

**ASSESSMENT OF FEED UTILIZATION IN BUFFALO CALVES
TO INVESTIGATE THE VARIATION IN
RESIDUAL FEED INTAKE**



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

**MASTER OF SCIENCE
IN
ANIMAL NUTRITION**

**BY
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NATIONAL DAIRY RESEARCH INSTITUTE
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KARNAL- 132001 (HARYANA), INDIA
2013**

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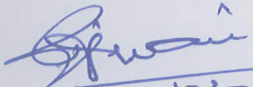
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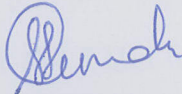
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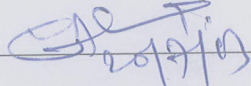
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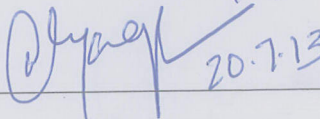

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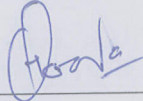

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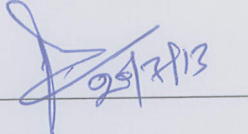
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This is to certify that the thesis entitled, “ASSESSMENT OF FEED UTILIZATION IN BUFFALO CALVES TO INVESTIGATE THE VARIATION IN RESIDUAL FEED INTAKE” submitted by NGUYEN THI BE THO towards the partial fulfilment of the requirements for the award of the degree of MASTER OF SCIENCE IN ANIMAL NUTRITION of the NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY), Karnal (Haryana), India, is a bonafide research work carried out by him under my supervision and guidance, and no part of the thesis has been submitted for any other degree or diploma.

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Date July, 2013

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**Dedicated to
My
Family**

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ABBREVIATIONS

%	Percentage
⁰ C	Degree celsius
ADF	Acid detergent fiber
ADG	Average daily gain
ADICP	Acid detergent insoluble crude protein
ADIN	Acid detergent insoluble nitrogen
ADL	Acid detergent lignin
ADS	Acid detergent solution
AOAC	Association of Official Analytical Chemists
BW	Body weight
BW ^{0.75}	Metabolic body weight
Cal	Calorie
CHO	Total carbohydrate
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude protein
CSC	Cotton seed cake
CTAB	Cetyl trimethyl ammonium bromide
DCP	Digestible crude protein
DE	Digestible energy
dL	Decilitre
DM	Dry matter
DMI	Dry matter intake
DORB	De-oiled rice bran
DOMC	De-oiled mustard cake
DMD	Dry matter digestibility
DDGS	Distillers dried grain solubles
d	day
EDTA	Ethylene diamine tetraacetic acid

EE	Ether extract
FA	Fatty acid
FCR	Feed conversion ratio
g	Gram
GE	Gross energy
GNC	Groundnut cake
hrs	Hour
IVDMD	<i>In vitro</i> dry matter digestibility
IVOMD	<i>In vitro</i> organic matter digestibility
J	Joule
Kcal	Kilocalorie
kg	Kilogram
LSC	Linseed cake
L	Litre
M	Molar
Mcal	Mega calorie
MGM	Maize gluten meal
MOC	Mustard oil cake
ME	Metabolizable energy
MEI	Metabolizable energy intake
ME _m	Metabolizable energy for maintenance
ME _g	Metabolizable energy for growth
mg	Milligram
MJ	Mega joule
ml	Mililiter
mm	Milimeter
mM	mil molar
μl	micro litter
MOC	Mustard oil cake
N	Nitrogen
(N)	Normal

NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein
NDIN	Neutral detergent insoluble nitrogen
NDRI	National Dairy Research Institute
NDS	Neutral detergent solution
NFC	Non fibrous carbohydrate
NFE	Nitrogen free extract
ng	Nanogram
nm	Nanometer
NRC	National Research Council
NSC	Non structural carbohydrates
OM	Organic matter
OMD	Organic matter digestibility
RFI	Residual feed intake
RE	Retained energy
SBM	Soybean meal
TA	Total ash
td CP	Truly digestible CP
td FA	Truly digestible FA
TDMD	True dry matter digestibility
td NDF	Truly digestible NDF
td NFC	Truly digestible NFC
TDN	Total digestible nutrients
TMR	Total mixed rations
w/v	Weight by volume
w/w	Weight by weight
Wt.or wt.	Weight

ABSTRACT

The present study was undertaken for assessment of feed utilization and variations in residual feed intake in Murrah buffalo calves was conducted in two phases. Evaluation of forty feed stuffs for chemical composition, *in vitro* digestibility and gas production was carried out in phase I. Oil cakes and meals contained highest CP ranged from 24.15 to 48.99% in CSC and guar meal. TDN content was estimated using fiber fraction data, and ranged in grains (78.67-86.27%) oil seed cakes (69.13 - 88.46%), green fodders (60.00-68.36%), range grasses (53.21-58.92%) and in agro industrial by-products (44.28-89.12%). Digestible and metabolizable energy were higher, whereas cell wall content was lower in grains compared to others feedstuffs. Green fodders and grasses contained highest fiber fractions among all feed stuffs. IVDMD (%) of oil cakes were higher (57.18-87.60) than green fodders (56.54-71.40) and grasses (38.40-64.17). In phase II, a feeding trial of fifty two days was conducted on eighteen male Murrah buffalo calves of 4-6 months age including a metabolism trial of eight days. The calves were offered *ad libitum* total mixed ration (Concentrate: Roughage = 40:60) and the variations in maintenance requirement of animals were estimated to work out residual feed intake. Animals with low RFI were having low ME_m compared to animals with high RFI. Based on $ME_m/100\text{kg BW}$ of calves were grouped into high (3.92 Mcal) and low ME_m (3.04 Mcal) groups. Mean DMI (g/kg BW) during the feeding trial period was significantly ($P<0.05$) lower in low ME_m group (2642.98 g) compared to high ME_m group (2788.28 g). Average initial BW, final BW, average daily gain and feed conversion ratio did not differ ($P>0.05$) between low and high ME_m groups. Nutrient digestibility coefficients were similar in both groups. Over the course of trial period animals with low ME_m consumed 5.34 % less feed compared to high ME_m group of animals, yet performed in similar way in terms of growth rate. Efficiency of ME utilization for growth did not differ between low and high ME_m groups. The individual variation in maintenance requirements and residual feed intake provides opportunity to select feed efficient buffalo calves and thus improve profitability of buffalo production systems without any negative impact on growth performance.

कटडों में अवशिष्ट खाने की मात्रा में बदलाव की जांच के लिए खाद्य उपयोग का आंकलन

शोधकर्ता: न्युयन थी बी थो

मुख्य मार्गदर्शक : डा. एस. एस. कुण्डू

सारांश

वर्तमान अध्ययन का उद्देश्य मुर्गाह भैंस के कटडों में पशु आहार का उपयोग एवं अवशिष्ट खाद्य सेवन की विविधताओं का विश्लेषण करना है। यह अध्ययन में दो चरणों में आयोजित किया गया। प्रथम चरण में चालीस पशु आहारों का मूल्यांकन रासायनिक संरचना के लिए सामान, इन विट्रो पाचनशक्ति और गैस उत्पादन का अध्ययन किया गया। खली पशु आहारों में सर्वाधिक अपरिष्कृत प्रोटीन की मात्रा 24.15 से 48.99 बिनौला खली एवं ग्वार चारा में पाई गई। कुछ पचनीय पोषक तत्वों का अध्ययन किया गया एवं इसकी सीमा दानों में 78.67 – 86.27 प्रतिशत खली पशु आहारों में 69.13–88.46 प्रतिशत, हरे चारे में 60.00–68.36 प्रतिशत एवं घास में 38.40–64.17 प्रतिशत से अधिक है। द्वितीय चरण में, 4–6 महीने की उम्र के अठारह कटडों का चयन किया गया तथा बावन दिन का पशु आहार परीक्षण किया गया। जिसमें आठ दिनों की चयापचय परीक्षण भी शामिल था। पशुओं की इच्छानुसार कुल मिश्रित राशन (टीएमआर, दाना:हरा चारा 40:60) खिलाया गया एवं पशुओं में रखरचाव के लिए ऊर्जा के सेवन पर पशुओं का अवशिष्ट खाद्य सेवन निकाला गया। जिन पशुओं अवशिष्ट खाद्य सेवन कम पाया गया उन्ही पशुओं में ME_m की मात्रा भी कम पायी गयी। ME_m प्रति 100 कि.ग्रा. शारीरिक भार के आधार पर कटडो का दो समूह में बाटा गया। अधिक ME_m (3.92Mcal) एवं निम्न ME_m (3.04Mcal)समूह। औसत शुष्क पदार्थ सेवन (ग्रा./कि.ग्रा.शरीर भार) की मात्रा पशु आहार परीक्षण अवधि के दौरान निम्न ME_m समूह में कम (2642.98 ग्राम) थी यद्यपि अधिक समूह में यह (2788.28 ग्राम) थी। औसत प्रारम्भिक शरीरिक भार, अंतिम भार औसम दैनिक वृद्धि एवं आहार रूपांतरण अनुपात में दोनो समूह में कोई बदलाव नही पाया गया। फीड रूपांतरण अनुपात कम दोनों समूहों में समान पाया गया। पूरे परीक्षण के दौरान कम ME_m जानवरों ने उच्च ME_m पशुओं से 5.34 प्रतिशत कम भोजन खाया, यद्यपि दोनो समूह में वृद्धि दर समान रही। ME उपयोग दक्षता भी दोनो समूह में समान रही। ME आवश्यकता में व्यक्तिगत भिन्नता एवं अवशिष्ट खाने की मात्रा कटडों के चयन के लिए एवं भैंस उत्पादन प्रणालियों को सुधार लाने तथा उसको लाभप्रद बनाने का अवसर प्रदान करता है।

1. INTRODUCTION

Role of buffaloes as meat and milk animal is growing throughout the world. Feeding costs about 60-70% of input of buffaloes rearing. Thus, balance feeding is of paramount importance. Cost-effective production of quality meat and milk from buffalo depends upon energy and protein utilization. Improvement in growing and fattening performance of buffalo male calves could be achieved through nutritional and management manipulations. Singh *et al.* (2003) reported that nutrient digestibility and nitrogen balance were higher in buffalo calves when compared with cross-bred cattle. Udeybir *et al.* (2000) reported that dry matter intake is higher in growing cattle than in growing buffaloes but buffalo calves utilized dry matter, energy, and protein more efficiently for growth than cattle calves.

Since feed resources in India are limited, their efficient use is essential. Any wastage of higher intake will also have environmental consequences. Potential physiological mechanisms by which variation in efficiency of nutrient use may occur were identified, being those associated with intake of feed, digestion of feed, metabolism (anabolism and catabolism associated with and including variation in body composition), activity, and thermoregulation (Herd *et al.*, 2004). Selection for greater feed efficiency could potentially result in a reduction of 9 to 10% in maintenance costs for the cow herd, a 10 to 12% reduction in feed intake, a reduction in methane emissions by 25 to 30% (Nkrumah *et al.*, 2006; Hegarty *et al.*, 2007), and a reduction in manure production by 15 to 20% without affecting average daily gain (ADG) or slaughter weight of the animal (Basarab *et al.*, 2003).

Shahzad *et al.* (2011) investigated the performance response of different dietary protein and energy levels of Nili-Ravi buffalo calves. The findings revealed that 12–15-month-old buffalo calves performed adequately well when fed on diets containing 12.2% CP and 2.10 ME/kg. The efficiency of energy and protein utilization in cattle and buffalo is a determinant of the profitability of milk and meat production. Protein and energy are the two most important constituents of animal diets having vital role in their growth, production, and reproduction. There is individual animal variation in requirements for energy and protein, their utilization, as

well as in feed intake. Feed intake and its utilization by the animal involve a complex of biological processes and pathways, and interactions with the environment. In addition, feed intake is highly correlated with body size and level of production. This lays the basis for improving the efficiency of energy and protein utilization in livestock species. Considerable variation in feed intake, independent of size and growth rate, exists in animals and this trait is defined in terms of residual feed intake (RFI).

Residual feed intake (RFI), as first defined by Koch *et al.* (1963), is expressed as the difference between actual feed intake and the feed an animal is expected to consume based on its body size and growth rate. Thus, RFI a measure of the variation of feed intake beyond that which is needed for maintenance and growth requirements (Archer *et al.*, 1999). Residual feed intake is moderately heritable and phenotypically independent of growth rate and BW in growing cattle; however, RFI has been shown to be genetically independent of ADG, but in some cases weakly correlated with BW (Herd and Bishop, 2000; Arthur *et al.*, 2001a, 2001c).

Cattle identified as having low RFI have lower feed intakes and FCR when compared to cattle identified as having high RFI (Herd *et al.*, 2002; Basarab *et al.*, 2003). Similarly, cattle divergently selected for post-weaning RFI have demonstrated direct selection responses equating to substantial differences in feed intake between selection lines (Arthur *et al.*, 2001b; Richardson *et al.*, 1998) with no changes in body weight or growth rates observed. Although negative consequences of selection for RFI are uncertain, cattle selected for low RFI have shown small associations with a reduction in carcass fat content (Richardson *et al.*, 2001b).

Thus, the present study is proposed to assessment of feed utilization in buffalo calves to investigate the variation in residual feed intake with the following objectives:

- 1) Evaluation of feeds for chemical composition and *in vitro* digestibility.
- 2) To find out residual feed intake in growing Murrah buffalo calves.

2. REVIEW OF LITERATURE

Accurate prediction of feed nutritive value is of paramount importance, since the feeding cost remains as high as 60 -70 % of the total inputs. Cost-effective production of quality meat and milk from buffalo depends upon energy and protein utilization. Improvement in growing and fattening performance of buffalo male calves could be achieved through nutritional and management manipulations. Singh *et al.* (2003) reported that nutrient digestibility and nitrogen balance were higher in buffalo calves when compared with cross-bred cattle. Udeybir *et al.* (2000) reported that dry matter intake is higher in growing cattle than in growing buffaloes but buffalo calves utilized dry matter, energy, and protein more efficiently for growth than cattle calves.

Improved nutrition is the most important and most feasible way to increase animal productivity. The development of accurate feed composition information for the tropics that can be used to develop accurate feeding recommendations is extremely important for cattle and buffalo production that optimize use of available forages. The study “assessment of feed utilization in buffalo calves to investigate the variation in residual feed intake” has been presented below:

2.1 Proximate analysis

The estimation of feed and forage nutritive value and performance of animals fed these stuffs needs correct accounting for factors that affect animal requirements and feed utilization. Much of the existing information we have about the composition of food is based on a system of analysis described as the proximate analysis system of foods. According to this method the nutrients present in a sample or feed are analyzed and expressed in terms of 6 broad fundamental groups or principles (empirical categories), namely water, ether extract (EE), crude fiber (CF), crude protein (CP), total ash (TA) and nitrogen free extract (NFE).

2.1.1 Proximate constituents of feedstuffs

The proximate system for analysis of feedstuffs has changed little in the last 150 years (Van Soest, 1982). The energy and protein yielding components of the dry matter

are described by ether extract (EE), crude protein (CP), crude fiber (CF), and nitrogen free extracts (NFE). The division of the carbohydrate fractions into NFE and CF was designed to reflect digestible and indigestible carbohydrate respectively. While this division may be a reasonable empirical approximation to the truth for cereals and other concentrate feeds given to simple stomach species, it has long been accepted that it is not true for ruminants. Proximate analysis system involves chemical techniques designed to differentiate nutritional components to non-nutritive materials. The components of the proximate analysis are dry matter (DM), crude protein (CP), ether extract (EE), total ash (TA) and nitrogen free extract (NFE). Dry matter (DM) determination involves drying the feed sample in a drying oven to constant weight. Crude protein (CP) is defined as the nitrogen content of the feed multiplied by a factor of 6.25. The fat contents of feeds are determined by extracting a feed sample with petroleum ether is known as ether extract (EE). Ash is total mineral content of feedstuffs is determined by igniting the feed sample in a muffle furnace at 550-600⁰C for 2- 2.5 hours.

Bhar *et al.* (1999) evaluated CP, EE and total ash of green maize (9.02, 9.44 and 1.22, respectively) and wheat straw (1.57, 3.29, 8.58, respectively). These values of natural grass (11.7, 2.68 and 13.45) and rice bran (15.74, 14.74 and 10.9) were observed by Pal *et al.* (1998). Balaraman (1995) estimated CP, EE and ash of hybrid Napier grass (13.5, 6.4 and 11.1%, respectively). Khandekar *et al.* (1997) estimated CP, EE and ash % as 4.25, 2.04 and 8.16 in wheat straw and 14.98, 2.81 and 2.54 in barley respectively. Concentration of CP, EE and ash of cowpea were 20.7, 3.8 and 16.1% respectively (Gupta *et al.*, 1989), while value of these nutrients reported by Mangat Ram and Gupta (1989) were 17.22, 3.20 and 10.12 in cowpea 15.20, 3.50 and 11.89% in berseem respectively. Dutta and Singh (1994) reported CP and ash % as (43.05 and 9.10), (35.76 and 9.60) and (30.80 and 5.80) in GNC, mustard cake and CSC, respectively. The values of CP, EE and ash contents of wheat straw and sorghum green were 3.5, 1.2, 11.7 and 7.4, 34.9, 1.5, respectively (Punia and Dahiya 1998). Radhakrishna *et al.* (2002) observed that gram churi had 16.45, 3.05 and 7.86% CP, EE and total ash content respectively. Sudhakar *et al.* (2002) reported that CP and EE of sorghum straw (3.72 and 1.29) were higher than rice straw (2.78 and 1.19) while total ash was comparable. Das and Singh (1999) reported CP,

EE and ash content of berseem as 17.29, 2.33, and 13.92 % respectively. CP, EE and TA for guinea grass were 7.94, 2.75 and 7.55% respectively (Kumar and Garg, 1998). The proximate composition in wheat straw and wheat bran were evaluated by Maity *et al.* (1999). CP (12.74%), EE (3.70%) was highest in wheat bran. Batajoo *et al.* (1998) estimated CP value of soybean meal (62.9%), barley (60.0%), soybean hulls (58.2%) and distillers dried grains (39.6%). Mondal *et al.* (2008) determined CP content (%) of some common Indian feedstuffs enlisted as mustard cake (33.18), groundnut cake (43.19), barley grain (10.25), wheat bran (13.56), wheat (8.94), de-oiled coconut cake (25.5), guar churi (55.12), soybean meal (54.81), maize grain (12.56), cotton seed cake (29.19) and maize gluten meal (70.31). Pugalenthi *et al.* (2004) evaluated CP (31.08%) content of *Tamarindus indica*.

2.1.2 Cell wall constituents

In recent years the proximate analysis has been severely criticized by many nutritionists as being archaic and imprecise, and in the majority of laboratories it is partially replaced by other analytical procedures. Most criticism of proximate analysis system has been focused on the crude fiber, ash and nitrogen free extractives fractions. Inadequacies in the nitrogen free extractives fractions have been addressed by the development of methods to quantify the non-structural carbohydrates, which are mainly starches and sugars.

