present study than in Buffel grass, Bermuda grass and Crysopogon grass (Prusty *et al.,* 2013) which indicates presence of more soluble sugars indicating that COBN-5 is highly digestible. On the other hand, Das *et al.* (2015) reported higher CA, CB_1 and CC values and lower CB_2 values in Hybrid Napier grass as compared to the present findings.

The protein fractions (% CP) of the COBN-5 fodder *viz*. PA, PB_1, PB_2, PB_3 and PC fractions were presented in Table 4. Yu *et al.* (2003) reported lower PA, PB_2 values and higher PB_1, PB_3 and PC values in alfalfa as compared to the present study. However, the present study indicated that PA and PB_3 fractions were higher, while PB_1, PB_2 and PC fractions were lower when compared to the values of trees and shrubs (*Azadirachta indica, Albizia amara, etc*.) as reported earlier (Bhadauria *et al.,* 2002; Chaurasia *et al.,* 2006; Prusty *et al.,* 2013). The PA and PC values of the present study were in agreement with those reported by Sharma *et al.* (2004) in Napier varieties. However, the present findings were lower in PB_2 and higher in PB_3 as compared to the values reported by Sharma *et al.* (2004) in Napier varieties (Napier-7, Napier-3). The PA, PB_2 and PB_3 values of present study corroborated to the reports of Das *et al.* (2015) in hybrid Napier fodder.

5.2 *IN VITRO* STUDIES

The *in vitro* digestibility (%) of DM, CP, NDF and ADF of COBN-5 fodder at different stages (30, 35, 40, 45 and 50 days) of harvest studied using rumen liquor collected from cannulated buffalo bulls maintained on COBN-5 fodder is presented in Table 5.

5.2.1 *In vitro* DM digestibility (%) of COBN-5 fodder at different stages of harvest

The IVDMD of COBN-5 fodder at different stages (30, 35, 40, 45 and 50 days) of harvest ranged from 60.69 to 70.40 per cent. The IVDMD was higher ($P<0.05$) in earlier stage and lower ($P<0.05$) as plant matures. Earlier reports on IVDMD of Napier grass (Zewdu *et al.,* 2002; Morenz *et al.,* 2012 and Bora *et al.,* 2011) corroborated with values of the present study. Lower digestibilities reported in late cut fodder might be due to higher lignin content as the harvesting stage advances. This is in agreement with the observations of earlier workers (Aganga *et al.,* 2005; Bora *et al.,* 2011 and Budiman *et al.,* 2012) in hybrid Napier grass.

The IVDMD of different Napier accessions showed complementary values (Zewdu, 2005) to the present study. The *in vitro* true digestibility (IVTD) of Napier grass (*Pennisetum purpureum*) varies due to their relationships in chemical composition, leaf to stem ratio, plant height, geographic location, climatic factors, different varieties and harvest interval (Bora *et al.,* 2011). Further, low dry matter digestibility may be attributed due to increase in the ADF content in fodder which will increase as the maturity of the fodder (Budiman *et al.,* 2012).

5.2.2 *In vitro* CP digestibility (%) of COBN-5 fodder at different stages of harvest

The *in vitro* CP digestibility (%) of COBN-5 fodder at different stages (30, 35, 40, 45 and 50 days) of harvest is presented in table 5. The IVCPD of COBN-5 fodder showed ($p<0.05$) significant differences between all stages of harvest and highest digestibility was observed in 30th day harvested fodder. The present study reported higher CP digestibility in early cut fodder than the late cut or matured fodder due to increase in the lignin content (NARC, 2006). Hence, feeding early cut fodder with high protein content supplies better protein to animals (Lounglawan *et al.,* 2014).

5.2.3 *In vitro* NDF digestibility (%) of COBN-5 fodder at different of harvest

The *in vitro* NDF digestibility (%) of COBN- 5 at different stages (30, 35, 40, 45 and 50 day) of harvest is presented in table 5. The IVNDFD of COBN-5 fodder showed ($P<0.05$) significant differences between all stages of harvest and highest ($P<0.05$) digestibility was observed at 30th day harvested fodder as compared to fodder harvested at other intervals. Similar values were also reported by some workers (Kondo *et al.*, 2015; Gallardo *et al.*, 2010) for Napier grass. On the other hand, Kondo *et al*. (2015) reported higher NDF digestibility in Para grass and Gamba grass as compared to the values reported for COBN-5 in the present study.

5.2.4 *In vitro* ADF digestibility (%) of COBN-5 fodder at different stages of harvest

The *in vitro* ADF digestibility (%) of COBN- 5 at different stages (30, 35, 40, 45 and 50 days) of harvest is presented in table 5. Higher ($P<0.05$) digestibility was reported in 30th day harvested fodder and it decreased ($P<0.05$) as the plant matures. Significant differences ($p<0.05$) were observed between different stages of harvest. The values reported in the present study were antithetical to the values reported by Gallardo *et al.* (2010) in elephant grass. The higher ADF digestibility in early cut fodder may be due to its lower lignin content compared to matured fodder.

5.3 *IN SACCO* STUDIES

Based on the oxalate content and data on *in vitro* digestibility, 40th and 45th day harvested fodder was selected for *in sacco* studies. The *in sacco* disappearance (%) and degradation kinetics of DM, CP, NDF and ADF of COBN-5 fodder was evaluated using nylon bag technique (Tables 6 to 9).

5.3.1 *In sacco* DM disappearance (%) and degradation kinetics of COBN-5 fodder

The average *in sacco* dry matter disappearance (%) of COBN-5 fodder at two different stages (40 and 45 days) of harvest was evaluated in the rumen of cannulated buffalo bulls (Table 6). Data revealed that as the period of incubation extended from 0 to 72 h in the rumen, the *in sacco* DM disappearance (%) values increased progressively.

The rapidly soluble fraction (a) was higher in the fodder harvested at 40th day. While, the insoluble but degradable fraction (b), potentially degradable fraction (a + b) and rate constant/h (c) were higher in the fodder harvested at 45th day. Data in the present study corroborated with the findings (Abdulrazak *et al.,* 1996) in Napier grass. In contrast to the findings of the present study, higher fraction ‘a’ and lower fraction ‘b’ was reported by some earlier workers (Kariuki *et al.,* 2001; Tessema and Baars. 2004; Valente *et al.,* 2011) in Napier grass.

The fractions a, b and a + b of the present experiment were antithetical to the values reported (Kariuki *et al.,* 2001) for Napier grass, while the fraction ‘c’ is in agreement. However, EDDM and PD (a + b) values were lower in Napier grass compared to the values in present experiment.

5.3.2 *In sacco* CP disappearance (%) and degradation kinetics of COBN-5 fodder

In sacco CP disappearance (%) and degradation kinetics of COBN-5 fodder in cannulated buffalo bulls at two different stages (40 and 45 days) of harvest is presented in table 7. The protein disappearance up to 72 h of incubation in the ventral sac of rumen was slightly higher in the fodder harvested at 40th day than on 45th day. The rapidly soluble fraction 'a' and EDCP (%) was higher in fodder harvested at 40 days, while the insoluble but degradable fraction 'b' was higher in the fodder harvested at 45 days.

Muia *et al.* (2001) reported similar values for fractions 'b' and 'c' but lower values for 'a' and EDCP in Napier fodder compared to the values in present study. The higher fraction 'a' fraction and lower 'b' fraction in the present experiment indicates presence of more soluble nitrogen content as compared to other Napier varieties. The effective degradability of COBN-5 fodder was higher than the other Napier grasses suggesting high degradable nitrogen content (Kabi *et al.,* 2005) in COBN-5 fodder.

5.3.3 *In sacco* NDF disappearance (%) and degradation kinetics of COBN-5 fodder

In sacco NDF disappearance (%) and degradation kinetics of COBN-5 fodder in fistulated buffalo bulls at two different stages (40 and 45 days) of harvest is presented in table 8. The NDF (%) disappearance up to 72 h of incubation in the ventral sac of rumen was higher in the fodder harvested at 40 days. Higher fraction 'a', (a+b) and EDNDF (%) were reported in the fodder harvested at 40 days compared to fodder harvested at 45 days.