Van Soest developed alternative procedures for fiber. The neutral detergent fiber (NDF), which is the residue after extraction with boiling neutral detergent solution of sodium lauryl sulphate, ethylene diamine tetra acetic acid (EDTA), disodium hydrogen phosphate and borax consists mainly of lignin, cellulose and hemicellulose and can be regarded as a measure of the plant cell wall material. The term non-structural carbohydrate (NSC) is sometimes used for the fraction obtained by subtracting the sum of the amounts (g/100 g) of CP, EE, and NDF from 100. The acid detergent fiber (ADF) is the residue obtained after refluxing the sample with cetyltrimethyl-ammonium bromide (CTAB) solution in 1N sulphuric acid and represent the crude lignin and cellulose fractions of plant material but also includes silica. In UK the ADF method has been modified slightly, the

duration of boiling increased (2n) and acid strength being decreased (1%). The term modified acid detergent fiber (MADF) is used to describe this determination. The acid detergent lignin determination involves the preparation of acid detergent fiber as the preparatory step. The ADF is treated with 72 % sulphuric acid, which dissolves cellulose.

Table 2.1.2 Classification of forage fractions using the detergent methods of Van Soest (1963)

Fraction	Components
Cell contents (soluble neutral detergent)	Lipids
	Sugars, organic acids and water soluble matter
	Starch
	Non-protein N
	Soluble protein
Cell wall constituents(fiber insoluble in neutral detergent)	Cellulose, hemicellulose, pectin, lignin, fiber bound nitrogen/protein.
Soluble in acid detergent	Hemicelluloses Fiber bound protein
Acid detergent fiber Acid detergent lignin	Cellulose Lignin Lignified N Silica
Acid insoluble Ash	Silica etc.

Singh *et al.* (2000) studied eighteen genotypes of hedge lucerne for cell wall constituents. The cell wall content (NDF, ADF and cellulose) varied significantly ($P < 0.05$) in evaluated germplasm. The mean of concentration of NDF, ADF, cellulose, hemicellulose and lignin was 42.31, 34.03, 21.17, 11.64 and 1.37 respectively. Singh *et al.* (2003) reported cell wall concentration of 25 sorghum genotypes. Evaluated lines

significantly varied ($P < 0.05$) in NDF, ADF, cellulose and hemicellulose concentration. Average contents of these nutrients observed were 57.09, 48.18, 39.12 and 8.91%, respectively. Mean lignin concentration of different lines was 9.04 % although it varied from 7.56 to 10.37%. Mahanta *et al.* (1999) evaluated diploid and tetraploid line of berseem for fiber fractions and observed that NDF, ADF, hemicellulose, cellulose and ADL ranged from 35.06-41.84, 21.75-27.94, 12.98-16.29, 15.53-20.67 and 4.72-8.14% respectively among 10 lines of diploids, while average concentration of these nutrients were 38.88, 24.01, 14.87, 14.87, 19.07 and 4.96% respectively among 10 lines of tetraploid. Singh *et al.* (2001) estimated twenty-two genotypes of *Stylosanthes* for cell wall composition (NDF, ADF, cellulose and hemicellulose) and found that fiber fractions varied significantly ($P < 0.05$) among the evaluated lines. The average concentration of these cell wall fractions were 59.02, 46.12, 37.41 and 12.90% respectively. Lignin concentration varied from 5.63 to 11.68% in the different genotypes.

Khandaker *et al.* (1997) reported NDF, ADF, lignin and hemicellulose of wheat straw and barley as 84.28, 59.27, 7.80, 25.01 and 23.4, 8.11, 1.13, 15.23 respectively. Mishra *et al.* (1999) evaluated cell wall constituent of various feedstuffs and observed that NDF was 52.98, 80.59, ADF was 22.32, 54.87 and ADL was 5.27, 7.40 in DORB and ammonia treated wheat straw, respectively. Ramteke *et al.* (2002) reported decrease in CP and NFE and increase in CF, NDF, ADF, hemicellulose and cellulose contents in diseased as compared to healthy sorghum stover. NDF, ADF, hemicellulose, cellulose and lignin in healthy sorghum were 66.96, 47.59, 19.37, 35.03 and 10.17%, respectively. These values in berseem were 47.87, 38.92, 14.56, 26.40 and 8.88% (Tewatia and Khirwar 2002a) against 47.60, 34.00, 13.60, 22.60 and 7.20% recorded by Gupta *et al.* (2002). However Jaiswal *et al.* (1988) reported 58.5, 41.6 and 16.9% NDF, ADF and hemicellulose respectively in berseem. Jaiswal *et al.* (1988) analyzed untreated paddy straw, ammonia treated paddy straw, berseem, GNC, fish meal, CSC, Subabool leaf meal and crushed maize. They observed that CP and ADF content in ammoniated paddy straw were higher than untreated paddy straw, while NDF, hemicellulose content were lower in former than later. Among the protein sources CP, NDF, ADF and hemicellulose (%) varied from 2.79-40.3, 18.6-33.0, 12.7-24.6, and 0.4-18.8 respectively.

Pachauri *et al.* (1997) analyzed two improved varieties of berseem. They reported that NDF, ADF, hemicellulose, cellulose and ADL in the berseem varieties ranged from 52.28 - 56.55, 33.10 - 36.05, 19.11-20.50, 24.56 -2 6.66 and 5.20 - 8.65% respectively. They also analyzed three varieties of Anjan grass for fiber components. Across the varieties NDF and ADF varied from 76.06-82.94 and 52.16-52.62, respectively. NDF, ADF, CP in five different cuts of berseem revealed that maturity increased NDF, ADF, and decreased CP content (Chaturvedi *et al.*, 2000). Gupta *et al.* (1975) estimated cell wall constituents of berseem at different cuttings. The cell wall components (except hemicellulose) increased during subsequent cuts. NDF content in plants increased from 37.18 to 41.13, ADF from 26.26 to 32.89, lignin from 7.31 to 9.97 and cellulose from 18.19 to 22.05% while hemi cellulose varied from 8.24 in 4th cutting to 10.92 in over subsequent cutting. The CP, NDF, ADF, hemicelluloses, lignin and cellulose contents of berseem, cowpea, guar at different stage of their growth ranged from 13.86-25.66, 31.92-55.54, 24.30 -46.65, 5.07-13.24, 8.23-14.31 and 14.45-36.75% respectively (Gupta and Pradhan 1975a). Lucerne had the lowest amount of cell wall constituents, while cowpea was richest.

The average content of NDF, ADF, cellulose and lignin (%) in maize germplasm was 56.32, 28.57, 29.69 and 10.28, respectively (Singh and Katiyar 1999). NDF, ADF, cellulose, hemicellulose and lignin (%) in green maize, CSC, maize grain and GNC, were 60.9, 41.5, 32.0, 19.4 and 6.8; 38.1, 29.9, 20.5, 8.2 and 4.3; 19.9, 6.3, 4.0, 13.6 and 2.0; and 29.9, 17.1, 7.0, 12.8, and 3.9, respectively (Sharma and Verma 1993). The concentrations of fiber fractions namely NDF, ADF and hemicellulose were 62.00, 41.85 and 20.15 in untreated rice straw, 27.60, 11.45 and 16.25 in GNC and 32.15, 21.70, and 10.45 in CSC respectively (Singh *et al.*, 1995). Dutta and Singh (1994) observed a number of oil cakes for NDF and ADF contents. The values of these nutrients were 22 and 12.00, 30.50 and 16.80, 40.50 and 30.20, 48.50 and 40.30% in GNC, MSC, LSC and CSC, respectively. The NDF, ADF, hemicellulose, cellulose and ADL of gram churi observed by Radha Krishna *et al.* (2000) were 42.79, 31.11, 11.68, 26.32 and 4.79 % respectively.

Singh and Srinivasa (1998) estimated that NDF and ADF of grasses were higher than legume while ADL in legume was higher than grasses. In grasses NDF, ADF and

ADL ranged from 77.40-79.14, 44.85-45.90 and 5.01-5.07, while these values in legumes ranged from 51.68-74.20, 31.68-43.48 and 7.31-11.20, respectively. Tewatia and Khirwar (2002b) observed the NDF, ADF, hemi cellulose, cellulose and lignin in sorghum as 71.35, 46.80, 24.55, 33.60 and 7.45 respectively. NDF (57.71) and ADF (37.08) contents of sorghum at half flowering stage were lower than dough stage (63.68 and 41.54) (Kumar *et al.*, 1994). NDF, ADF, CP and ash contents in sorghum (61.00, 33.70, 6.91 and 7.43%) were lower than in Dinanath (65.90, 44.0, 7.88 and 12.09%) grass (Kishore and Verma, 2000). Gupta and Pradhan (1975b) observed 6 legumes and 7 non-legumes for chemical constituents. They reported that mean concentration of cell wall fractions was lower in legumes than grasses. The cell wall in the legume had considerably less hemicellulose but was more lignified. Legume had about 2-3 times more CP content than grasses.

Gupta and Pradhan (1975b) reported chemical composition of seven non-legumes forages at three stages of their maturity. The NDF ranged from 47.24 to 71.69 %, ADF from 29.96 to 48.66%, hemicellulose from 22.28 to 34.88 %, lignin from 4.22 to 9.33 %, and cellulose from 22.28 to 34.88 % respectively. Among non-legume forages teosinte contained highest and oats lowest amount of cell wall constituent. Oats and sorghum were comparatively richer in CP than other non-legume forages whereas pearl millet contained the highest amount of cellulose. Yadav and Bhadoria (2001) found that NDF, ADF, lignin and hemicellulose varied from 35.07-43.87, 26.81-39.02, 9.05 - 14.75 and 4.85-8.17% respectively in shrubs and tree leaves.

Singh *et al.* (1999) observed that CP content in tree leaves were highest in rainy season (16.94%) followed by summer (15.02%) and winter (13.63%) season. NDF, ADF and ADL of different seasons varied between 16.01-42.66, 14.65-32.86 and 8.36-11.59, respectively. The amount of NDF, ADF, hemicellulose, cellulose and lignin (%) of cowpea was 57.53, 44.5, 12.94, 35.72 and 10.86 respectively (Tekleab and Agrawal 2000). Shakil *et al.* (1999) evaluated cell wall constituents in legumes, grasses and fodder tree leaves. The NDF and ADF (%) were highest in range grasses (68.39-79.74 and 29.42-49.11) followed by legumes (47.62-69.48 and 29.42-46.93) and lowest in fodder tree leaves (22.76-44.0 and 19.38 and 30.43), however lignin contents in grasses was lower than legumes and tree leaves. Cellulose and hemicellulose were higher in grasses than

legumes and tree leaves. Singh *et al.* (1995) evaluated tree leaves and range legumes for chemical constituents. They found that tree leaves and range legumes were comparable in their CP content. The legumes were comparable in their CP content except that of *Stylosanthes hamata* which had lower CP than others. Tree leaves were lower in NDF, ADF and higher in lignin than range legumes.

2.2. *In vitro* digestibility and rumen fermentation parameters

The digestibility of feeds for ruminants can be measured quite accurately in the laboratory by treating them with rumen liquor. Generally, digestibility determined *in vitro* is slightly lower than that determined *in vivo* and corrective equations are required to relate one measure to the other (Getachew *et al.*, 2002). The quantity of gas produced during the *in vitro* incubation of a substrate is closely related to its digestibility and consequently to its energetic value. The gas production data provide a prediction of the effective organic matter degradation. A better correlation was achieved when the equation includes CP, crude fat and ash content (Menke and Steingass, 1988).

Srinivas and Gupta (1994) concluded that gas production was influenced by the CP, OM as well as cell wall constituents of the ingredients. Total gas production (ml/g DM) of mustard, groundnut, cottonseed cake was 208.0, 208.6 and 179.6, respectively. The relative proportion of concentrate and forage in the diet will have a considerable influence on *in vitro* gas production. The difference in donor animal's diet and interaction with type of feed incubated reflected variation in total gas volume produced *in vitro* (Getachew *et al.*, 2002; Nagadi *et al.*, 2000). The diet of donor animal exerted considerable influence on bacterial concentration and so influenced *in vitro* gas production. Since different feeds can affect the relative proportion of microbes in the rumen, this may influence the extent of fermentation of feeds (Nagadi *et al.*, 2000).

Kiran and Krishnamoorthy (2007) reported that total gas production (ml/ g DM) at 24 hrs incubation of cottonseed, groundnut cake, mustard cake, maize grain, wheat bran and paddy straw were 190.5, 211.5, 204.5, 377.0, 286.5 and 145.2, respectively. Ayyappan and Tomar (2006) reported that the total gas production (ml/ 300mg DM) from

wheat straw, paddy straw, maize grain, and mustard cake and wheat bran at 48 hrs incubation was 15.0, 14.3, 73.0, 38.7 and 43.3, respectively.

Sallam *et al.* (2005) used *in vitro* gas production technique to evaluate some feed resources like rice straw, tropical shrubs (*Artiplex semibacata*, *Artiplex halimus*, *Leucaena*), artichoke leaves and stems in comparison with berseem hay. The maximum gas volume was highest for artichoke stems followed by artichoke leaves, bereem hay, rice straw, *Atriplex semibacata* and *Leucaena* and lowest for *Atriplex halimus*. The maximum rate of gas production was highest for both artichoke stems and leaves and berseem hay and lowest for rice straw. The organic matter digestibility values ranged from 41.48 - 67.57% with the highest value in artichoke stems and the lowest value in rice straw. The leaves or stems of artichoke showed the highest ($P < 0.05$) values for ME, NE and OMD than all of the other roughages used in this study.

Ali *et al.* (2007) compared *in vitro* gas production and OMD of widely used legume hays. Total gas production after 96 hrs incubation ranged between 61.67 and 76.00 ml/0.2 g of substrate. At 24, 72 and 96 hrs incubation the total gas production for common vetch was significantly ($p < 0.01$) higher than that of other legume hays. The OMD of legume hays ranged from 61.30 to 75.54%. *In vitro* OMD of oats ranged from 62.7 to 65.5% (Getnet Assefa *et al.*, 2001). The dry matter and organic matter digestibility values ranged from 74.40-73.33% and the highest value was that of artichoke stems and the lowest value was that of *L. leucocephala* (44.7, 40.71%) (Gregorio, 2005).

The DMD was 90.65% in wheat and 66.61% in maize fodder DMD was statistically similar in lucerne and berseem; fenugreek and white clover; berseem and leuceana leaves. Kamble *et al.* (2010) reported that the highest DMD among the energy, protein and by-products were observed in white sorghum (94.67%) soybean cake (93.72%) and rice bran (86.34%) and oats (77.36%), cotton seed cake (65.05%), rice polish (60.89%). There was no significant difference observed in the gas production on DM basis, whereas on % digestible DM basis differed significantly (Datt *et al.*, 2009; Kamble *et al.*, 2010; Gupta *et al.*, 2011).

2.3 Residual feed intake

2.3.1 Definition of RFI

Residual feed intake is defined as the difference between the actual feed intake of an animal and its expected feed requirements for maintenance and growth (Basarab *et al.*, 2003). RFI is phenotypically independent of the production traits (Archer *et al.*, 1999). A phenotypic linear regression equation, computed using intake and performance data from a contemporary set of animals, was used to determine an animal's expected feed intake. The RFI is an individual measurement; therefore, animals must be fed individually or in groups using electronic devices that measure each animal's intake (Sainz and Paulino, 2004).

The animal's actual feed intake against (more or less) its expected feed intake is referred to as residual feed intake (RFI). Efficient animals are those that consume less feed than expected based on their size and growth rate, thus efficient animals will have negative RFI. Conversely, inefficient animals will consume more feed than expected and have positive RFI.

Residual feed intake has been shown to be phenotypically independent of ADG and BW in growing cattle (Arthur *et al.*, 2001c). However, Kennedy *et al.* (1993) found that when RFI is calculated by phenotypic regression of production on feed intake, the resulting measure of efficiency is not necessarily genetically independent of production. Selection responses to RFI based on genotypic regression would be expected to be independent of production, and be more likely to reflect genetic differences in inherent relationships between feed intake and production. In young beef cattle, feed efficiency is usually evaluated in relation to growth, and some of the most common measures used, such as partial efficiency of growth, feed conversion ratio (FCR) and residual feed intake (Arthur *et al.*, 2001b).

2.3.2 Calculation of RFI

Calculation of RFI begins with an individual record of each animal taken over a long term trial, normally lasting between 70 and 84 days. Animals may be housed individually where accurate daily measurements of the amount of food offered and the amount eaten, and average daily gain and body weight are monitored. More recently, to

determine the amount of feed an animal is expected to consume, researchers have taken the phenotypic regression approach (Archer *et al.*, 1997; Arthur *et al.*, 2001; Crews *et al.*, 2003). Intake is adjusted for level of production by regressing intake on average daily gain (ADG) and mid-test body weight ($BW^{0.75}$). RFI should be phenotypically independent of growth and the weight traits used in the regression procedure, because variation from those traits has been removed (Herring and Bertrand, 2002). The statistical model becomes:

$$Y = \beta_0 + \beta_1 (ADG) + \beta_2 (WT)^{0.75} + \text{residual error}$$

Where Y is expected DMI, β_0 is the regression intercept, β_1 is the partial regression of daily intake on average daily gain (ADG), β_2 is the partial regression of daily intake on mid-test body weight raised to the 0.75 power ($WT^{0.75}$) and RFI is the residual. Mid-test body weight raised to the power 0.75 is used instead of actual weight to balance the difference in maintenance requirement of cattle caused by differences in mature size (BIF, 1986).

The properties of RFI can be defined using standard statistical procedures. One central feature is the distributional property ($RFI \sim N(0, \beta_2 RFI)$), showing RFI has a mean of zero (Searle, 1982). The partial regressions in the estimation model are independent of RFI. Because the estimation method causes RFI to be independent of production, the variation in RFI is probably due to metabolic processes.

The expected intake of the animal is determined by the estimation equation. Once determined, RFI is calculated by subtracting expected feed intake from the observed feed intake. Efficient animals, with negative RFI values, have daily feed intakes less than that predicted for their level of production and body weight. Conversely, an animal with a positive RFI daily feed intake trait is greater than expected based on growth and body weight.