The lower EDNDF (%) in the fodder harvested at 45 days might be due to higher cellulose and acid detergent lignin content. The potentially degradable fraction of elephant grass was in agreement with the present study (Valente *et al.*, 2011). But lower values were reported than the value in the present study (Gallardo *et al.,* 2010).

5.3.4 *In sacco* ADF disappearance (%) and degradation kinetics of COBN-5 fodder

In sacco ADF disappearance (%) and degradation kinetics of COBN-5 fodder in fistulated buffalo bulls at 40 and 45 days of harvest is presented in table 9. The ADF (%) disappearance up to 72 h of incubation in the ventral sac of rumen was higher in the fodder harvested at 40 days. The rapidly soluble fraction (a), insoluble but degradable fraction (b) and PD (a+b) were higher in the fodder harvested at 40 days. Further, the effective degradability of ADF (EDADF %) was higher in the fodder harvested at 40 days. Lower potential degradability values reported for elephant grass than the values in the present study (Gallardo *et al.*, 2010; Singh *et al.*, 1992). Decreased ADF degradability in late cut forages, may be attributed to presence of higher lignified fibre.

5.4 RUMEN FERMENTATION STUDIES

In the present study, four rumen fistulated Murrah buffalo bulls were fed *ad libitum* COBN-5 fodder harvested at 40 and 45 days. At the end of collection period, rumen liquor was collected from buffalo bulls just prior to feeding (0) and at 2, 4, 6 and 8 h post feeding to study the effect on rumen fermentation pattern (Table 10).

5.4.1 Rumen pH

The mean pH values in ruminal fluid of buffalo bulls as affected by time of sampling are presented in Table 10. Time of sampling had a significant ($P<0.01$) on pH of rumen liquor. Further, the present study revealed that the pH of rumen liquor showed a decreasing trend up to 6 h post feeding in all the buffalo bulls fed COBN-5 fodder and increased thereafter. The results of the present study were in agreement with the findings of Abdulrazak *et al.* (1996) in buffalo steers, Mahr-un-nisa and Sarwar, (1998) in buffalo Calves Mahr-un-nisa *et al.* (2000) in buffalo bulls, Puga *et al.* (2001) in sheep, Srinivas Kumar *et al.* (2011) in buffalo bulls fed Napier fodders. In contrast, some reports showed antithetical values to the present study (Kariuki *et al.,* 2001 in cattle; Muia *et al.*, 2001 in Friesian steers; Kozloski *et al.*, 2006 in cattle; Bandeswaran *et al.,* 2013 in cattle; Ganai *et al.*, 2015 in Bucks) fed different Napier varieties. This post prandial decline in pH values observed in the present study might be attributed to increased microbial fermentation and accumulation of organic acids in the rumen.

5.4.2 Ammonia nitrogen

The ammonia nitrogen concentration in SRL of buffalo bulls fed *ad libitum* COBN-5 fodder is presented in Table 10. Time of sampling significantly ($P<0.01$) affected the rumen ammonia nitrogen concentration in buffalo bulls. The rumen ammonia nitrogen concentration increased linearly up to 2 h post feeding beyond which there was a decline in its concentration. The mean ammonia nitrogen concentration in SRL of buffalo bulls was highest at 2 h of post feeding and lowest was recorded at 8 h post feeding.

The results of the present study are in agreement with the findings of previous workers (Abdulrazak *et al.,* 1996 in buffalo steers; Mahr-un-nisa and Sarwar., 1998 in buffalo calves; Mahr-un-nisa *et al.,* 2000 in buffalo bulls, Kariuki *et al.,* 2001 in cattle and Puga *et al.,* 2001 in sheep) fed Napier fodders. Lower values compared to those observed in the present study were reported by Muia *et al.* (2001) in Friesian steers, Kozloski *et al.* (2006) in cattle, Bandeswaran *et al.* (2013) in cattle fed different Napier varieties.

5.4.3 Total volatile fatty acids (TVFA)

The total volatile fatty acid (TVFA) concentration in SRL of buffalo bulls fed *ad libitum* COBN-5 fodder are presented in Table 10. Time after feeding linearly increased ($P<0.01$) the TVFA concentration up to 6 h post feeding beyond which there was a decline in its concentration. The mean TVFA concentration in

SRL of buffalo bulls was higher at 6 h of post feeding and lowest was reported at 0 h of feeding respectively.

Similar findings were reported in buffalo bulls fed CO-1 fodder (Srinivas Kumar *et al.,* 2011). The total volatile fatty acids of the present study were in range with the earlier workers (Kariuki *et al.,* 2001 in cattle; Ganai *et al.,* 2015 in bucks) fed Napier fodders. However, higher TVFA concentration was reported by Muia *et al.* (2001) in Friesian calves and Puga *et al.* (2001) in sheep than the values in the present study.

5.5 METABOLISM STUDIES IN MURRAH BUFFALO BULLS

5.5.1 Apparent nutrient digestibility coefficients

Based on the oxalate content, *in vitro* study and *in sacco* degradability of COBN-5 fodder harvested between 40 to 45 days was fed to six buffalo bulls (357.95 ± 8.26 kg) to study the nutrient digestibility by conducting a metabolism trial.

The apparent nutrient digestibility coefficients of DM, OM, CP, EE, CF and NFE were presented in Table 11. The digestibility of DM and OM observed in the present study corroborated with the findings of earlier workers (Abdul razak *et al.,* 1996 in buffalo steers; Kozloski *et al.,* 2006 in cattle) fed Napier grass. The digestibility values for DM, CP, CF, OM and NFE in the present study were higher than the other Napier varieties reported by earlier workers (Balaraman. 1995; Gwayumba *et al.,* 2002; Nyambati *et al.,* 2003; Reddy *et al.,* 2009;

Wadhwa *et al.,* 2010 and Chandra *et al.,* 2012). Similarly, the digestibility of CP for COBN-5 fodder observed in the present study corroborated with the digestibility of CP in bajra variety (PHBF-1) as reported earlier (Singh *et al.,* 2009). The digestibility of DM and CP observed in the present study corroborated with those of Perez *et al.* (2010) in Napier grass at different stages of harvest. However, Bandeswaran *et al.* (2013) reported lower digestibility of DM and OM for Napier grass compared to the values in the present study indicating the superiority of COBN-5 fodder with more digestible nutrients.

The apparent nutrient digestibility coefficients of NDF, ADF, hemi-cellulose and cellulose were presented in Table 11. The NDF digestibility of COBN-5 fodder as reported in the present study was in agreement with the findings of Gwayumba *et al.* (2002) and Chandra *et al.* (2012) in Napier fodder. In contrast, higher NDF digestibility in Napier fodder as compared to that observed in the present study was reported by Kozloski *et al.* (2006). The ADF digestibility of COBN-5 fodder reported in the present study corroborated with findings of Wadhwa *et al.* (2010) in Napier grass. However, lower (Reddy *et al.,* 2009) and higher (Gwayumba *et al.,* 2002 and Chandra *et al.,* 2012) ADF digestibility as compared to that observed in the present study were reported earlier. The digestibility of cellulose and hemi-cellulose for COBN-5 fodder as observed in the present study were antithetical to the values reported in Napier fodder by Reddy *et al.* (2009) and Wadhwa *et al.* (2010).

5.5.2 Nitrogen balance

Nitrogen intake, outgo and retention (g/d) in buffalo bulls fed of the COBN-5 fodder are shown in the Table 12. Positive nitrogen balances are recorded indicating that the buffalo bulls fed *ad libitum* COBN-5 fodder met the nitrogen requirements.

The nitrogen intake was high in the present experiment reflecting sufficient nitrogen content in the COBN-5 fodder. Amount of nitrogen outgo was lower due to the better digestibility of the nitrogen in the fodder. The values for nitrogen intake (%) corroborated with values reported by Wadhwa *et al.* (2010) in bajra fodder. Further, the nitrogen intake observed in the present study was higher than that reported in other Napier varieties by earlier workers (Kariuki *et al.,* 1998; Kaitho and Kariuki, 1998). The nitrogen intake expressed as % absorbed was higher in COBN-5 fodder than that reported in Napier grass by other workers (Reddy *et al.,* 2009 in goats; Chandra *et al.,* 2012 in goats) which might be attributed to the higher CP digestibility of COBN-5 fodder (Table 11).