2.4. Necessity for selection of animals as per RFI

The limitation of using feed efficiency to measure performance and the lack of economic improvements in the breeding herd has led to a shift in focus in recent times in using a concept known as residual feed intake (RFI) or net feed efficiency (NFE) in

identifying efficient animals. As indicated previously, feed efficiency (FE) is highly correlated with growth rate and may be confused with maturity patterns, body size, composition of gain and appetite of cattle (genetic correlations of -61 to -95%) (Mrode *et al.*, 1990; Fan *et al.*, 1995; Liu *et al.*, 1998, Arthur *et al.*, 2001). On the other hand, RFI is the difference between the metabolizable energy intake (MEI) and metabolizable energy required for maintenance and gain (MER) (Fan *et al.*, 1995; Liu *et al.*, 1998). Mathematically, RFI or residual metabolizable feed consumption (RMFC) in kg d⁻¹ is calculated as $RFI = MEI - MER$. RFI is independent of growth and maturity patterns, and is a more sensitive and precise measure of feed quality based on energy intake and energy requirements (Fan *et al.*, 1995; Liu *et al.*, 1998). Practically, RFI estimates efficiency of use of feed consumed by subtracting observed DMI of an individual from DMI predicted by an equation developed from the relationship between DMI, daily gain and metabolic mean weight across fed contemporaries (Basarab *et al.* 2003). When $MEI = MER$ the $RFI = 0$ and means that the energy requirements of the animal are completely met. A positive RFI means that $MEI >$ than MER and means the animal's energy intake exceeds its requirement for maintenance and growth. A negative RFI means $MEI <$ MER and that the animal either requires less energy than what is estimated or is eating less to produce the same weight gain. The concept of residual feed intake has been used to identify efficient test station bulls and a genetic basis for RFI has been identified with the heritability of the trait estimated to be between 0.16 and 0.46 (Fan *et al.*, 1995; Liu *et al.*, 1998). This suggests that RFI can be improved through selection of traits like average daily gain. The concept of RFI to measure efficiency of beef production needs to receive more attention as it has the potential to recognize genotypes or individual animals whose requirement equals ME, ME intake is greater than requirement and those whose ME intake is less than requirement.

2.4.1 Feed efficiency in growing cattle

Differences in feed efficiency have the ability to impact the profitability of an integrated production system, which has led to the universal use of FCR by livestock producers to select for more efficient poultry, swine and cattle. It has been widely

demonstrated that FCR is moderately heritable (Table 2.4.1.1).

Table 2.4.1.1 Summary of studies reporting heritability estimates of residual feed intake (RFI) and feed conversion ratio (FCR) in growing calves

	RFI	FCR	
Breed	Heritability	Heritability	Reference
British	0.28 ± .11	0.35 ± .24	Koch <i>et al.</i> , 1963
Swedish Red and White	0.27 ± .33	0.32 ± .02	Berlin & Brannang, 1982
Holstein and Brown Swiss	0.28 ± .11	ND	Jennen <i>et al.</i> , 1992
Beef cattle	ND	0.17 ± .02	Koots <i>et al.</i> , 1994 ^b
British	0.46 ± .07	ND	Archer <i>et al.</i> , 1998
Hereford	0.16 ± .04	0.17 ± .09	Herd and Bishop, 2000
Angus	0.39 ± .03	0.29 ± .04	Arthur <i>et al.</i> , 2001 ^a
Charolais	0.39 ± .04	0.46 ± .04	Arthur <i>et al.</i> , 2001 ^c

^aND = not determined

^b Weight averaged for 23 studies

Heritability estimates for FCR in growing cattle ranged from 0.17 ± 0.09 (Herd and Bishop, 2000) to 0.46 ± 0.04 (Arthur *et al.*, 2001c). It was well documented that FCR is both phenotypically and genetically correlated with production aspects among livestock species. Bishop *et al.* (1991) found that FCR was negatively correlated with ADG ($r = -0.33$) and back fat ($r = -0.33$) and positively correlated with feed intake ($r = 0.49$) and BW ($r = 0.15$) suggesting that progeny with lower (more desirable) feed conversion ratios were fatter, gained faster and yielded carcasses with higher quality grades and less desirable yield grades. In a similar study, Brelin and Brannang (1982) reported negative phenotypic correlations between feed efficiency (ratio of feed energy to live weight gain) and carcass muscle content ($r = -0.45$) and daily gain ($r = -0.55$), but only a weak correlation with carcass fat content ($r = 0.06$). Arthur *et al.* (2001a) reported strong genetic and phenotypic correlations between FCR and ADG ($r_g = -0.62$; $r_p = -0.74$), but weak correlations between

FCR and back fat ($r_g = 0.03$; $r_p = 0.08$) and longissimus muscle area ($r_g = -0.12$ $r_p = 0.03$). These studies demonstrated that strong genetic and phenotypic correlations exist between FCR and growth rate and stage of maturity. A negative correlation between feed efficiency and fat may exist in younger growing cattle, and a positive correlation may exist in older cattle when fat deposition is considerable (Brelvi and Brannang, 1982).

The strong genetic correlation between FCR and growth (Table 2.4.1.2) suggested that selection for growth would produce correlated improvements in FCR, thus reducing the justification for measuring feed intake in order to improve feed efficiency. However, it is well-known from the literature that FCR increases as animals get older (Hansson *et al.*, 1967) which is explained by the fact that, as animals mature, maintenance energy requirements increase as a proportion of the feed consumed and the energy content of gain increases, due to greater fat deposition.

Table 2.4.1.2. Genetic and phenotypic correlations between performance and feed efficiency traits with measures of efficiency in growing steers and bulls

Traits	Arthur <i>et al</i> 2001a		Arthur <i>et al</i> 2001c		Herb & Bishop 2000		Archer <i>et al</i> 1998		Jensen <i>et al</i> 1992	
	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g
Residual feed intake:										
ADG	-0.06	-0.04	0.01	-0.01	-0.1	0.09	0.02	0.02	-0.04	0.42
BW ^b	0.02	-0.06	0.03	0.32	-0.01	0.15	-0.03	-0.25	ND	ND
Feed intake	0.72	0.69	0.60	0.79	0.7	0.64	0.56	ND	0.09	0.43
FCR	0.53	0.60	0.57	0.85	0.61	0.70	ND	ND	ND	ND
Feed conversion ratio:										
ADG	-0.74	-0.62	-0.46	ND ^c	ND	ND	ND	ND	ND	ND
BW ^b	0.16	-0.01	-0.08	0.24	ND	ND	ND	ND	ND	ND
Feed intake	0.23	0.31	0.48	0.64	ND	ND	ND	ND	ND	ND

r_g = genetics correlation and r_p = phenotypic correlation

^b correlation reported in literature of RFI and FCR with BW are approximately yearling

with the exception metabolic mid-test body weight report by Arthur *et al* (2001a)

^cND = Not determined

2.4.2. Variation in maintenance energy requirements and feed efficiency

Variation in energy requirements for maintenance appeared greater than variation in energy requirements for growth, gestation or lactation (Ferrell and Jenkins, 1985). The method used to improve feed efficiency was selection for improved FCR. There are a number of studies demonstrating that maintenance requirements and feed conversion ratio differed between breeds of cattle. Ferrell and Jenkins (1984) found that Angus and Herford cattle had lower maintenance requirements than Simmental and Charolais cows. Frisch and Vercoe (1984) found that 15-month-old Herford x Shorthorn bulls required approximately 20% more feed to maintain the same body weight as Brahman bulls. Differences in energy required for maintenance may be associated with differences among animals in their level of production (Ferrell and Jenkins, 1985; Frisch and Vercoe, 1984; Taylor *et al.*, 1986) or the proportion of metabolically highly active organs (Ferrell and Jenkins, 1985). Hotovy *et al.* (1991) suggested that there is a genetic component to variation in fasting heat production and maintenance energy requirements in beef cattle. Protein turnover is the balance of relative rates of protein synthesis and protein degradation. Increased protein turnover results in greater energy required for re-synthesis of the peptide bond, thus, decreased efficiency in conversion of nutrients to muscle and fat.

2.4.3. Different measures of feed efficiency in buffaloes

Traditionally, attempts to improve genetic potential for feed efficiency in cattle and buffaloes have been accomplished by selection for feed conversion ratio (FCR). Feed conversion ratio is generally depicted as units of feed required for one unit of gain, or as its inverse, units of gain for every one unit of feed consumed. Feed conversion ratio is a gross measure of efficiency in that it does not account for feed requirements needed for maintenance and growth. The strong genetic correlation between FCR and growth suggests that selection for growth will produce correlated improvements in FCR, thus reducing the justification for measuring feed intake in order to improve feed efficiency. However, it is established that FCR increases as animals get older (Hansson *et al.*, 1967),

which is explained by the fact that, as animals mature, maintenance energy requirements increase as a proportion of the feed consumed and the energy content of gain increases, due to greater fat deposition. For FCR, a lower numerical value would identify a more efficient animal and for gain: feed, a larger number is indicative of greater efficiency. Many times genotypes with improved FCR will also have increased ADG, and therefore tend to have heavier mature cow weights, consequently requiring more feed inputs (Archer *et al.*, 1999). While FCR is moderately heritable (Arthur *et al.*, 2001a; Arthur *et al.*, 2001b, Herring and Bertrand, 2002), it often has unfavorable genetic and phenotypic correlations associated with it that would offset any profitability a producer could gain from improvements in FCR, such as increases in mature BW and DMI. Even so, results can be misleading, because these ratios are closely correlated to the intake and rate of gain of the animal (Carstens *et al.*, 2004). So, two animals might have similar gain and still be very different in their feed intakes. Conversely, the same animal at different intakes would certainly have different gain: feed ratios, even though the genetics of the animal hadn't changed. Therefore, gain: feed has never taken off as a criterion for genetic selection. Therefore, selection for FCR will likely increase feed costs of the breeding herd and not necessarily improves feed efficiency of integrated beef operations. Several other feed efficiency traits have also been examined viz., Kleiber ratio, partial efficiency of growth and relative growth rate. These feed efficiency traits are also related to growth rate (Arthur *et al.*, 2001b; Nkrumah *et al.*, 2004) suggesting that selection for improved efficiency would result in increased cow mature size and feed requirements for maintenance of the cow herd.

2.4.4 Relationship between RFI and feed efficiency

Basarab *et al.* (2003) reported that the correlation coefficient between FE and ADG and between RFI and ADG were -0.61 and -0.95, respectively, indicating that RFI may be an indicator of the animal's maintenance requirements rather than growth. There are several possible reasons for these results as follows. Differences in maintenance requirements of the efficient and inefficient steers whereas steers with low ME intakes tended to have low RFI ($r=0.51$, $P<0.0001$). Ferrell and Jenkins (1998) reported that the efficiency of ME use for retained energy was not constant, but decreased as ME intake

increased. Partial non-linearity in the relationship of retained energy on ME intake is due to a depression in metabolizability of the diet at high levels of intake, higher maintenance cost due to heavier organ weights of stomach complex, intestines, heart, lung, kidney and spleen, and/or and heat increment of feeding of certain steers all leading to higher heat production for the inefficient steers.

2.5. Digestibility

It is known from the literature that as the level of intake relative to maintenance increases the digestion of feed decreases (Oddy and Herd, 2001). Richardson *et al.* (1996) found small but significant differences in digestibility between cattle of high and low RFI. Richardson *et al.* (2001b) found that low RFI cattle were better able to digest a pelleted roughage ration and a feedlot ration when compared to high RFI cattle. The apparent decrease in digestibility for high RFI cattle could contribute up to at least 10% of the difference in ME intake (Richardson *et al.*, 2001b). However, Katle (1991) examined chickens for causal factors of variation in RFI and concluded that results for digestibility were unclear. The lack of a relationship between digestibility and RFI have been confirmed in chickens by Luiting *et al.* (1994) and in growing pigs by the Haer *et al.* (1993).

2.5.1 Feed utilization

Until recent times the efficiency of feed utilization has been quite difficult and expensive to measure and quantify compared to the measurement of growth rate. It was thus practical to measure gross feed intake and gain as measures of feed efficiency. However, Ferrell and Jenkins (1998) showed that the relationship between feed (energy) intake and gain is not linear (Figure 1). They indicated that the maximum efficiency in daily gain may occur at less than maximum energy or feed intake. Decreases in efficiency at higher metabolizable energy (ME) intake have been attributed to depression of ME of diet at higher levels of intake, higher heat increment of feeding at higher intakes and heavier visceral organ weights (Ferrell and Jenkins 1998). The factors that influence average daily gain and impact negatively on feed intake are as follows: feed is not stale or moldy, as cattle tend to

eat less when feed is not fresh, amount of concentrate is enough to meet a targeted average daily gain, protein content of the diet is adequate to meet requirements for maintenance and for the targeted rate of growth and there are no limiting factors to negate the targeted growth rate. Some researchers have attempted to use the concepts of Ferrell and Jenkins (1998) to increase efficiency of cattle production through the practice of limit feeding.

2.5.2 RFI classifications for digestibility and nutrient utilization

The variation in RFI reflects differences in digestion process, metabolic utilization of feed intake, or both in animals of similar BW and production levels (Nguyen *et al.*, 2005). It is known from the literature that as the level of intake relative to maintenance increases the digestion of feed decreases (Oddy and Herd, 2001). Differences in feed intake between animals could impact RFI status because as feed intake increases, the amount of energy needed for digestion also increases, thereby increasing the maintenance requirement. This is due to an increased mass of digestive tissues needed for digestion. As the mass of digestive tissues increase, the metabolism of these tissues may be altered, reducing digestive efficiency.

In growing cattle divergently selected for low and high RFI after one generation, digestibility estimates were examined on roughage - and grain based diets (Richardson *et al.*, 2001a). When fed roughage-based diets, low RFI bulls and heifers tended ($P < 0.10$) to have higher digestibility compared to the high RFI bulls and heifers (68.1 vs. 67.1%). Likewise, low RFI steers had higher digestibility than high RFI steers when a grain-based diet was fed (79.5% vs. 77.3%). Herd *et al.* (2002) estimated that cattle divergently selected for low RFI after two generations would produce 15% less methane than cattle divergently selected for high RFI. Reductions in methane could be a consequence of lower DMI and (or) lower rates of methane production per unit of DMI. An increase in ME can result from increases in digestibility, or reductions in urinary and gaseous (e.g. methane) energy losses. Redirecting feed fermentation products to decrease methane emission would improve efficiency of feed utilization, allowing for lower feed intake and less fecal waste.

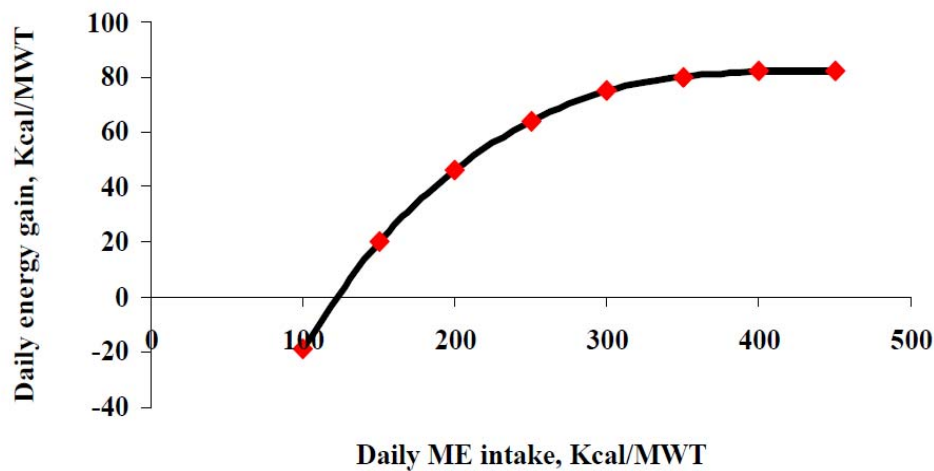


Figure 1. Relationship between energy gain and metabolizable energy intake (adapted from Ferrell and Jenkins 1998).

Channon *et al.* (2004) observed negative correlations between fecal dry matter and RFI in Angus, Shorthorn, and Hereford crossbred steers fed a 75% rolled barley diet, indicating that more efficient cattle would have higher fecal dry matter contents. The higher fecal dry matter content is an indicator of reduced hindgut fermentation and more efficient starch digestion in the rumen and small intestine. They also observed negative correlations between fecal dry matter and DMI, indicating more efficient starch digestion by cattle with lower DMI. Additionally, fecal pH can be a reflection of the site of starch digestion. The lower fecal pH reflected the higher concentration of acid resulting from more hindgut fermentation. Fecal nitrogen content was not correlated with RFI, but fecal dry matter was negatively correlated with fecal nitrogen content. Increased fecal nitrogen resulted from increased starch flow to the lower intestinal tract and microbial protein excreted in fecal material. The higher starch digestion in low RFI cattle could indicate differing rumen bacterial populations or host characteristics compared to high RFI cattle.

Differences in RFI status may also be affected by site of digestion or utilization of digestive products like differences in microbial protein synthesis between affected the supply of microbial protein to the small intestine (Herd *et al.*, 2004). Nkrumah *et al.* (2005) reported that low RFI steers produced 28% less methane compared to high RFI steers. Nkrumah *et al.* (2005) reported that RFI was ($P < 0.10$) correlated with apparent

DM digestibility ($r = -0.33$) and CP ($r = -0.34$), indicating that steers with low RFI had higher DM and CP digestibility compared to steers with high RFI. In contrast, FCR was not correlated with apparent DM or CP digestibility.

Nkrumah *et al.* (2006) found low RFI steers tended to have greater apparent digestibility of DM (high: 70.87 ± 1.97 vs. low: 75.33 ± 2.10 ; $P = 0.10$) and CP (high: 69.76 ± 2.17 vs. low: 74.70 ± 2.29 ; $P=0.09$) when compared to high RFI steers. Low RFI steers lost less methane as a percent of gross energy (GE) intake than both the medium and high RFI steers (high: 4.28 ± 0.26 vs. medium: 4.25 ± 0.35 vs. low: 3.19 ± 0.34 ; $P=0.04$) (Nkrumah *et al.*, 2006). RFI was correlated with daily fecal output ($r = 0.33$; $P < 0.10$), daily methane production ($r = 0.44$; $P < 0.05$), daily heat production ($r = 0.68$; $P < 0.001$), daily retained energy ($r = -0.67$; $P < 0.001$), apparent DM digestibility ($r = -0.33$; $P < 0.10$), and CP digestibility ($r = -0.34$; $P < 0.10$) (Nkrumah *et al.*, 2006). The relationship between energy partitioning and RFI was examined by Nkrumah *et al.* (2006). Apparent digestibility of DM and CP was 5% greater for the low RFI animals. These same animals were restricted to 2.5 x their estimated maintenance requirements while dietary energy flows were measured. There was no difference in energy intake; however DE, ME, and retained energy were all greater for the low RFI steers. Heat production, and methane energy was greater for the high RFI steers. The difference in heat production was due to differences in metabolic rate and efficiency.

2.6 Body composition and metabolism

Residual feed intake is based on energy and energy requirement and RFI is due to differences in maintenance requirements (Nkrumah *et al.*, 2006). Maintenance requirement is defined as the feed energy required for zero body components (Ferrell and Jenkins., 1985). Other way it can be defined as the ratio of body weight to feed intake at zero body weight change (Archer *et al.*, 1999). The gastrointestinal tract and liver contribute approximately 50% of the maintenance energy requirements in ruminants (Lobley, 2003), gastrointestinal tract 16 to 29% and metabolism 20 to 26% (Johson *et al.*, 1990). Therefore, studies focusing on gastrointestinal tract and live metabolism may be lead to better understanding of the genetic variation underlying RFI.

According to Nkrumah *et al* (2006), variation in feed efficiency is primarily related to differences in dietary energy losses (fecal, methane, and urinary), heat production, and energy retention. To better understand these losses, they suggested studies that are typically related to variation in the efficiency of conversion of gross energy (GE) to metabolism energy (ME).