5.5.3 Calcium balance

Calcium intake, outgo and retention (g/d) in buffalo bulls fed *ad libitum* COBN-5 fodder are presented in Table 13. All the animals were in positive calcium balance due to presence of appropriate amount of calcium in the COBN-5 fodder and higher calcium retention.

The calcium intake (g/d) was high in the present experiment because of moderate content when compared to the other Napier varieties. Amount of calcium outgo was lower due to the better digestibility of the calcium present in the fodder. The values reported by Kaitho and Kariuki (1998) were similar to the values of present study in Napier grass. In contrast, the calcium (% of intake) was lower in the present study compared to the values reported by the Chandra *et al.* (2012) and Reddy *et al.* (2009) in goats fed Napier bajra fodder. However, the calcium requirement of buffalo bulls for maintenance was met by feeding *ad libitum* COBN-5 fodder as a sole diet (Ranjhan, 1998).

5.5.4 Phosphorus balance

Phosphorus intake, outgo and retention (g/d) in buffalo bulls fed *ad libitum* COBN-5 fodder are presented in Table 14. All the animals were in positive phosphorus balance due to presence of appropriate amount of phosphorus in the COBN-5 fodder and higher phosphorus retention in the animals.

The phosphorus intake (g/d) was high in the present experiment because of moderate content of phosphorus in the fodder. Phosphorus retention (g/d) was higher in the animal fed COBN-5 fodder because of better availability of phosphorus. The values reported by Kaitho and Kariuki. (1998) were similar to the values of present study in Napier grass. In contrast, the phosphorus (% of intake) was lower in the present study compared to the values reported by the Chandra *et al.* (2012) and Reddy *et al.* (2009) in goats fed Napier bajra fodder.

The phosphorus requirement for the buffalo bulls for maintenance was met by feeding *ad libitum* COBN-5 fodder as a sole diet (Ranjhan, 1998).

5.5.5 Plane of nutrition

Data on plane of nutrition of buffalo bulls fed ad libitum COBN-5 fodder (Table 15) revealed that the DMI expressed as kg/d or as % of body weight met the requirements for maintenance.

The average DMI of buffalo bulls fed COBN-5 fodder *ad libitum* was comparable to the values recommended by ICAR (2013) and Kearl (1982) standards. The average recommended DMI (kg/d) of buffalo bulls (350 kg) by ICAR (2013) and Kearl (1998) were 5.0 and 5.7 kg, respectively and the DMI intake through COBN-5 fodder is 6.01 kg. This indicates that COBN-5 fodder is highly palatable. On the other hand, Abdulrazak *et al.* (1996) and Kariuki *et al.* (1998) reported lower DMI for Napier grass as compared to that of COBN-5 fodder indicating that COBN-5 fodder is more palatable when compared to Napier grass. On the contrary, higher DMI (% BW) and (kg/day) in Napier grass harvested in early stages compared to that observed in the present study were also reported (Kaitho and Kariuki, 1998; Gwayumba *et al.*, 2002 and Nyambati *et al.*, 2003).

The DCP expressed as kg/d or as % in the fodder consumed was more than the requirement for maintenance of buffalo bulls. The average recommended DCP (kg/d) of buffalo bulls (350kg) by ICAR (2013) and Kearl (1998) was 0.23 kg and the DCP intake through COBN-5 fodder is 0.53. This may be attributed to higher digestibility of crude protein in the COBN-5 fodder. The DCP values reported in the present study are antithetical to the reports of Pachauri and Pathak, (1989) in hybrid Napier fodder and Ajaib Singh *et al.* (2009) in hybrid Bajra fodder.