Ferrell and Jenkins (1998) showed that differences in water, protein, and fat deposition influence efficiency and rate of body weight gain. Even though energy expense for fat is more than for protein deposition, maintenance of protein requires more energy than maintenance of fat. The deposition of the same weight of lean tissue and fat occur at different energy costs, and this variation in efficiency is primarily due to the variation in protein turnover. Protein turnover required high energy usage whereas variation in protein metabolism was associated with genetic selection for growth and other traits in domestic animals (reviewed by Oddy, 1999).

It has been observed in several other species that the rate of protein degradation is association with selection for growth leanness. In chickens (Tomas *et al.*, 1991) protein degradation rate was associated with difference in the net efficiency of protein utilization and decreased degradation rates.

Basarab *et al.* (2003) reported that approximately 4% of the variation in daily feed intake was due to difference in empty body fat, compared to 67.9 and 8.6% attributed to body weight and daily gain respectively. They further showed that the rate of deposition of fat, measured as ultrasound subcutaneous fat gain and ultrasound intramuscular fat gain, increased the variance of daily feed intake explained by regression on weight and gain alone from 78% to 80.9%.

2.7 Activity

Studies on non-ruminant animals have shown that variation in heat production (energy available for maintenance and growth) was associated with an animal's activity, and with RFI. De Haer *et al.* (1993) found positive correlation of total feeding time ($r = 0.64$) and number of visits to the feeding station ($r = 0.51$) with RFI in pig. Richardson *et al.* (2000) reported a phenotypic correlation ($r = 0.32$) between RFI and daily

pedometer count. Arthur *et al.* (2001) conducted a similar study that showed high RFI steers took 6 percent more steps than low RFI steers. High RFI steers also were assumed to spend 13% longer time in feeding stall and ruminating. The increase in distance walked and time spent standing and ruminating accounted for approximately 5% of the increase in feed energy intake by high RFI (low efficiency) group compared to the low RFI group.

2.7.1 Differences between RFI classifications for growth and weights

Most literature agrees that RFI is phenotypically uncorrelated with its indicator traits weight and ADG since RFI is calculated using a regression model. However, there may be genetic relationships. Selecting for improved i.e. lower RFI could essentially result in decreased BW and ADG (Kennedy *et al.*, 1993). Cows expressing low RFI post weaning tended to produce more body weight of calf at weaning per unit of intake compared to cows expressing high RFI post weaning (Herd *et al.*, 1998). Arthur *et al.* (1999) reported a positive correlation between post weaning RFI and RFI in four year old cows. Therefore selection for decreased RFI post weaning will produce more efficient cows that consume less feed per unit weight of calf produced. RFI was genetically independent from mature cow weight ($r_g = -0.09 \pm 0.26$) (Herd and Bishop, 2000). In beef cattle, Arthur *et al.* (2001a) found significant genetic relationships between RFI with BW (0.32 ± 0.10) and ADG (-0.10 ± 0.08). In another study by Arthur *et al.* (2001b), genetic correlations were observed between RFI and 200 day weight ($r_g = -0.45 \pm 0.17$), 400 day weight ($r_g = -0.26 \pm 0.13$), 200 day weight maternal ($r_g = 0.22 \pm 0.20$), and 400 day weight maternal ($r_g = 0.14 \pm 0.25$).

Carstens *et al.* (2002) found ADG to be similar across the RFI groups. When comparing RFI classified cattle, Herd *et al.* (2003) observed no differences ADG and body weight between the Angus steers progeny whose parents were selected for low RFI or high RFI. Basarab *et al.* (2003) observed no differences in ADG among the high, medium, and low RFI groups. Cost of gain was correlated moderately and positively with DMI and RFI, and strongly and negatively correlated with gain: feed and ADG.

Kolath *et al.* (2006) observed no differences ($P=0.80$) in final body weight or ADG between the low and high RFI groups. In agreement, Baker *et al.*, 2006 reported no

differences in ADG, initial body weight, and 71days body weight among the high, mid, and low RFI steers. However, they observed a greater dry matter intake (DMI, $P=0.004$) and feed conversion ratio for the high RFI steers when compared to the low RFI steers. Golden *et al.* (2008) found no significant differences between the high and low RFI steers for initial body weight, final body weight, or ADG. Kelly *et al.* (2010) examined the effect of divergence in RFI of 86 Limousin crossed Holstein-Freisan growing heifers. The results indicated that low RFI heifers consumed 8.5 and 15.9 % less feed than their counterparts ranked as either medium or high RFI, respectively. The average daily gain (ADG), initial body weight, and final body weight did not differ ($P > 0.10$) between the high, medium, and low RFI groups.

2.7.2 Phenotypic and genetic correlations of RFI with feed intake

RFI is only useful as a selection tool if there is potential for improvement in feed intake and feed conversion ratio (FCR). Residual feed intake of growing dairy heifers had a high positive genetic correlation with roughage and metabolizable energy intake of lactating dairy heifers (Nieuwhof *et al.*, 1992). Carstens *et al.* (2002) reported high RFI steers consumed more feed ($P<0.001$) and had higher FCR ratios than the low RFI steers (Carstens *et al.*, 2002). In another study, Basarab *et al.* (2003) reported significant differences across the three RFI classifications for DM, FCR (high: 5.95 vs. medium: 5.70 vs. low: 5.39). DMI also differed across the groups with the low RFI cattle having the most favorable values (8.00 kg/d) and the high RFI cattle having the most unfavorable values (8.93 kg/d) (Basarab *et al.*, 2003). Nkrumah *et al.* (2004) also found high RFI steers had higher FCR and consumed more DM than low RFI steers. RFI was estimated to have a strong phenotypic correlation with feed intake (0.65 and 0.67) in both growing and finishing cattle (Carstens and Tedeschi, 2006). Baker *et al.* (2006) reported low RFI Angus steers consumed significantly less DM than high RFI steers (9.3 kg/d vs. 10.3 kg/d; $P<0.05$) and had better FCR (6.7 vs. 7.7; $P<0.05$) (Baker *et al.*, 2006). Kolath *et al.* (2006) compared 8 high RFI steers to 9 low RFI steers. The low RFI steers had higher gain: feed (0.20 vs. 0.16; $P<0.001$) and less average daily feed intake (7.40 vs. 8.94; $P<0.001$) than the high RFI steers (Kolath *et al.*, 2006). Low RFI steers consumed 19.1% less dry matter than high RFI steers (Gomez *et al.*, 2007). Also low RFI bulls and heifers exhibited a 17%

decline in feed intake compared to high RFI bulls and heifers (Ribeiro *et al.*, 2007). Golden *et al.* (2008) reported that low RFI steers ate fewer times and consumed less feed than high RFI steers. Cattle with lower RFI may be capable of greater DM digestibility, spend less time at the bunk and had lower feed intake. Genetic correlations between RFI and feed intake have been reported from 0.64 to 0.79 (Arthur *et al.*, 2001a; Nkrumah *et al.*, 2007; Herd and Bishop, 2000). RFI most often had moderate to high correlations with feed intake. Phenotypic correlations between RFI and feed intake ranged from 0.52 to 0.72 (Arthur *et al.*, 1997, 2001a,b; Baker *et al.*, 2006; Carstens *et al.*, 2002, Nkrumah *et al.*, 2007c; Herd and Bishop, 2000; Lancaster, *et al.*, 2005). Likewise, genetic correlations were strong as well.

2.8. Effect of residual feed intake on concentrations of urine metabolites

2.8.1 Creatinine

Creatinine is an end product formed in muscles from creatinine phosphate, the high- energy storage compound. Creatinine production is primary a function of muscles mass and the amount of produced is fairly constant. Creatinine is removed from plasma by glomerular filtration and excretion in urine. Therefore, creatinine clearance is measure of Glomerular Filtration Rate (GFR) and its measurement is useful in assessing renal function. Serum creatinine is more specific and sensitive indicator of renal function than serum urea. The urine/plasma creatinine ratio is greater than 40 in pre-renal uremia and less than 20 in renal uremia.

The excretion rate of creatinine is relatively constant in healthy animals (Chen *et al.*, 1995). The use of creatinine as an internal marker of urinary output relies on the assumption that the creatinine excretion through urine is affected neither by diet nor by the physiological status of the animal, but is excreted in proportion to body weight. It is conceivable that species differences in musculature and variations in body weight could account for the lower urinary creatinine level in rabbit. A number of studies in ruminant species (Daniels *et al.*, 1994; Chen *et al.*, 1995) indicated that purine derivatives: creatinine ratio (purine derivatives/creatinine) could predict microbial nitrogen supply with reasonable accuracy as it was little affected by sampling time. Higher allantoin/creatinine and purine derivatives/creatinine ratio in cattle and goat was attributed

to higher allantoin/ purine derivatives level and similar creatinine when compared to other ruminants. The excretion of creatinine was independent of diet when straw was supplemented either with *Gava Thripasha*, tree fodders or both (Thakshala *et al.*, 2008). The concentrations of allantoin or total PD when expressed as molar proportions of creatinine i.e. allantoin: creatinine and purine derivatives: creatinine were highest (1.81- 1.98, 1.55- 2.02 mmol/L) in cattle and goat and lowest (0.52- 0.61 mmol/L) in buffaloes (George *et al.*, 2011).

2.8.2 Hydroxy proline

Hydroxy proline is produced by hydroxylation of the amino acid proline by enzyme prolyl hydroxylase following protein synthesis as a post-translational modification. The enzyme catalyzed reaction takes place in the lumen of the endoplasmic reticulum. Although it is not directly incorporated into proteins, hydroxy proline comprises roughly 4% of all amino acid found in animal tissue. Hydroxy proline is a major component of the protein collagen. Hydroxy proline and proline play key roles for collagen stability. They permit the sharp twisting of the collagen helix.

Hydroxy proline in urine (Prockop & Kivirikko, 1967) has been widely used for the assessment of bone metabolism and bone disease. Urinary excretion of hydroxy proline was difficult to interpret as an index of bone resorption because a portion of the hydroxyl proline in urine is derived from peptides related to collagen synthesis and many of the hydroxyl proline containing peptides released during degradation of collagen were further metabolized and did appear as urinary hydroxyl proline (Prockop & Kivirikko, 1967).

3. MATERIALS AND METHODS

The present study was carried out to evaluate feeds and fodders for their nutrient composition as per proximate fractions and cell wall fractions, *in vitro* gas production, *in vitro* digestibility and *in vitro* methane production. *In vivo* experiments were conducted assessment of feed utilization in buffalo calves to investigate the variation in residual feed intake in Murrah buffalo calves. The techniques and methods adopted during experimental period are as follows.

IN VITRO EXPERIMENT

3.1 Sample collection and preparation

The samples of feeds and forages were collected from forage farm section of NDRI, Karnal, local market Karnal. The samples are divided into five groups as grains, green fodders (leguminous and non-leguminous), range herbage, oil cakes and agricultural byproducts (list is presented in table 3.1) Samples of feed and fodder were dried in hot air oven at 60°C till a constant weight was attained. The dried samples were ground through 1 mm sieve using electrically operated willey mill. The ground samples were stored in sample bottles (250 ml capacity), after proper labeling and kept for further chemical analysis.

Table 3.1 Common and botanical names of the feed ingredients evaluated in present study

S. No.	Common Name	Botanical name
Grains		
1	Maize (yellow)	<i>Zea mays</i>
2	Maize (white)	<i>Zea mays</i>
3	Wheat	<i>Triticum aestivum</i>
4	Pearl millet	<i>Pennisetum typhoides</i>
5	Sorghum (white)	<i>Sorghum bicolor</i>
6	Sorghum(red)	<i>Sorghum bicolor</i>

7	Barley	<i>Hordeum vulgare</i>
8	Oats	<i>Avena sativa</i>
Green fodders (Non leguminous and leguminous)		
9	Maize	<i>Zea mays</i>
10	Sorghum	<i>Sorghum bicolor</i>
11	Pearl millet	<i>Pennisetum typhoides</i>
12	Oats	<i>Avena sativa</i>
13	Berseem	<i>Trifolium alexandrinum</i>
Grasses		
14	Hybrid napier	<i>Pennisetum purpureum</i>
15	Bermuda grass/Dhoob grass	<i>Cynadon dactylon</i>
16	Setaria grass	<i>Setaria pumila</i>
17	Para grass	<i>Brachiaria mutica</i>
18	Guinea grass	<i>Panicum maximum</i>
19	Lemon grass	<i>Cymbopogon citrates</i>
20	Vetiver grass	<i>Vetiveria zizanioides</i>
Oil cakes and meals		
21	Ground nut oil cake	<i>Arachis hypogaea</i>
22	Groundnut cake(deoiled)	<i>Arachis hypogaea</i>
23	Mustard oil cake	<i>Brassica juncea</i>
24	Mustard cake(deoiled)	<i>Brassica juncea</i>
25	Soya bean meal	<i>Glycine max</i>
26	Cotton seed cake	<i>Gossypium spp.</i>
27	Guar meal	<i>Cyaopsis tetragonoloba</i>

Agricultural industrial by products and cereal crop residues		
28	Wheat bran	<i>Triticum aestivum</i>
29	Rice bran	<i>Oryza sativa</i>
30	Deoiled rice bran	<i>Oryza sativa</i>
31	Rice polish	<i>Oryza sativa</i>
32	Maize gluten meal	<i>Zea mays</i>
33	Gram churi	<i>Cicer arietinum</i>
34	Gram husk	<i>Cicer arietinum</i>
35	Gram khanda	<i>Cicer arietinum</i>
36	Wheat straw	<i>Triticum aestivum</i>
37	Paddy straw	<i>Oryza sativa</i>
38	Rice gluten meal	<i>Oryza sativa</i>
39	Beet pulp	<i>Beta vulgaris</i>
40	Distillers dried grain solubles	

3.2 Chemical analysis of the feed ingredients

The quantitative chemical analysis of various constituents in feed and forage is the first step and essential prerequisite to estimate the nutritive value of feeds. Feedstuffs are composed of chemically measurable carbohydrate, protein, fat, ash and water. The method of analysis followed in the present investigations for the estimation of dry matter (DM), organic matter (OM), crude protein (CP), total ash (TA), and ether extract (EE) were essentially those recommended by AOAC (2005). Methods employed were as follows:-

3.2.1 Dry matter (DM)

A known quantity of ground sample (about 5-10g) was taken in a pre-weighed moisture cup and placed in hot air oven at $100 \pm 5^{\circ}\text{C}$ for 24 hrs. The loss in moisture content after drying was estimated and dry matter was calculated as follows:

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

3.2.2 Total ash (TA)

A known quantity of sample (about 3-5g) was taken in pre-weighed silica crucible. After charring the sample (till the smoke disappeared), the crucible was kept in muffle furnace for ignition at 550⁰C for 2-3 hrs. The crucible was removed on cooling and kept in a desiccator and weighed again to find out weight of ash. The ash content was calculated as given below:

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

3.2.3 Organic matter (OM)

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

$$\text{OM (\%)} = 100 - \text{Total ash (\%)}$$

3.2.4 Crude protein (CP)

Apparatus: Kjeldahl flasks, digester, Kjeldahl distillation apparatus, erlenmeyer flask, titration assembly.

Reagent: Digestion mixture (K₂SO₄ and CuSO₄ in the ratio of 9:1), 40% NaOH solution (400g NaOH pellets dissolved in distilled water and volume made to 1000 ml), concentrated H₂SO₄ (98% purity gravity 1.84), 2% acid boric indicator solution (20 g boric acid dissolved to 1L and added with 10 ml 0.2% bromocresol green and 20 ml 0.1% methyl red indicator), N/100 H₂SO₄.

Total nitrogen is measured by Kjeldahl method (AOAC, 2005). A known quantity of sample (about 0.5-1g) was taken in Kjeldahl flask and digested with concentrated H₂SO₄ and 2-3 g of digestion mixture till the solution became colorless. After digestion, the contents were cooled and volume was made to 100 ml. Ten ml of aliquot was distilled in Kjeldahl distillation apparatus (KELPLUS Nitrogen Analyzer) after adding 10-15 ml. About 60-75 ml of distillate was collected into a conical flask containing 10 ml of 2% boric acid solution having mixed indicator. The distillate was then titrated against N/100 H₂SO₄

$$N (\%) = \frac{0.0014 \times 0.01 \times \text{Volume of N/100 H}_2\text{SO}_4 \text{ used} \times \text{Volume made (ml)}}{\text{Aliquot taken (ml)} \times \text{Sample taken (g)}} \times 100$$

The crude protein (%) of sample was calculated by multiplying the N-content with factor 6.25. This was based on the principle that protein contains 16% nitrogen

3.2.5 Ether extract (EE)

A known quantity of ground sample (about 3g) was taken in a cellulose thimble and extracted for 10 -12 hrs with petroleum ether (40-60⁰C) in Soxhlet's extraction apparatus attached to a pre-weighed oil flask. The oil flask was removed and after evaporating the excess of ether, it was dried overnight in an oven (temperature 100 ± 5⁰C). The flask was cooled in a desiccator and weighed. The difference in two weights gave the amount of ether extract in the sample.

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

3.3 Estimation of cell wall constituents

The fraction of cell wall constituents such as neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose and lignin were estimated as per Van Soest *et al.* (1991). The method adopted were the modification of original procedures described by Goering and Van Soest (1970).

3.3.1 Neutral detergent fiber (McQueen and Nicholson, 1979)

Apparatus: Spoutless beaker, sintered crucible, vacuum pump, hot air oven, muffles furnace, weighing balance, desiccator.

Preparation of neutral detergent solution (NDS)

Sodium lauryl sulphate	-30.00 g
Disodium ethylene diamino tetra acetate (EDTA)	-18.61 g
Sodium borate decahydrate	-6.81 g
Disodium hydrogen phosphate (anhydrous)	-4.56 g

Triethylene glycol	-10 ml
Distilled water	-To make 1L solution

Solution Preparation

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

Preparation of amylase solution

Reagents: Diastase/Amylase, thermolabile (Thomas baker-12095)-Activity (starch acc. Zulkowsky) -130UI/mg, potassium di-hydrogen phosphate orthophosphate, sodium phosphate anhydrous, sodium azide.

Phosphate buffer solution (0.067M)

Potassium dihydrogen phosphate orthophosphate	-3.56 g
Sodium phosphate anhydrous	-7.22 g
Sodium azide	-0.2 g

Mix the above in 1L distilled water and adjust the pH to 7.

Amylase solution: Dissolve 8 g amylase in 1L phosphate buffer solution.

Procedure

Feed sample weighing 0.5 g was taken in a 500 ml spoutless beaker. 30 ml amylase solution was added into the beaker and mixed with swirling action. Covered the beaker (to avoid evaporation) and incubate at 40⁰C over night (12-18 hrs). Next day, 50 ml NDS and 0.5 g sodium sulphite were added and the contents of spout less beaker were refluxed for an hour (one hour after the initial onset of boiling). Then the contents of beaker were filtered through pre-weighed 50 ml sintered glass crucible (G-I) using oil-free vacuum pump. The contents were washed repeatedly with hot boiling water and then acetone to remove all salts. The

sintered crucible-containing residue was dried in hot air oven ($100 \pm 5^{\circ}\text{C}$) for overnight, cooled and weighed to a constant value. The crucibles were kept for ash in a muffle furnace at 500°C for 2-3 hrs and crucible along with ash was weighed again.