The TDN values of COBN-5 fodder observed in the present study corroborated with the TDN value reported by Pachauri and Pathak (1989) in Napier grass. In contrast, antithetical values when compared to the present findings were reported by earlier workers (Pratap Reddy *et al.,* 1989; Balaraman. 1995; Reddy *et al.,* 2009; Chandra *et al.,* 2012) in goats fed different Napier varieties. Similarly, the TDN content expressed as % in the fodder consumed or kg/d met the requirements for maintenance. The higher nutrient digestibilities recorded in the present experiment may be attributed to the higher TDN content of the fodder (Table 15).

The ME (Mcal) intake by buffalo bulls in the present experiment was 12.18 and the recommended levels by ICAR (2013) was 9.4 and by Kearl (1998) was 9.5, respectively. The values for ME (Mcal) and TDN (% in diet consumed) were higher than the earlier report (Garg *et al.,* 2012) in animals fed hybrid Napier x bajra fodders. Meanwhile, the ME content of COBN-5 fodder was higher compared to the ME content reported by Perez *et al.* (2010) in ten re-growth ages of Napier grass. Increased amount of ME (Mcal) in COBN-5 fodder may be attributed to easily soluble and degradable fraction of carbohydrates and protein. The DM, DCP, TDN and ME intakes per $Wkg^{0.75}$ as well as calorie protein ratio in buffalo bulls fed *ad libitum* of COBN-5 fodder were higher than the values recommended in ICAR (2013) and Kearl (1982) standards.

5.6 Conclusion

The present study indicated that feeding *ad libitum* COBN-5 fodder to buffalo bulls met the adequacy of nutrients (ICAR, 2013).

Further, it is concluded that feeding *ad libitum* COBN-5 fodder starting from 40 days of harvest as a sole diet to the buffalo bulls had no adverse effects of oxalates and evinced better nutrient utilization, rumen fermentation pattern and plane of nutrition.

Chapter - VI

Summary

CHAPTER – VI

SUMMARY

The COBN-5 fodder procured from the cultivated fields of ILFC, NTR College of Veterinary Science, Gannavaram was fed *ad libitum* to Murrah buffalo bulls (357.97 ± 8.26 kg) and evaluated for its nutrient digestibility, balance of nutrients and rumen fermentation pattern. The fodder was evaluated for its chemical composition, cell wall constituents, oxalate content and carbohydrate and protein fractionation, *in vitro* nutrient digestibility at different stages (30, 35, 40, 45 and 50 days) of harvest. Further, was evaluated *in sacco* for DM, CP, NDF and ADF degradability at 40 and 45 days of harvest.

The chemical composition at different days of harvest (30, 35, 40, 45 and 50 days) revealed that the CP and NFE content gradually decreased as the days of harvest increased but the CF, NDF, ADF and lignin content increased with increase in days of harvest. The carbohydrate and protein fractions indicated that carbohydrate fraction C (unavailable cell wall fraction) was lower and CB_2 fraction was higher in the fodder harvested at 30 days. Further, protein fraction 'B_3', largely a bypass protein is higher and protein fraction 'C', which cannot be degraded by rumen bacteria and cannot provide amino acids post ruminally was lower in the COBN-5 fodder, indicating its superiority compared to other Napier varieties.

In vitro digestibility (%) of COBN-5 fodder at different stages (30, 35, 40, 45 and 50 days) of harvest was in range of 60.69 to 70.40; 69.64 to 81.01; 55.82

to 64.88; 49.30 to 56.96 for DM, CP, NDF and ADF, respectively. Fodder harvested at 30 and 35 days showed higher digestibility than the fodder harvested at later intervals. Because of increase in fibre and lignin content in the mature fodder, the digestibility of DM, CP, NDF and ADF decreased.

The *in sacco* degradability of DM, CP, NDF and ADF of COBN-5 fodder harvested at 40 and 45 days was evaluated in three rumen cannulated buffalo bulls. Data revealed that fraction 'a' PD (a+b) and EDDM (0.05 %) were higher ($p<0.05$) in the fodder harvested at 40 days compared to fodder at 45 days of harveste. The *in sacco* CP degradability values revealed that the fractions 'a', PD (a + b) and EDCP (0.05 %) were higher ($p<0.05$) in the fodder harvested at 40 days.