The NDF was calculated as follows:

$$\text{NDF (\%)} = \frac{\text{Weight of crucible with residue} - \text{Weight of crucible with ash}}{\text{Weight of sample}} \times 100$$

3.3.2 Acid detergent fiber (ADF)

Preparation of acid detergent solution (ADS): Twenty-gram cetyl-trimethyl-ammonium bromide (CTAB) was dissolved in one liter of 1N H_2SO_4 .

Procedure

Approximately 1 g of sample was taken in a spout less beaker of 1 L capacity. To this, 100 ml acid detergent solution was added and the contents were refluxed for exactly 1 hour. After refluxing the residue was filtered through pre-weighed sintered glass crucible (grade-I) using vacuum pump, washed with hot water 2-3 times and followed by acetone to remove all salts. The ADF was calculated as follows:

$$\text{ADF (\%)} = \frac{\text{Weight of crucible with residue} - \text{weight of empty crucible}}{\text{Weight of sample taken}} \times 100$$

3.3.3 Hemicellulose

Hemicellulose was soluble in ADS and was calculated by subtraction of ADF from NDF as follows:

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

3.3.4 Cellulose

Principle

For estimation of cellulose the acid detergent fiber (ADF) procedure was used as a preparatory step. The ADF residue consisted of cellulose, lignin, cutin and acid insoluble ash (mainly silica). Cellulose was dissolved when ADF was treated with 72% (w/w) H₂SO₄.

Preparation of 72% H₂SO₄ (w/w basis)

Reagent grade H₂SO₄ (specific gravity 1.84 and 98% purity) was standardized to specific gravity 1.634 at 20⁰C or 12 M. For this 1200g H₂SO₄ about 654 ml reagent grade H₂SO₄ was added to 440 ml distilled water in 1L capacity volumetric flask while cooling under running tap water. The weight was standardized to 1634 g/L at 20⁰C by removing solution and adding distilled water or H₂SO₄ as required.

Procedure

Sintered crucible grade (G-I) containing ADF contents was placed in enamel tray in such a manner that one end of the enamel tray was at about 2 cm height than the other end, so that acid could drain away from the crucible. The crucible was then filled with 72% H₂SO₄ (w/w basis) and the contents were stirred with glass rod to break all the lumps. The crucible was refilled with 72 % H₂SO₄ after 1hour interval. After 3 hours, the crucible was removed from the tray and filtration of acid was done by using vacuum pump. The material was washed with hot water until it become free from acid and kept into the oven (100 ± 5⁰C) overnight and weighed.

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

Where,

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

W2= wt. after extraction

Y = wt. of initial sample (DM)

3.3.5 Acid detergent lignin (ADL)

Apparatus and reagents: Same as that of ADF estimation.

Procedure

The procedure for estimation of ADL content was exactly same up to the filtering and drying of ADF contents of sintered crucible after treating with 72% H₂SO₄ (w/w) in the cellulose estimation procedure. Then the crucible with dry residue was kept in muffle furnace for ignition at 550 to 600⁰C for 2-2.5 hours, cooled and weighed again. The acid detergent lignin was calculated as follows:-

$$\text{ADL (\%)} = \frac{\text{wt. of crucible with dry residue} - \text{wt. of crucible with ash}}{\text{Weight of sample}} \times 100$$

3.4 Estimation of nitrogen fractions

Acid detergent insoluble nitrogen (ADIN), neutral detergent insoluble nitrogen (NDIN), non-protein nitrogen and soluble protein were estimated as per Licitra *et al.* (1996).

3.4.1 Determination of acid detergent insoluble nitrogen (ADIN)

The procedure was exactly as that for ADF estimation (Van Soest, 1991). Nitrogen in ADF residue was estimated following the standard Kjeldahl method. ADIN of sample was express as percent of total nitrogen and acid detergent insoluble crude protein (ADICP) was either expressed as % of DM or as % CP.

3.4.2 Determination of neutral detergent insoluble nitrogen (NDIN)

The procedure was exactly as that for NDF estimation (Van Soest, 1973). Nitrogen in NDF residue was estimated following the standard Kjeldahl method. NDIN of sample was express as percent of total nitrogen. Neutral detergent insoluble crude protein (NDICP) was expressed either as % of DM or as % CP.

3.5 Total gas production

Menke *et al.* (1979) and Steingass (1988) developed a system for feed evaluation based on *in vitro* gas production in Germany. This system is a Tilley and Terry (1963) system but in this method gas production rather than dry matter loss is measured.

The amount of gas (CO₂ and CH₄) released when feeds are incubated *in vitro* with rumen liquor is closely related to digestibility and to the energetic feed value of diets for

ruminants. The feeds of different digestibility introduced into a calibrated glass syringe with buffered rumen liquor medium and incubated at 39⁰C. At the end of incubation, the gas produced was recorded.

Preparation of solution

1. Macro mineral solution

Na₂HPO₄ 2.70 g

KH₂PO₄ 6.20 g

MgSO₄ 0.60 g

Make up to 1L with distilled water

2. Micro mineral solution

CaCl₂·2H₂O 13.2 g

MnCl₂·4H₂O 10.0 g

CoCl₂·6H₂O 1.00 g

FeCl₂·6H₂O 0.80 g

Make up 100ml with distilled water

3. Buffer solution

NaHCO₃ 35 g

NH₄CO₃ 4 g

Make up 1L with distilled water

4. Resazurine solution

Resazurine 100 mg

Distilled water 100 ml

5. Reduction solution

1N NaOH	2 ml
Na ₂ S.7H ₂ O	0.285 mg
Distilled water	47.5 ml

It should be prepared fresh at the time of use.

Procedure

The incubation of each ingredient (200mg) was carried out in 100 ml calibrated plastic syringes as described by Menke and Steingass (1988). The substrate was weighed on a plastic boat with removable stem and placed into the bottom of the plastic syringe without sticking to the sides of syringe. The piston was lubricated with vaseline and pushed inside the glass syringe. Each ingredient was taken in triplicate.

Rumen liquor was collected from a donor buffalo bull, fitted with permanent rumen fistula. Rumen liquor was collected before feeding the donor animal into a pre-warmed thermo-flask and brought to the laboratory. The rumen liquor was bubbled with CO₂ for about two minutes and filtered through four layers of muslin cloth.

The medium was prepared by mixing 730 ml distilled water, 365 ml rumen buffer solution and 365 ml macro minerals solution, 1.90 ml resazurine solution, 0.185 ml micro minerals solution and 76.10 ml reducing solution (prepared fresh and added just prior to incubation). The medium was pre-warmed to 39⁰C and bubbled with CO₂ until the blue color of the medium vanished.

After medium became colorless, the required amount of strained rumen liquor (SRL) was added. The ratio of medium to rumen liquor was 2:1. Then, 30ml of incubation medium was injected to each syringe using automatic dispenser. The syringes were shaken gently, residual air or air bubble, if any, was removed, and the outlet was closed with the clamp. The level of piston was recorded as a initial reading and the syringes were placed in an incubator (39 ± 0.5⁰C). The syringes were shaken 4 to 5 times during incubation. Gas produced (ml) during fermentation was measured after 24 hours in all feeds except green and dry roughages where it was measured after 48 hours. Net gas produced (mM/200 mg substrate) were calculated after deducting the blank and considering the volume of 1 mM of gas at 39⁰C in Karnal is equal to 24.97 ml.

3.6 Methane production

After the incubation of feeds, suitable aliquot of gas was withdrawn from the tip of the syringe using Hamilton gas tight syringe and was analyzed for its methane using gas chromatograph (Nucon 5700, India) fitted with stainless steel column packed with Porapak-q (length 6'; o.d. 1/8"; i.d. 2 mm; mesh range 80-100) and thermal conductivity detector. The temperature of injection port, column and detector was 150, 60 and 130°C, respectively. The flow rate of carrier gas (N₂) through the column was 40 ml/ min. The standard gas used for CH₄ estimation (Spantech Calibration gas, Surrey, England) composed of 50% CH₄ and 50% CO₂. The peak of CH₄ gas was recognized based on of retention time of standard CH₄ gas and the area obtained was used to calculate CH₄ percentage in the gas sample.

$$\text{CH}_4 (\%) = \frac{\text{Area of sample}}{\text{Area of standard}} \times 50$$

CH₄ produced from the substrate during incubation was corrected for the blank values. The net volume of CH₄ (ml) produced was calculated and then the amount of CH₄ (g/kg DM incubated, g/kg IVDMD and g/kg IVOMD

$$\text{CH}_4 (\text{ml}) = \frac{\text{Total gas produced (ml)} \times \% \text{ CH}_4 \text{ in the sample}}{100}$$

$$\text{Net CH}_4 (\text{ml}) = \text{CH}_4 \text{ in sample} - \text{CH}_4 \text{ in blank}$$

$$\text{CH}_4 (\text{g/kg DM incubated}) = \frac{\text{Total gas produced (ml)} \times \% \text{ CH}_4 \text{ in the sample}}{\text{DM incubated (g)} \times 24.97 \times 1000}$$

$$\text{CH}_4 \text{ (g/kg IVDMD) } = \frac{\text{Net CH}_4 \text{ (ml) } \times 1000 \times 16}{\text{TDMD (g) } \times 24.97 \times 1000}$$

$$\text{CH}_4 \text{ (g/kg IVOMD) } = \frac{\text{Net CH}_4 \text{ (ml) } \times 1000 \times 16}{\text{TOMD (g) } \times 24.97 \times 1000}$$

3.7 *In vitro* dry matter and organic matter digestibility

Reagents

The McDougall buffer solution was prepared (composition of McDougall buffer is presented below) adding the CaCl₂ solution at the end. Thereafter, buffer was saturated with CO₂ until become clear (pH= 6.9).

Composition of McDougall buffer

Sodium hydrogen carbonate (NaHCO ₃)	-9.80 g
Disodium hydrogen phosphate (Na ₂ HPO ₄)	-9.30 g
Sodium chloride (NaCl)	-0.47 g
Potassium chloride (KCl)	-0.57 g
Calcium chloride (CaCl ₂)	-0.04 g
Magnesium sulphate (MgSO ₄ .7H ₂ O)	-0.12 g
Distilled water	-Up to 1 liter

Procedure

Samples of each feed (0.5g) were weighed into 100 ml glass bottles in triplicate and stored at 38⁰C until required. Rumen liquor, was collected from each animal was mixed thoroughly and strained through four layers of muslin cloth. Strained rumen liquor (SRL) was exposed to CO₂ and maintained at 38 –39⁰C.

The required quantities of buffer and SRL in the ratio of 40:10 were mixed and 50 ml of this mixture was transferred to each bottle including those without sample (blank). The space above the liquid in each bottle was flushed with CO₂ and bottles were sealed immediately with cork and tightened with aluminum cap. Thereafter, bottles were incubated at 38⁰C for 48 hrs, and shaken 4 times a day, manually. Gas produced during fermentation,

was released with the help of syringe from each incubation bottle. Finally, the bacterial activity was stopped by adding 1 ml of 5% HgCl₂.

Contents of each bottle were filtered through pre-weighed 50 ml capacity sintered glass crucible (G1) and contents were dried at 100⁰C till constant weight. The dry weight of residue was calculated. Dry weight of blank was subtracted from those recorded for the test samples. Following equation was used to determine IVDMD:

$$\text{IVDMD (\%)} = \frac{\text{Wt. of dried sample} - \text{Wt. of residue}}{\text{Wt. of dried sample}} \times 100$$

The residue in each crucible was ashed in a muffle furnace at 550⁰C to determine its organic matter content. *In vitro* organic matter digestibility (IVOMD) was calculated as follows:

$$\text{IVOMD (\%)} = \frac{\text{Wt. of OM in sample} - \text{Wt. of OM in residue}}{\text{Wt. of OM in sample}} \times 100$$

***IN VIVO* EXPERIMENT**

3.1 Selection and distribution of animals

Eighteen male Murrah buffalo calves (4-6 months) purchased from village Bannat (Tehsil Shamali, Utter Pradesh) were selected for the present study. Details of their age and body weight at the starting of experimental feeding are given in table 3.2

Table 3.2 Description of experimental animals

Animal no	BW(kg)
6566	79.40
6567	95.20
6568	73.20
6570	71.40
6571	79.80
6572	88.20
6573	91.00
6574	67.20

6575	69.20
6576	60.60
6577	48.80
6578	60.20
6579	76.40
6580	63.20
6583	81.00
6587	50.60
6588	60.00
6589	59.60

3.2 Feeding and housing of animals

All the eighteen buffalo calves were fed *ad libitum* with total mixed ration prepared with concentrate mixture, green fodder (oats) and dry roughage (wheat straw) in the ratio of 40: 40: 20 (on DM basis) for twenty one day prior to the actual feeding period to allow acclimation to diet. After the adaptation period, a growth trial was carried out for 52 days with *ad libitum* feeding of the same TMRs for estimating the variations in maintenance energy requirements and residual feed intake (RFI) of each calves. A metabolism trial was conducted during the last 8 days of growth trial period. Ingredient composition of experimental diets is given in table 3.3.

Table 3.3 Feed ingredients (%DM) of the experimental ration

Sr.no.	Ingredient	Parts per 100 (%)
1	Maize	33
2	MOC	12
3	SBM	21
4	Wheat bran	20
5	DORB	6
6	Pearl millet	5
7	Mineral mixtures	2
8	Salt	1

3.3 RFI measurement

ME required for maintenance and growth was calculated as per feeding standards (ICAR, 2013) for buffalo calves. Actual ME intake of the animals were estimated during the growth trial period. The calculated (expected) ME for growth (ME_g) was deducted from both the calculated (expected) and actual ME intake to find out the ME for maintenance. Maintenance variability coefficient ($ME_m/100\text{kg BW}$) was found out for every animal. The individual variation in maintenance requirements was estimated and its relationship with RFI was found out. Different methods were followed to find out the RFI in buffalo calves in present study.

RFI was calculated as per the following formulae

$$\text{RFI} = \text{Actual } ME_m - \text{Expected } ME_m$$

When ME_m actual = ME_m expected, $\text{RFI} = 0$ which means the energy requirements of the animal are completely met. A positive RFI means that the animal's energy intake exceeds its requirement for maintenance. A negative RFI means that the animal either requires less energy than estimated or is eating less to produce the same weight gain.

Residual feed intake was calculated in terms of ME intake also. RFI is the difference between the metabolizable energy intake (MEI) and metabolizable energy required for maintenance and gain (MER) (Fan *et al.* 1995; Liu *et al.* 1998). Mathematically, RFI or residual metabolizable feed consumption in kg d^{-1} is calculated as $\text{RFI} = \text{MEI} - \text{MER}$.

Residual feed intake was computed for each animal and was assumed to represent the residuals from a multiple regression model regressing DMI on ADG and mid test metabolic body weight (MBW) (Archer *et al.* 1997).

The base model used was $Y_j = \beta_0 + \beta_1 \text{MBW}_j + \beta_2 \text{ADG}_j + e_j$

Where, Y_j is the DMI of the j^{th} animal

β_0 is the regression intercept

β_1 is the regression coefficient on MBW

β_2 is the regression coefficient on ADG

e_j is the uncontrolled error of the j^{th} animal.

3.4 Metabolism trial

Animals were shifted into the metabolism shed three days prior to the metabolism trial for their adaptation to the surroundings. Animals were weighed before and after the trial consecutively for two days. Animals were fed ad libitum with TMR. Fresh drinking water

was provided twice a day, and the quantity was measured each time to calculate the total water intake.

3.5 Sampling, processing and storage

The feed samples were daily taken for DM estimation during metabolism trial. These samples were pooled at the end of the collection period and ground to pass through 1 mm sieve and stored in airtight containers. The samples were analyzed for proximate principles and cell wall constituents (NDF, NDICP, ADF, ADICP and ADL) as discussed in earlier and to calculate the TDN content of rations (NRC, 2001). Daily residue left was weighed and treated the same manner as that of the fodder components to determine the intake of nutrients.

Faeces voided during 24 hrs, was collected daily for six days and weighed at 9:00 AM daily. After thorough mixing, an approximately 2% of total sample on weight basis was kept for DM estimation. Dried pooled dung samples were ground to pass through 1 mm sieve size and analyzed for proximate principles and cell wall constituents (NDF and ADF) as per standard procedure.

Total urine voided from each buffalo calf was collected using plastic buckets. Total urine output for 24 hrs was measured daily at 8:30 AM and an aliquot (0.5% of total output) was taken for the nitrogen estimation. The aliquots were stored in plastic containers containing 20 ml of 25% H₂SO₄ for total nitrogen estimation.

3.6 Analysis of feed, faeces and urine

Dry matter, organic matter, crude protein, ether extract, and total ash in feeds and faeces samples were determined as per AOAC(2005). The fractions of cell wall constituents (NDF, ADF and ADL) were also determined (Van Soest *et al.*, 1991). Neutral detergent insoluble crude protein (NDCIP) and acid detergent insoluble crude protein (ADCIP) were estimated as per Licitra *et al.* (1996).

3.6.1 Estimation of total nitrogen in urine

Total nitrogen content of urine samples were estimated using KELPLUS- N analyzer (Pelican, India). From each sample, 10ml urine was transferred into Kjeldahl digestion tube and digested by adding 10 ml concentrated H₂SO₄ along with 2-3 g digestion mixture. Digested material was diluted with distilled water up to 100 ml in volumetric flask. An

aliquot (10 ml) of digested sample was taken in distillation tube and adequate amount of 40 % NaOH was added into the tube and distillation was done. The distillate was collected in a conical flask containing 2% boric acid mixed indicator solution, which was titrated against N/100 H₂SO₄. Total Nitrogen was calculated as below :

$$\text{Total N (mg/100 ml)} = \frac{V \times B \times DF \times 0.014 \times 100 \times 1000}{\text{Volume of sample taken for digestion}}$$

Where, V = Volume of H₂SO₄

B = Normality of H₂SO₄

DF = Dilution factor (total volume made/ aliquot taken for distillation)

3.6.2 Calculation of nutrient digestibility coefficients (%)

The nutrients (dry matter, organic matter, crude protein, ether extract, NFC, NDF and ADF) digestibility coefficients were calculated from the nutrient intake and nutrition output in faeces during metabolism trial as below :

$$\text{Digestibility (\%)} = \frac{(\text{Nutrient intake} - \text{Nutrient output in faeces}) \times 100}{\text{Nutrient intake}}$$

3.7 Calculation of total digestible nutrient (TDN)

1. Truly digestible NFC (tdNFC) = 0.98 (100 - [(NDF - NDICP) + CP + EE + Ash]) x PAF
2. Truly digestible CP for forages (tdCPf) = CP x exp [-1.2 x (ADICP/CP)]
3. Truly digestible CP for concentrates (tdCPc) = [1 - (0.4 x (ADICP/CP))] x CP
4. Truly digestible FA (tdFA) = FA Note: If EE < 1, then FA = 0
5. Truly digestible NDF (tdNDF) = 0.75 x (NDFn - L) x [1 - (L/NDFn)^{0.667}]

In Equations 1, 2, 3, 4 and 5, NDICP = neutral detergent insoluble N x 6.25, PAF = processing adjustment factor (i.e., 1 for fodders), ADICP = acid detergent insoluble N x 6.25,

FA = fatty acids (i.e., EE - 1), L = acid detergent lignin, and NDFn = NDF - NDICP. All values were expressed as a percent of dry matter (DM).