Further, the data on the *in sacco* degradability of NDF and ADF revealed higher ($p<0.05$) fraction 'a', PD (a+b), EDNDF and EDADF (0.05 %) in the fodder harvested at 40 days compared to the fodder at 45 days of harvest. The corresponding values for *in saaco* degradability of DM, CP, NDF and ADF were higher in COBN-5 compared to the values reported in the other Napier varieties.

Data on rumen fermentation in the buffalo bulls fed COBN-5 fodder revealed that mean rumen pH values were higher at 0 h (before feeding) and declined to minimum by 6 h post feeding, while TVFA concentration (meq/L) was the reverse of pH which increased gradually upto at 6 h post feeding and then declined. However, ammonia N (mg/100 ml SRL) concentration reached peak at 2 h post feeding and later followed a declining trend in COBN-5 fodder. Increased ammonia concentration observed in the present study may be attributed to high amount of soluble protein fractions.

The digestibility coefficient (%) for DM, OM, CP, EE, CF, NFE, NDF, ADF, hemi-cellulose and cellulose were 60.62, 62.38, 68.74, 62.60, 55.72, 62.74, 55.07, 46.18, 71.30 and 47 in buffalo bulls fed *ad libitum* COBN-5 fodder. The digestibility of nutrients inCOBN-5 fodder in the present study was reported to be higher than many other Napier varieties.

The nutrient balance studies indicated that all the buffalo bulls were in positive balance for N, Ca and P. The total nitrogen intake (g/d), nitrogen retention and nitrogen intake (%) were higher in the buffalo bulls fed COBN-5 fodder compared to the values reported for other Napier varieties. Similarly, the values reported in the present study for calcium and phosphorus retention (g/d) and % absorbed were also higher compared to other Napier varieties.

The average DMI (kg/d or % BW) of buffalo bulls fed COBN-5 fodder met the nutrient requirements. Further, the DE, DCP, TDN and ME intakes per $WKg^{0.75}$ were higher than the values recommended by ICAR (2013) and Kearl (1982) standards. Similarly, DE and ME intakes (Mcal) were also comparably higher in the present study while, the DCP: ME ratio was slightly higher than other Napier varieties.

The oxalate content of COBN-5 fodder in the present study revealed higher values in the early stages of harvest and decreased as the harvesting age increased. Oxalate content in the fodder harvested at 40 and 45 days is within safe zone for animal feeding and with better nutrient digestibilities. Hence, feeding fodder harvesting from 40 days will not cause any adverse effects to the animals and also proved to contain higher digestible nutrients.

Based on the results from the present study the following conclusions are drawn:

1. Chemical composition, cell wall constituents and oxalate content of COBN-5 fodder at different stages (30, 35, 40, 45 and 50 days) of harvest revealed that the fodder harvested at 40-45 days proved better and safe for animal feeding with permissible level of oxalate content and higher digestible nutrients.
2. Carbohydrate and protein fractions of the COBN-5 fodder revealed higher amount of soluble and degradable contents in the fodder harvested at 40-45 days.
3. *In vitro* and *in sacco* studies of COBN-5 fodder revealed higher digestibility of nutrients compared to other Napier varieties
4. Rumen pH, TVFA and ammonia nitrogen indicated that the COBN-5 fodder promoted better rumen fermentation in Murrah buffalo bulls.
5. The DMI in buffalo bulls was higher than that of recommended by ICAR (2013) and Kearl (1982) standards, indicating that the COBN-5 fodder is palatable and resulting in better digestibility.

6. The digestibility (%) of all nutrients (DM, M, CP, EE, CF, NDF, ADF, hemi-cellulose and cellulose) were higher in COBN-5 fodder compared to many other grasses and Napier varieties.
7. All the animals were in positive balance for N, Ca and P. Further, the Ca and P amount in the fodder were higher than that recommended by ICAR (2013) and Kearl (1982).

   Hence, it is suggested that the fodder can be harvested starting at 40 days and can be fed to ruminants without any adverse effects on the health and digestibility.

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