Equations 1, 2, 3, 4 and 5 were based on true digestibility, but TDN is based on apparent digestibility; therefore, metabolic fecal TDN was subtracted from the sum of the digestible fractions. Weiss *et al.* (1992) determined that, on average, metabolic fecal TDN is equal to 7. The TDN was calculated using equation 6 given below (NRC, 2001).

$$6. \text{ TDN (\%)} = \text{td NFC} + \text{td CP} + (\text{td FA} \times 2.25) + \text{td NDF} - 7$$

The TDN was also calculated from digestibility data of metabolism trial using the equation 7 given below

$$7. \text{ TDN (\%)} = (\% \text{ dig NFC}) + (\% \text{ dig CP}) + (\% \text{ dig. FA}) \times 2.25 + (\% \text{ dig NDF})$$

Where % digestibility nutrients (NFC, CP, FA and NDF) were calculated as below:

$$\% \text{ Dig nutrient} = \frac{\% \text{ nutrient in fodder} \times \text{digestibility (\%)}}{100}$$

DE and ME contents of feeds and TMRs were estimated as per the following equations of NRC

$$\text{DE (Mcal/kg)} = 0.04409 \times \text{TDN (\%)}$$

$$\text{ME (Mcal/kg)} = (1.01 \times \text{DE} - 0.45)$$

DE and ME contents were expressed a MJ/kg using the following formula i.e.
1 cal = 4.18joule

3.8 Estimation of creatinine in urine

Creatinine reacts with picric acid in an alkaline medium to form an orange coloured complex. The rate of formation of this complex is measured by reading the change in absorbance at 505 nm in a selected interval of time and is proportional to the concentration of creatinine.

Alkaline medium

Creatinine + Picric acid \longrightarrow orange coloured complex

Kit reagents were prepared and stored as per the instructions provided with the assay kit (Modified Jaffe's Reaction. Cogent, REF: 85LS200-60/85LS200-66)

Reagents Composition

Reagent No	Reagent	REF	Pack size	Composition	Concentrate
1	Picrate reagent	85LS200-60/66	1x50ml/1x125ml	Picric acid preservative	40mM/L Qs
2	Sodium hydroxide	85LS200-60/66	1x50ml/1x125ml	Sodium hydroxide	200mM/L
3	Creatinine standard	85LS200-60/66	1x1ml/1x2ml	Creatinine stabilizer	2mg/dl Qs

Working reagent preparation

Working reagent was prepared by mixing equal volume of reagent 1 (picrate reagent) with reagent 2 (sodium hydroxide) to make the desired volume and mixed gently for 2 minutes.

Reagent 3 was ready to use then.

Procedure

Pipette into tube marked	Blank	Standard	Test
Serum / Plasma	-	-	100 μ l
Reagent 3	-	100 μ l	-
Working creatinine reagent	-	1000 μ l	1000 μ l
Purified water	1000 μ l	-	-

- Sample, reagent, working creatinine reagent was added to the tubes and mixed well.
- Purified water was used as blank.

- Initial absorbance of the standard i.e. AS1 after 30 seconds and final absorbance i.e. AS2 after another 120 seconds was measured.
- After standard reading was noted, the reading of test i.e. AT1 and AT2 were noted accordingly.

Calculation

The blood creatinine was calculated as per formula and expressed in mg/dl

$$\text{Urine Creatinine (mg/day)} = \frac{\text{AT2} - \text{AT1}}{\text{AS2} - \text{AS1}} \times 2 \times \text{dilution factor} \times 24 \text{ hours urine volume in dl}$$

Concentration of standard = 2 mg/d

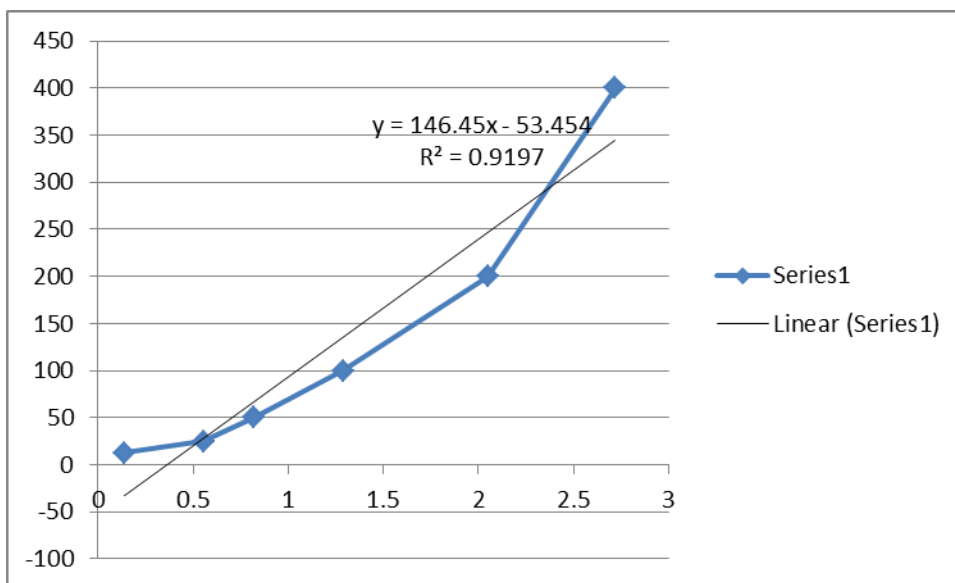
3.9 Estimation of hydroxy-proline in urine

Assay principle: Quantitative sandwich immunoassay using Bovine hydroxy proline – rich glycoprotein, HRGP ELISA kit

Assay procedure

1. All the standards were prepared before starting assay.
2. Sample diluents of 50 micro liter/well were added to 2 standard wells serving as the zero standard. The standards were taken and agitated gently and pre-diluted standard was added @ 50 micro liter/well to other standard wells following the sequence of S1 to S6. Fifty micro liter of urine sample was added to the sample wells.
3. Hundred micro liter conjugate was added to each well and mixed well. Then the plate was covered and incubated for 1 hour at 37⁰C.
4. The plate was washed 5 times with diluted wash solution using an auto washer and then dried.
5. Fifty micro liter chromogen substrate A and 50 micro lilter chromogen substrate B was added to each well, subsequently. Again plate was covered and incubated for 15 minutes at 37⁰C.
6. Then 50 micro ml stop solution was added to each well and mixed well.
7. Optical density (O.D) at 450 nm using microliter plate reader was read immediately.

Figure 2. Standard curve of hydroxyl - proline in urine



3.10 Statistical analysis

All the data generated during *in vitro* studies were subjected to statistical analysis by one way analysis of variance (ANOVA) and data of *in vivo* trial were analyzed by independent sample t-test and GLM procedure using software package SAS (version 9.3.1).

4. RESULTS AND DISCUSSION

4.1 Chemical analysis of the feed ingredients

4.1.1 Proximate compositions of grains (% DM)

Data on proximate constituents in grains are presented in table 4.1.1. The DM content was not different among the grains. The OM content varied significantly ($P < 0.01$) with the highest in sorghum red (97.82%) and lowest in wheat (96.11%). The CP content was maximum in wheat (14.50%) and minimum in white maize (8.03%). Oats (3.65%), yellow maize (3.37%) and white maize (3.39%) were having higher EE (%) as compared to wheat, barley and sorghum. Mondal *et al.* (2008) observed the CP content of some common Indian feedstuffs enlisted as barley grain (10.25), wheat bran (13.56), wheat (8.94) and maize grain (12.56). Similar chemical composition of maize, barley and wheat has been reported earlier (Shalini, 2008; Kamble *et al.* 2010).

4.1.2 Proximate compositions of green fodders (% DM)

Chemical constituents of cereals and legume fodders have been presented in table 4.1.2. The crude protein value varied from 9.63% (pearl millet) to 19.03% (berseem). Oats and berseem had EE of 3.62 and 2.37% respectively, while the corresponding values for sorghum, pearl millet and maize were 2.28, 2.44 and 1.45%, respectively. The ash content of maize, sorghum, pearl millet, oats and berseem were 9.49, 8.89, 13.47, 10.84 and 13.83%, respectively. The results obtained in present study were in accordance to the results reported by Prusty (2011) and Kamble *et al.* (2011).

4.1.3 Proximate compositions of range grasses (%DM)

Proximate constituents of grasses have been presented in table 4.1.3. The DM content varied significantly ($P < 0.01$) among grass samples and ranged from 14.89% in hybrid Napier to 32.34% in Bermuda grass. The CP content was maximum in hybrid Napier (10.24%) and minimum in Vetiver grass (5.57%). EE content was highest in lemon grass (2.47%) and lowest in Vetiver grass (1.16%). Ash content varied significantly ($P < 0.01$) among grasses, the highest was in hybrid Napier (12.33%) and lowest in Vetiver (5.63%). Kamble *et al.* (2010) reported significantly higher CP for hybrid Napier (17.01%) in comparison to present study. Similar proximate composition of Guinea grass has been reported earlier (Gupta *et al.* 2011; Prusty, 2011).

4.1.4 Proximate compositions of oil cakes and meals (%DM)

Proximate constituents of oil cakes and meals viz. GNC, de-oiled GNC, MOC, de-oiled mustard cake, CSC, SBM and guar meal have been given in table 4.1.4. The DM and OM content were significantly different and averaged 92.17 and 93.67%, respectively. The CP value varied significantly ($P < 0.01$) with SBM having highest (50.17%) and CSC the lowest CP (24.15%). The ash content of SBM, MOC, GNC, and de-oiled GNC were 7.52, 7.17, 6.67 and 6.73% respectively, which were higher than that of guar meal (5.25%) and CSC (4.48%). Mondal *et al.* (2008) reported the CP of mustard cake (33.18), GNC (43.19), soybean meal (54.81), and cotton seed cake (29.19), slightly higher than present values whereas Kamble *et al.* (2010) reported similar CP values for the cakes other than soybean meal. Shalini (2008) reported similar proximate composition in CSC, GNC and mustard cake.

4.1.5 Proximate compositions of agro industrial by-products and cereal crop residues (% DM)

Proximate constituents of thirteen by-products evaluated in the present study are presented in table 4.1.5. The DM content varied from 93.46% in rice bran to 10.39% in beet pulp. The OM varied ($P < 0.01$) from 95.83% in wheat bran to 96.80% in distillery bran. CP content was maximum in MGM (64.26%) and minimum in gram husk (3.51%). The EE contents were very low in all the by-products except in rice polish (13.31%). The TA was highest in paddy straw (17.01%) and the lowest in gram husk (3.79%). Rice polish was also high (8.84%) in ash content. The results of this study were in accordance to the findings of Prusty (2011), Kamble *et al.* (2010) and (2011).

4.1.6 Cell wall and cell wall bound protein constituents of grains (% DM)

The data pertaining to the cell wall and fiber bound protein in grains are presented in the table 4.1.6. NDF was highest in barley (25.90%) and lowest in sorghum white (12.16%). ADF, ADL, hemicellulose and cellulose % ranged between 2.87 (wheat) to 13.30 (barley), 0.77 (maize, white) to 3.96 (oats), 5.63 (maize, white) to 14.23 (wheat) and 1.66 (wheat) to 9.37 (barley) respectively. Shalini (2008) reported the comparable values of cell wall constituents. The ADF content of barley was similar to those reported by Ferarzani *et al.* (2004). Fiber bound protein fractions viz: NDICP, ADICP were highest in barley (2.07%) and pearl millet (0.75%) and lowest in white sorghum (1.22%) and yellow maize (0.39%), respectively.

Table 4.1.1 Proximate compositions of grains (% DM)

Feed	DM	OM	CP	EE	TA
Yellow maize	91.90 ±0.80	97.03 ^{ab} ±0.44	9.91 ^d ± 0.96	3.37 ^a ±0.66	2.96 ^{ab} ± 0.44
White maize	93.58 ±0.30	96.51 ^b ± 0.02	8.03 ^f ± 0.05	3.39 ^b ±0.01	3.49 ^a ± 0.02
Wheat	91.81 ±0.07	96.11 ^b ± 0.43	14.50 ^a ± 0.01	1.59 ^d ±0.06	3.38 ^a ± 0.04
Pearl millet	91.28 ±0.70	96.54 ^b ± 0.02	10.63 ^c ± 0.02	2.70 ^b ±0.06	3.45 ^a ±0.002
White jowar	91.87 ±0.30	96.71 ^b ± 0.00	9.29 ^e ± 0.01	2.06 ^c ± 0.07	3.28 ^a ±0.001
Red jowar	91.02 ±0.96	97.82 ^a ±0.007	9.10 ^e ± 0.01	2.35 ^c ± 0.05	2.17 ^b ±0.001
Barley	92.41 ±0.98	97.00 ^{ab} ± 0.14	9.09 ^e ± 0.01	2.21 ^c ± 0.05	3.00 ^{ab} ± 0.14
Oats	90.91 ±0.40	97.11 ^{ab} ± 0.40	11.67 ^b ± 0.06	3.65 ^a ± 0.17	2.89 ^{ab} ± 0.04

Means bearing different superscripts differ significantly along the column (P<0.01)

Table 4.1.2 Proximate compositions of green fodders (% DM)

Feed	DM	OM	CP	EE	TA
Maize	11.81 ^c ± 0.70	92.52 ^a ± 0.09	10.16 ^c ± 0.10	1.45 ^b ± 0.00	9.49 ^c ±0.70
Sorghum	19.64 ^a ± 0.40	91.01 ^a ± 0.09	9.78 ^d ± 0.01	2.28 ^b ± 0.20	8.98 ^c ±0.40
Pearl millet	16.40 ^b ± 0.08	86.53 ^c ± 0.40	9.63 ^d ± 0.00	2.44 ^{ab} ± 0.04	13.47 ^a ±0.08
Oats	10.78 ^c ± 0.64	89.15 ^b ± 0.13	16.22 ^b ± 0.03	3.62 ^a ± 0.03	10.84 ^b ±0.64
Berseem	12.58 ^c ± 0.07	86.4 ^c ± 0.01	19.03 ^a ± 0.10	2.37 ^{ab} ± 0.02	13.83 ^a ±0.07

Means bearing different superscripts differ significantly along the column (P<0.01)

Table 4.1.3 Proximate compositions of range grasses (% DM)

Feed	DM	OM	CP	EE	TA
Hybrid Napier	14.89 ^e ± 0.18	87.66 ^f ± 0.00	10.24 ^a ±0.00	1.56 ^{ab} ± 0.09	12.33 ^a ±0.00
Bermuda	32.34 ^a ± 0.17	87.82 ^f ± 0.08	10.21 ^a ± 0.02	2.09 ^a ± 0.03	12.17 ^a ± 0.08
Setaria	26.27 ^{cd} ± 0.28	90.55 ^d ±0.02	6.49 ^e ± 0.02	2.26 ^a ± 0.21	9.45 ^c ± 0.02
Para	24.41 ^d ± 0.28	89.05 ^e ± 0.09	8.72 ^c ± 0.00	1.85 ^{ab} ± 0.03	10.94 ^b ± 0.04
Guinea	27.92 ^{bc} ± 0.06	92.17 ^c ± 0.07	9.62 ^b ±0.00	1.86 ^{ab} ± 0.02	7.82 ^d ± 0.07
Lemon	28.28 ^b ± 0.05	93.73 ^b ± 0.00	8.17 ^d ± 0.01	2.47 ^a ± 0.02	6.26 ^e ± 0.00
Vetiver	28.45 ^b ± 0.12	96.36 ^a ± 0.17	5.57 ^f ± 0.11	1.16 ^b ± 0.05	5.63 ^f ± 0.17

Means bearing different superscripts differ significantly along the column (P<0.01)

Table 4.1.4 Proximate compositions of oil cakes and meals (%DM)

Feed	DM	OM	CP	EE	TA
GNC	92.29 ^b ± 0.38	93.33 ^c ± 0.20	41.37 ^d ± 0.23	7.78 ^b ± 0.04	6.67 ^a ± 0.45
De-oiled GNC	92.07 ^b ± 0.22	93.26 ^c ± 0.01	42.27 ^c ± 0.24	1.64 ^d ± 0.01	6.73 ^a ± 0.19
Mustard cake	92.64 ^b ± 0.21	92.83 ^c ± 0.39	35.47 ^f ± 0.03	9.74 ^a ± 0.02	7.17 ^a ± 0.19
DOMC	93.55 ^a ± 0.14	93.48 ^{bc} ± 0.32	39.24 ^e ± 0.21	1.64 ^d ± 0.06	6.51 ^{ab} ± 0.42
SBM	88.48 ^c ± 0.38	92.47 ^c ± 0.08	50.17 ^a ± 0.41	1.23 ^d ± 0.04	7.52 ^a ± 0.04
CSC	93.66 ^a ± 0.17	95.52 ^a ± 0.01	24.15 ^g ± 0.24	6.74 ^b ± 0.06	4.48 ^c ± 0.03
Guar meal	92.49 ^b ± 0.20	94.78 ^{ab} ± 0.01	48.99 ^b ± 0.06	5.28 ^c ± 0.10	5.25 ^{bc} ± 0.37

Means bearing different superscripts differ significantly along the column (P<0.01)

Table 4.1.5 Proximate compositions of agro industrial by- products and cereal crop residues (% DM)

Feed	DM	OM	CP	EE	TA
Wheat bran	91.31 ^{cd} ± 0.42	95.83 ^{bc} ± 0.11	16.03 ^d ± 0.00	1.62 ^g ± 0.26	4.19 ^{fg} ± 0.11
Rice bran	93.46 ^a ± 0.28	85.46 ^g ± 0.07	9.27 ^g ± 0.09	6.80 ^b ± 0.20	14.90 ^b ± 0.07
DORB	89.47 ^f ± 0.12	90.55 ^f ± 0.02	11.46 ^f ± 0.04	1.47 ^g ± 0.08	9.43 ^c ± 0.02
Rice polish	91.53 ^{bcd} ± 0.4	91.16 ^f ± 0.00	13.47 ^e ± 0.29	12.3 ^a ± 0.15	8.84 ^c ± 0.00
MGM	91.59 ^{bc} ± 0.23	94.58 ^d ± 0.09	64.26 ^a ± 0.55	5.86 ^c ± 0.19	5.42 ^e ± 0.09
Gram churi	92.49 ^{ab} ± 0.21	95.30 ^c ± 0.01	16.65 ^d ± 0.00	3.55 ^d ± 0.46	4.70 ^f ± 0.01
Gram husk	91.22 ^{ecd} ± 0.16	95.87 ^{ab} ± 0.11	3.51 ⁱ ± 0.08	0.25 ^h ± 0.02	3.79 ^{gh} ± 0.11
Gram khanda	90.23 ^{ef} ± 0.15	95.76 ^{bc} ± 0.10	9.96 ^g ± 0.28	1.41 ^g ± 0.11	4.23 ^{fg} ± 0.01
DDGS	90.57 ^{cd} ± 0.14	95.13 ^{dc} ± 0.10	44.05 ^b ± 0.07	3.46 ^d ± 0.02	4.69 ^{ef} ± 0.11
Wheat straw	89.48 ^f ± 0.23	92.56 ^e ± 0.46	4.62 ^h ± 0.01	1.72 ^{fg} ± 0.28	7.44 ^d ± 0.46
Paddy straw	92.94 ^a ± 0.14	82.97 ^h ± 0.21	4.39 ^h ± 0.07	2.71 ^{de} ± 0.10	17.01 ^a ± 0.21
Beet pulp	10.39 ^h ± 0.09	93.25 ^e ± 0.20	10.95 ^f ± 0.00	1.45 ^g ± 0.04	6.74 ^d ± 0.20
Distillery bran	18.56 ^g ± 0.31	96.80 ^a ± 0.20	18.16 ^c ± 0.09	2.57 ^{ef} ± 0.32	3.19 ^h ± 0.20

Means bearing different superscripts differ significantly along the column (P<0.01)

4.1.7 Cell wall and cell wall bound protein constituents of green fodders (% DM)

Data regarding cell wall constituents of cereal and legume fodders are presented in table 4.1.7. Sorghum had the highest NDF, ADF and cellulose content among green fodders (64.32, 41.04 and 34.22%, respectively). Prusty (2011) and Kamble *et al.* (2011) reported comparable values as revealed in the current study. Das *et al.*, (2007) reported similar values of NDF (68.65), lignin (3.97) and cellulose (26.28) for maize fodder and ADF (38.07) for sorghum fodder. NDICP and ADICP varied significantly ($P<0.01$) among green fodders, the lowest being in sorghum (3.10, 0.98%) and highest in maize and berseem (6.03, 2.83%, respectively).

4.1.8 Cell wall and cell wall bound protein constituents of range grasses (% DM)

The data are presented in table 4.1.8. Highest NDF was observed in Vetiver (86.33%) and lowest in Para grass (70.45%). ADF content (%) varied between 30.28 (Bermuda) and 45.48 (Vetiver) and hemicellulose between 4.22 (Setaria) and 8.67 (hybrid Napier). Cellulose and lignin content in Guinea grass and hybrid Napier reported by Shalini (2008) was higher than present findings. The fiber bound protein fractions viz: NDICP, ADICP varied significantly ($P<0.01$) the highest being in hybrid Napier and Para (6.87, 2.96%) and lowest in guinea and Setaria (2.52, 1.19%), respectively.

4.1.9 Cell wall and cell wall bound protein constituents of oil cakes and meals (% DM)

The cell wall and fiber bound protein constituents are presented in table 4.1.9 and differed significantly ($P<0.01$) in the group. NDF, ADF, lignin and cellulose content of CSC was the highest among the cakes (47.58, 37.33, 11.50 and 25.68%, respectively) whereas, in extracted SBM the NDF, ADF and lignin (13.93, 9.67 and 1.40%, respectively) was the lowest. Gupta *et al.* (2011) reported lower NDF value for GNC and CSC, but higher value for soybean cake. However NDF value for MOC was comparable to present findings. Prusty (2011) and Kamble *et al.* (2010) reported higher fiber fractions (NDF and ADF) of GNC, MOC and soybean meal. However their lignin, cellulose and hemicellulose content were lower than present reports. NDICP (%) varied from 1.08 (guar meal) to 4.46 (MOC). ADICP was also lowest in guar meal (0.59%) and highest in de-oiled GNC (1.36%).

4.1.10 Cell wall and cell wall bound protein constituents of agro industrial by-products and cereal crop residues (% DM)

The data regarding fiber fractions of agro industrial by product are presented in table 4.1.10. The NDF, ADF, lignin cellulose and hemicellulose content ranged from 15.14 (rice

bran) to 75.65 (gram husk), 5.35 (distillers dried grain soluble) to 71.23 (gram husk), 1.24 (distillers dried grain soluble) to 23.34 (gram husk), 2.81 (MGM) to 44.57 (wheat straw) and 1.67 (paddy straw) to 58.99 (MGM). Kamble *et al.* (2010) reported significantly higher NDF, ADF and lignin values of wheat bran, DORB and rice polish than the present results. Gupta *et al.* (2011) reported higher NDF and ADF values of DORB, but the corresponding values for wheat bran were comparable to present study. Jeya Prakash *et al.* (2003) reported similar NDF, ADF and hemicellulose values for wheat straw. Among agro industrial by products, distillers dried grain soluble had highest NDICP (9.69%) and ADICP (8.70%) content whereas gram khanda (1.03%) and beet pulp (0.36%), respectively.

4.2 Energy values of the feed ingredients

4.2.1 Energy values of grains

Energy values of grains are presented in table 4.2.1. TDN content of all grains was recorded more than 80% except barley, which recorded 78.67%. DE and ME were slightly differed between grains with highest in yellow maize (15.90 and 15.50 MJ/kg) and lowest in barley (14.18 and 12.76 MJ/kg). NRC (2001) suggested similar TDN, DE and ME values of the tested grains except higher TDN value than present findings. Feed library of CNCPS (2003) reported similar TDN, DE and ME values as that of present study, but the TDN value of oat was lower as compared to the present findings.

4.2.2 Energy values of green fodders

The data pertaining to TDN, DE and ME content of green fodders have been presented in table 4.2.2. The td NFC and td CP content of berseem (30.84 - 17.90%) was highest among the green fodders and that of pearl millet (16.80, 9.07%) was lowest. The lowest to highest values of td NDF ranged from 17.29 (berseem) to 38.37 (sorghum) %. Oats had highest TDN, DE and ME (68.36, 12.60 and 10.84) value in the group. Prusty *et al.* (2012) reported lower TDN values for berseem and oat fodder as that of present study. Similar lower TDN content of green fodders was also reported both NRC (2001) and CNCPS (2003).

4.2.3 Energy values of range grasses

TDN, DE and ME contents of grasses are depicted in table 4.2.3. Para grass had the highest td NFC (10.79%) while Vetiver had the lowest. The td NDF (%) of the grasses ranged from 40.56 (Bermuda) to 54.24% (Vetiver). TDN, DE and ME of Setaria was highest (58.92, 10.86 and 9.09) and was comparable to that of Bermuda grass (50.21, 9.81 and 8.02). NRC

Table 4.1.6 Cell wall and cell wall bound protein constituents in grains (% DM)

Feed	NDF	ADF	ADL	Cellulose	Hemi-cellulose	NDICP	ADICP
Yellow maize	13.67 ^e ± 0.32	4.58 ^e ± 0.26	0.87 ^d ± 0.11	3.54 ^{ef} ± 0.01	6.53 ^{bc} ± 0.05	1.22 ^d ± 0.11	0.36 ^c ± 0.04
White maize	14.90 ^c ± 0.43	4.38 ^c ± 0.12	0.77 ^d ± 0.16	3.81 ^e ± 0.04	5.63 ^c ± 0.00	1.34 ^c ± 0.11	0.36 ^e ± 0.04
Wheat	14.63 ^c ± 0.19	2.87 ^d ± 0.03	1.19 ^c ± 0.04	1.66 ^g ± 0.08	14.2 ^a ± 0.01	1.41 ^c ± 0.10	0.68 ^b ± 0.05
Pearl millet	12.31 ^{de} ± 0.17	4.77 ^c ± 0.10	1.49 ^{bc} ± 0.24	2.85 ^f ± 0.01	7.93 ^b ± 0.00	1.64 ^b ± 0.11	0.75 ^a ± 0.05
White jowar	11.26 ^e ± 0.04	5.51 ^c ± 0.13	1.62 ^b ± 0.16	5.00 ^d ± 0.12	7.23 ^{bc} ± 0.00	1.17 ^d ± 0.11	0.57 ^c ± 0.04
Red jowar	12.16 ^{de} ± 0.14	5.41 ^c ± 0.02	1.47 ^{bc} ± 0.93	8.08 ^b ± 0.01	6.75 ^{bc} ± 0.01	1.19 ^d ± 0.12	0.68 ^b ± 0.05
Barley	25.90 ^a ± 0.15	13.3 ^a ± 0.02	3.92 ^a ± 1.36	9.37 ^a ± 0.01	6.88 ^{bc} ± 0.00	2.07 ^a ± 0.11	0.44 ^d ± 0.05
Oats	22.10 ^b ± 0.13	9.70 ^b ± 0.19	3.96 ^a ± 0.34	6.92 ^c ± 0.03	8.01 ^b ± 0.01	1.74 ^b ± 0.11	0.39 ^e ± 0.05

Means bearing different superscripts differ significantly along the column (P<0.01)

Table 4.1.7 Cell wall and cell wall bound protein constituents in green fodders (% DM)

Feed	NDF	ADF	ADL	Cellulose	Hemi-cellulose	NDICP	ADICP
Maize	62.56 ^b ± 0.01	39.36 ^a ± 0.50	5.79 ^c ± 0.03	33.94 ^a ± 0.00	8.70 ^c ± 0.30	6.03 ^a ± 0.00	2.01 ^b ± 0.15
Sorghum	64.32 ^a ± 0.27	41.04 ^a ± 0.70	6.30 ^b ± 0.01	34.22 ^a ± 0.05	7.49 ^c ± 0.15	3.10 ^e ± 0.00	0.98 ^d ± 0.15
Pearl millet	63.01 ^{ab} ± 0.60	35.29 ^b ± 0.30	4.85 ^d ± 0.50	30.72 ^c ± 0.01	7.18 ^d ± 0.45	5.68 ^c ± 0.01	1.38 ^c ± 0.15
Oats	54.13 ^c ± 0.18	33.70 ^b ± 0.08	1.47 ^e ± 0.02	32.23 ^b ± 0.07	12.60 ^b ± 0.45	3.97 ^d ± 0.01	1.32 ^c ± 0.15
Berseem	39.19 ^d ± 0.20	31.85 ^b ± 0.04	6.66 ^a ± 0.06	25.32 ^d ± 0.20	16.66 ^a ± 0.09	5.92 ^b ± 0.01	2.83 ^a ± 0.15

Means bearing different superscripts differ significantly along the column (P<0.01)

4.1.8 Cell wall and cell wall bound protein constituents in range grasses (% DM)

Feed	NDF	ADF	ADL	Cellulose	Hemi-cellulose	NDICP	ADICP
Hybrid napier	75.45 ^e ± 0.00	40.85 ^c ± 0.56	4.74 ^f ± 0.08	35.48 ^e ± 0.03	8.67 ^a ± 0.14	6.87 ^a ± 0.00	2.13 ^a ± 0.01
Bermuda	74.16 ^f ± 0.00	30.28 ^d ± 0.05	8.86 ^b ± 0.01	21.47 ^g ± 0.00	8.00 ^a ± 0.04	6.08 ^b ± 0.00	1.32 ^b ± 0.01
Setaria	79.55 ^b ± 0.00	42.64 ^b ± 0.00	4.21 ^g ± 0.04	38.43 ^b ± 0.00	4.22 ^d ± 0.27	3.36 ^e ± 0.01	1.19 ^b ± 0.01
Para	70.45 ^g ± 0.00	43.21 ^b ± 0.05	5.99 ^e ± 0.09	37.18 ^c ± 0.00	6.87 ^b ± 0.03	2.96 ^g ± 0.01	1.41 ^b ± 0.02
Guinea	77.63 ^d ± 0.00	42.55 ^b ± 0.8	6.33 ^d ± 0.09	35.97 ^d ± 0.01	7.76 ^{ab} ± 0.00	5.43 ^c ± 0.01	2.52 ^a ± 0.02
Lemon	77.84 ^c ± 0.00	39.67 ^c ± 0.04	11.18 ^a ± 0.07	27.93 ^f ± 0.00	5.68 ^c ± 0.51	4.60 ^d ± 0.01	2.42 ^a ± 0.02
Vetiver	86.33 ^a ± 0.00	45.48 ^a ± 0.27	6.71 ^c ± 0.17	38.97 ^a ± 0.00	4.41 ^d ± 0.24	3.20 ^f ± 0.01	1.52 ^b ± 0.03

4.1.9 Cell wall and cell wall bound protein constituents in oil cakes and meals (% DM)

Feed	NDF	ADF	ADL	Cellulose	Hemi-cellulose	NDICP	ADICP
GNC	9.62 ^g ± 0.00	9.39 ^d ± 0.12	1.80 ^e ± 0.12	7.50 ^g ± 0.48	33.59 ^e ± 0.45	3.28 ^b ± 0.01	0.71 ^c ± 0.13
De-oiled GNC	21.03 ^d ± 0.01	19.55 ^b ± 0.17	5.20 ^c ± 0.37	10.22 ^c ± 0.09	40.60 ^c ± 0.19	2.56 ^d ± 0.01	1.36 ^a ± 0.14
Mustard cake	21.34 ^b ± 0.00	19.45 ^b ± 0.13	4.05 ^d ± 0.06	11.50 ^b ± 0.42	25.72 ^f ± 0.40	4.46 ^a ± 0.01	1.35 ^a ± 0.14
DOMC	21.25 ^c ± 0.00	16.97 ^c ± 0.38	6.64 ^e ± 0.06	10.07 ^d ± 0.05	37.59 ^d ± 0.02	2.63 ^c ± 0.02	1.16 ^b ± 0.13
SBM	13.93 ^f ± 0.00	9.67 ^d ± 0.27	1.40 ^e ± 0.25	8.54 ^f ± 0.08	48.44 ^a ± 0.02	1.91 ^b ± 0.01	1.31 ^a ± 0.14
CSC	47.58 ^a ± 0.00	37.33 ^a ± 0.22	11.50 ^a ± 0.48	25.68 ^a ± 0.35	17.41 ^g ± 0.03	1.40 ^f ± 0.02	1.06 ^b ± 0.15
Guar meal	14.29 ^e ± 0.00	9.91 ^d ± 0.45	0.97 ^f ± 0.48	8.62 ^e ± 0.00	43.52 ^b ± 0.62	1.08 ^g ± 0.01	0.59 ^d ± 0.13

Means bearing different superscripts differ significantly along the column (P<0.01)

4.1.10 Cell wall and cell wall bound protein constituents in agro industrial by products and cereal crop residues (% DM basis)

Feed	NDF	ADF	ADL	Cellulose	Hemi-cellulose	NDICP	ADICP
Wheat bran	33.25 ^c ± 0.07	13.31 ^h ± 0.19	6.35 ^{de} ± 0.00	6.83 ^g ± 0.00	14.40 ^d ± 0.27	3.03 ^f ± 0.22	0.75 ^{def} ± 0.22
Rice bran	15.14 ^d ± 0.00	7.73 ⁱ ± 0.36	4.50 ^{ef} ± 0.01	2.89 ⁱ ± 0.00	2.47 ^{hi} ± 0.29	1.80 ^h ± 0.22	0.99 ^{cd} ± 0.22
DORB	35.39 ^c ± 0.02	15.06 ^g ± 0.2	8.14 ^{cd} ± 0.03	6.78 ^g ± 0.00	9.98 ^f ± 0.08	5.84 ^d ± 0.23	1.84 ^b ± 0.22
Rice polish	31.53 ^c ± 0.02	19.17 ^f ± 0.12	7.11 ^{dc} ± 0.02	12.05 ^f ± 0.06	12.26 ^e ± 0.13	1.27 ^j ± 0.22	0.77 ^{cdef} ± 0.23
MGM	16.43 ^d ± 0.00	6.26 ^j ± 0.08	3.39 ^{fg} ± 0.04	2.81 ⁱ ± 0.00	58.99 ^a ± 0.75	7.25 ^c ± 0.23	2.05 ^b ± 0.23
Gram churi	30.35 ^c ± 0.01	24.34 ^e ± 0.27	3.96 ^{fe} ± 0.00	21.08 ^d ± 0.04	13.10 ^c ± 0.45	1.52 ⁱ ± 0.22	0.95 ^{cde} ± 0.23
Gram husk	75.65 ^a ± 0.00	71.23 ^a ± 0.26	23.34 ^a ± 0.01	6.60 ^g ± 0.00	3.24 ^h ± 0.45	1.84 ^h ± 0.21	0.88 ^{cdef} ± 0.24
Gram khanda	48.88 ^b ± 0.00	42.29 ^c ± 0.00	15.06 ^b ± 0.01	27.28 ^c ± 0.05	8.55 ^g ± 0.06	1.03 ^k ± 0.23	0.86 ^{cdef} ± 0.24
DDGS	20.43 ^d ± 0.27	5.35 ^j ± 0.15	1.24 ^g ± 0.00	20.43 ^{de} ± 0.03	40.59 ^b ± 0.08	9.69 ^a ± 0.24	8.70 ^a ± 0.23
Wheat straw	74.47 ^a ± 0.00	53.48 ^b ± 0.44	9.23 ^c ± 0.00	44.57 ^a ± 0.01	2.89 ^h ± 0.04	1.65 ^{hi} ± 0.25	1.01 ^c ± 0.23
Paddy straw	72.36 ^a ± 0.10	38.86 ^d ± 0.02	4.37 ^{ef} ± 0.00	32.22 ^b ± 0.07	1.67 ⁱ ± 0.28	2.59 ^e ± 0.22	0.68 ^f ± 0.23
Beet pulp	43.77 ^b ± 0.02	23.12 ^c ± 0.34	4.07 ^{ef} ± 0.01	19.09 ^e ± 0.01	9.50 ^{fg} ± 0.04	3.63 ^e ± 0.25	0.36 ^g ± 0.25
Distillery bran	44.53 ^b ± 0.02	7.63 ⁱ ± 0.26	2.84 ^{fg} ± 0.01	4.67 ^h ± 0.05	15.59 ^c ± 0.02	8.24 ^b ± 0.24	0.71 ^{ef} ± 0.21

Means bearing different superscripts differ significantly along the column (P<0.01)

4.2.1 Energy values of grains

Feed	td NFC	td CP	td FA	td NDF	TDN (%DM)	DE (MJ/kg)	ME (MJ/kg)
Yellow maize	69.96 ^b ± 0.29	9.76 ^{bcd} ± 0.03	2.38 ^a ± 0.01	8.28 ^c ± 0.19	86.27 ^a ± 0.02	15.90 ^a ± 0.03	14.18 ^a ± 0.00
White maize	71.37 ^{ab} ± 0.03	7.88 ^d ± 0.04	1.40 ^c ± 0.04	9.00 ^c ± 0.23	84.39 ^b ± 0.17	14.81 ^b ± 0.03	13.08 ^b ± 0.03
Wheat	64.67 ^c ± 1.06	15.55 ^a ± 0.00	0.64 ^e ± 0.00	8.48 ^c ± 0.10	83.14 ^{bc} ± 0.00	15.32 ^{bc} ± 0.00	13.59 ^{bc} ± 0.00
Pearl millet	71.22 ^{ab} ± 0.17	10.33 ^{bc} ± 0.05	1.70 ^b ± 0.05	3.63 ^e ± 0.08	82.01 ^{cd} ± 0.14	15.11 ^{cd} ± 0.02	13.38 ^{cd} ± 0.03
White jowar	73.76 ^a ± 0.10	9.06 ^{cd} ± 0.06	1.02 ^d ± 0.01	3.41 ^e ± 0.15	81.54 ^d ± 0.18	15.03 ^d ± 0.03	13.30 ^d ± 0.03
Red jowar	72.58 ^a ± 0.59	8.83 ^{cd} ± 0.13	1.35 ^c ± 0.07	4.46 ^d ± 0.09	81.90 ^{cd} ± 0.58	15.09 ^{cd} ± 0.10	13.36 ^{cd} ± 0.10
Barley	60.64 ^c ± 0.01	8.92 ^{cd} ± 0.02	1.26 ^c ± 0.06	13.29 ^a ± 0.01	78.67 ^c ± 0.03	14.50 ^e ± 0.00	12.76 ^e ± 0.00
Oats	60.19 ^d ± 0.32	11.52 ^b ± 0.04	2.53 ^a ± 0.04	10.70 ^b ± 0.04	81.10 ^d ± 0.23	14.95 ^d ± 0.04	13.22 ^d ± 0.04

Means bearing different superscripts differ significantly along the column (P<0.01)

4.2.2 Energy values of green fodders

Feed	td NFC	td CP	td FA	td NDF	TDN (%DM)	DE (MJ/kg)	ME (MJ/kg)
Maize	21.90 ^b ± 0.20	9.36 ^c ± 0.01	0.63 ^e ± 0.01	35.46 ^c ± 0.01	61.37 ^{bc} ± 0.00	11.15 ^a ± 0.01	9.27 ^a ± 0.01
Sorghum	17.39 ^{cd} ± 0.30	9.37 ^c ± 0.01	1.18 ^b ± 0.00	38.37 ^a ± 0.26	60.79 ^{bc} ± 0.07	11.20 ^a ± 0.01	9.43 ^a ± 0.03
Pearl millet	16.80 ^d ± 0.60	9.07 ^d ± 0.01	1.77 ^b ± 0.06	37.13 ^b ± 0.04	60.00 ^{bc} ± 0.14	11.06 ^a ± 0.04	9.29 ^a ± 0.10
Oats	18.76 ^c ± 0.40	15.70 ^b ± 0.05	2.27 ^a ± 0.01	35.80 ^c ± 0.10	68.36 ^a ± 0.41	12.60 ^a ± 0.02	10.84 ^a ± 0.01
Berseem	30.84 ^a ± 0.30	17.90 ^a ± 0.06	1.38 ^c ± 0.02	17.29 ^d ± 0.19	62.13 ^{bd} ± 0.01	11.45 ^a ± 0.00	9.68 ^a ± 0.00

Means bearing different superscripts differ significantly along the column (P<0.01)

4.2.3 Energy values of range grasses

Feed	td NFC	td CP	td FA	td NDF	TDN (%DM)	DE (MJ/kg)	ME (MJ/kg)
Hybrid Napier	7.13 ^d ± 0.07	9.39 ^a ± 0.01	0.67 ^f ± 0.01	45.68 ^d ± 0.03	56.69 ^{bc} ± 0.06	10.45 ^{bc} ± 0.01	8.67 ^b ± 0.09
Bermuda	7.18 ^d ± 0.11	9.68 ^a ± 0.02	1.24 ^b ± 0.02	40.56 ^g ± 0.00	53.21 ^d ± 0.23	9.81 ^d ± 0.02	8.02 ^d ± 0.01
Setaria	5.68 ^e ± 0.26	6.02 ^d ± 0.00	1.05 ^c ± 0.00	51.85 ^b ± 0.01	58.92 ^a ± 0.04	10.86 ^a ± 0.01	9.09 ^a ± 0.04
Para	10.79 ^a ± 0.08	8.16 ^b ± 0.00	0.84 ^d ± 0.00	43.38 ^c ± 0.00	57.22 ^b ± 0.06	10.55 ^b ± 0.01	8.77 ^b ± 0.00
Guinea	8.33 ^c ± 0.07	8.62 ^b ± 0.00	0.86 ^d ± 0.01	46.51 ^c ± 0.01	58.39 ^a ± 0.31	10.76 ^a ± 0.05	8.99 ^a ± 0.01
Lemon	9.67 ^b ± 0.38	7.18 ^c ± 0.00	1.85 ^a ± 0.01	41.81 ^f ± 0.02	55.81 ^c ± 0.32	10.29 ^c ± 0.05	8.51 ^c ± 0.05
Vetiver	4.41 ^f ± 0.11	4.96 ^e ± 0.00	0.22 ^f ± 0.00	54.24 ^a ± 0.01	57.10 ^b ± 0.28	10.52 ^b ± 0.05	8.75 ^b ± 0.05

Means bearing different superscripts differ significantly along the column (P<0.01)

4.2.4. Energy values of oil cakes and meals

Feed	td NFC	td CP	td FA	td NDF	TDN (%DM)	DE (MJ/kg)	ME (MJ/kg)
GNC	37.09 ^a ± 0.12	41.08 ^d ± 0.00	6.46 ^b ± 0.01	2.76 ^f ± 0.00	88.46 ^a ± 0.13	16.30 ^a ± 0.02	14.59 ^a ± 0.02
De-oiled GNC	30.29 ^c ± 0.16	41.73 ^c ± 0.00	0.75 ^f ± 0.01	4.78 ^c ± 0.01	74.79 ^d ± 0.16	13.17 ^d ± 0.03	11.43 ^d ± 0.03
Mustard cake	30.30 ^c ± 0.13	34.92 ^f ± 0.00	8.61 ^a ± 0.02	4.40 ^e ± 0.00	85.55 ^b ± 0.11	15.11 ^b ± 0.02	13.38 ^b ± 0.02
DOMC	33.30 ^b ± 0.55	38.78 ^e ± 0.02	0.94 ^e ± 0.01	6.85 ^e ± 0.08	74.04 ^d ± 0.54	13.65 ^d ± 0.10	11.90 ^d ± 0.10
SBM	28.47 ^d ± 0.56	49.65 ^a ± 0.03	0.22 ^g ± 0.01	7.34 ^d ± 0.01	78.95 ^c ± 0.49	14.55 ^c ± 0.09	12.81 ^c ± 0.09
CSC	18.08 ^e ± 0.02	23.45 ^g ± 0.01	5.74 ^c ± 0.02	21.69 ^a ± 0.00	69.13 ^c ± 0.05	12.74 ^e ± 0.01	11.02 ^e ± 0.01
Guar meal	26.87 ^d ± 0.11	48.57 ^b ± 0.18	4.02 ^d ± 0.01	8.73 ^b ± 0.06	86.21 ^b ± 0.30	15.89 ^b ± 0.06	14.22 ^b ± 0.06

Means bearing different superscripts differ significantly along the column (P<0.01)

4.2.5 Energy values of agro industrial by- products and cereal crop residues

Feed	td NFC	td CP	td FA	td NDF	TDN (%DM)	DE (MJ/kg)	ME (MJ/kg)
Wheat bran	46.98 ^b ± 0.36	15.72 ^d ± 0.01	0.81 ^{df} ± 0.01	15.40 ^{fg} ± 0.01	72.92 ^{de} ± 0.27	13.44 ^{de} ± 0.05	11.69 ^{de} ± 0.07
Rice bran	53.16 ^a ± 0.11	8.83 ^h ± 0.06	5.94 ^b ± 0.00	5.40 ^{hi} ± 0.02	75.36 ^{cd} ± 0.07	13.81 ^{cd} ± 0.01	12.07 ^{cd} ± 0.03
DORB	47.23 ^b ± 0.04	10.72 ^f ± 0.01	5.77 ^b ± 0.01	13.12 ^g ± 0.01	76.06 ^{cd} ± 0.04	14.17 ^{cd} ± 0.05	11.53 ^{cd} ± 0.07
Rice polish	27.52 ^c ± 0.02	13.16 ^c ± 0.21	18.20 ^a ± 0.21	14.29 ^{fg} ± 0.03	89.12 ^a ± 0.12	16.42 ^a ± 0.02	14.71 ^a ± 0.03
MGM	15.01 ^f ± 0.09	63.58 ^a ± 0.03	4.41 ^c ± 0.04	3.27 ⁱ ± 0.04	85.12 ^b ± 0.13	15.69 ^b ± 0.20	13.97 ^b ± 0.02
Gram churi	45.34 ^{bc} ± 0.46	16.28 ^d ± 0.01	2.22 ^d ± 0.03	14.65 ^f ± 0.00	76.56 ^{cd} ± 0.31	14.11 ^{cd} ± 0.06	12.37 ^{cd} ± 0.08
Gram husk	18.25 ^f ± 0.00	3.16 ^j ± 0.07	0.22 ^h ± 0.00	29.87 ^c ± 0.00	44.28 ^h ± 0.07	8.36 ^h ± 0.01	6.05 ^h ± 0.01
Gram khanda	36.01 ^d ± 0.23	9.61 ^g ± 0.03	0.33 ^{gh} ± 0.00	19.29 ^e ± 0.01	58.68 ^f ± 0.06	10.81 ^f ± 0.01	9.04 ^f ± 0.01
DDGS	36.14 ^d ± 0.04	40.57 ^b ± 0.13	2.44 ^d ± 0.01	6.57 ^h ± 0.01	81.78 ^b ± 0.07	15.07 ^b ± 0.02	13.34 ^b ± 0.03
Wheat straw	13.33 ^f ± 0.25	4.22 ⁱ ± 0.00	0.52 ^{fg} ± 0.01	43.60 ^b ± 0.06	55.18 ^g ± 0.13	10.17 ^{fg} ± 0.02	8.39 ^{fg} ± 0.02
Paddy straw	5.91 ^g ± 0.10	4.12 ⁱ ± 0.01	1.79 ^c ± 0.01	47.00 ^a ± 0.01	54.05 ^g ± 0.07	9.96 ^g ± 0.01	8.18 ^g ± 0.01
Beet pulp	40.65 ^{cd} ± 0.02	10.85 ^f ± 0.01	0.45 ^{fg} ± 0.04	25.24 ^d ± 0.78	68.82 ^e ± 0.13	12.85 ^e ± 0.06	25.24 ^e ± 0.60
Distillery bran	39.83 ^d ± 0.29	17.88 ^f ± 0.07	1.80 ^c ± 0.01	23.82 ^d ± 0.17	77.16 ^c ± 0.32	77.16 ^c ± 0.06	11.41 ^c ± 0.06

Means bearing different superscripts differ significantly along the column (P<0.01)

(2001) and CNCPS (2003) reported slightly lower values of TDN, DE and ME compared to current results.

4.2.4. Energy values of oil cakes and meals

The data on TDN, DE and ME content of oil cake and meal are presented in table 4.2.4 and varied significantly ($P < 0.01$). GNC had the highest td NFC, TDN, DE and ME content (37.09, 88.46, 15.30 and 14.59, respectively), whereas CSC had the lowest TDN, DE and ME (69.13, 12.74 and 11.02) and highest td NDF (21.69%). Highest td CP was of SBM and MOC (49.65%) while lowest was of MOC (34.92%). In NRC (2001) comparable DE and ME but less TDN has been reported. Under CNCPS (2003) lower TDN for CSC but similar TDN for other cakes is reported.

4.2.5 Energy values of agro industrial by-products and cereal crop residues

Energy values of agro industrial by-products and straw have been presented in table 4.2.5. Highest td NFC was rice bran (53.16%) and lowest in paddy straw (5.91%). The td CP (%) of agro industrial by products ranged from 3.16 (gram husk) to 63.58 (MGM). The td FA was very high in rice polish (12.20%). Rice polish had highest TDN, DE and ME (89.12, 16.42 and 14.17, respectively). The lowest to highest values was content of td NDF from 3.27 (MGM) to 47.00 (paddy straw). NRC (2001) reported comparable value of TDN for wheat bran.

4.3 Rumen fermentation pattern of feed ingredients under *in vitro* condition

Nineteen feed ingredients were divided into three groups: green fodders, grasses and oil cakes. These feed ingredients were screened for IVDMD, IVOMD, net gas production and methane. Rumen fermentation pattern of ingredients are presented as below:

4.3.1 IVDMD%, IVOMD%, net gas production and methane production in green fodders incubated for 24 hrs

Data on digestibility and gas production parameters have been presented in table 4.3.1, revealed significant ($P < 0.05$) difference among green fodders. The IVDMD and IVOMD were maximum in pearl millet (71.40, 72.41%, respectively), whereas minimum in sorghum (56.54, 57.34%). Net gas production (ml/200mgDM and mmol/200mgDM) was recorded highest in berseem (36.60, 1.45) and lowest in oats (27.58, 1.09). Methane production (g/kg DM incubated and g/ kg IVDMD) ranged from 21.26 (maize) to 28.11 (sorghum) and 30.79 (oats) to 37.41 (sorghum). Khandaker *et al.* (1994) reported similar IVDMD and IVOMD values for maize fodder whereas Assefa *et al.*, (2001) reported lower

IVDMD AND IVOMD of oats. Krishnamoorthy *et al.* (1995) recorded higher methane production in maize than the present study. The digestibility of DM and OM was directly related with methane production and with the increase in IVDMD the methane production was reduced. The data revealed the superiority of oats over maize as a choice of feeding for methane reduction.

4.3.2 IVDMD%, IVOMD%, net gas production and methane production in range grasses incubated for 24 hrs

Data are presented in table.4.3.2. Rumen fermentation pattern of feed ingredients under *in vitro* condition differed significantly ($P<0.05$) among of grass groups. The lowest IVDMD and IVODM were observed in Vetiver (38.40, 38.85%, respectively) and highest in hybrid Napier (64.17, 65.19%). Net gas production (ml/200mg DM and mmol/200mg DM) was lowest in Vetiver (8.84, 0.35) and highest in hybrid Napier (28.71, 1.15). Hybrid Napier also had methane production highest (14.30, 22.29) comparable to others. Khanum *et al.*, (2007) reported lower methane production of Para grass and hybrid Napier than the present study.

4.3.3 IVDMD%, IVOMD%, net gas production and methane production in oil cakes and meals incubated for 24 hrs

Data pertaining to the digestibility and gas production have been presented in table 4.3.3. The highest values of IVDMD and IVOMD were in guar meal (87.60 and 88.48%) and the lowest in CSC (57.18 and 59.43%). However net gas production (ml/200mgDM and mmol/200mgDM) was highest in DOMC (42.69, 1.71) and lowest in CSC (29.71, 1.19). DOMC also had lowest methane production (16.13, 19.10) while it was highest in SBM (25.59, 27.23). In support of present findings Srinivas and Gupta (1994) reported methane emissions of 24.6, 23.6 and 39% of total gas production in GNC, mustard cake and CSC respectively. Lee *et al* (2003) reported the methane emissions of 7.14 and 4.48 ml/0.2 g DM in SBM and CSC.

4.4 *In vivo* experiment

4.4.1 Chemical composition of experimental diets (%DM)

Detailed chemical composition of concentrate mixtures, oats green, wheat straw and total mixed rations (TMR) are given in table 4.4.1. The concentrate mixture, oats green, wheat straw and TMR had CP content of 20.04, 10.69, 3.15 and 11.50%, respectively. The NDF, ADF, NDICP and ADICP content of the TMR was 54.33, 32.11, 2.1 and 0.72 %, respectively.

4.3.1 IVDMD%, IVOMD%, net gas production and methane production in green fodders incubated for 24 hrs

Feed	IVDMD%	IVOMD%	Gas production (ml/200mg)	Gas production (mmol/200mg)	Methane (g/kg DM)	Methane (g/kg IVDMD)
Maize	65.12 ^{bc} ±0.35	65.79 ^{bc} ±0.29	29.63 ^c ±0.50	1.18 ^c ±0.04	21.26 ^d ±0.40	32.60 ^c ±0.14
Sorghum	56.54 ^d ±0.24	57.34 ^c ±0.13	35.74 ^{ab} ±0.13	1.40 ^b ±0.03	28.11 ^a ±0.53	37.41 ^a ±0.70
Pearl millet	71.40 ^a ±0.06	72.41 ^a ±0.48	35.20 ^b ±0.71	1.41 ^b ±0.00	23.10 ^{bc} ±0.83	35.87 ^b ±0.28
Oats	66.45 ^b ±0.13	67.84 ^b ±0.05	27.58 ^d ±0.08	1.09 ^d ±0.01	24.14 ^b ±0.28	30.79 ^{bc} ±0.41
Berseem	68.33 ^b ±0.18	71.30 ^{ab} ±0.07	36.60 ^a ±0.25	1.45 ^a ±0.05	23.37 ^{bc} ±0.48	38.14 ^{bc} ±0.75

Means bearing different superscripts differ significantly along the column (P<0.05)

4.3.2 IVDMD%, IVOMD%, net gas production and methane production in range grasses incubated for 24 hrs

Feed	IVDMD%	IVOMD%	Gas production (ml/200mg)	Gas production (mmol/200mg)	Methane (g/kg DM)	Methane (g/kg IVDMD)
Hybrid napier	64.17 ^a ±0.20	65.19 ^a ±0.41	28.71 ^a ±0.82	1.15 ^a ±0.03	14.30 ^a ±0.47	22.29 ^a ±0.73
Bermuda	48.49 ^d ±0.65	51.42 ^c ±0.57	22.71 ^{cd} ±0.41	0.91 ^{bc} ±0.02	12.55 ^b ±0.34	21.45 ^{ab} ±0.30
Setaria	55.27 ^c ±0.43	56.11 ^c ±0.33	14.37 ^d ±0.25	0.58 ^d ±0.22	7.10 ^d ±0.03	12.85 ^d ±1.24
Para	60.52 ^b ±0.40	61.15 ^b ±0.95	27.04 ^b ±0.77	0.88 ^c ±0.19	10.10 ^c ±0.75	16.69 ^c ±0.89
Guinea	55.04 ^c ±0.24	56.54 ^c ±0.30	23.04 ^c ±0.12	0.92 ^b ±0.08	11.39 ^{bc} ±1.11	19.62 ^b ±1.92
Lemon	48.22 ^d ±0.68	48.38 ^d ±0.93	11.46 ^e ±0.16	0.46 ^e ±0.25	6.17 ^e ±1.12	12.80 ^d ±0.48
Vetiver	38.40 ^e ±0.20	38.85 ^e ±0.16	8.84 ^f ±0.04	0.35 ^f ±0.08	6.25 ^f ±0.96	16.28 ^c ±0.50

Means bearing different superscripts differ significantly along the column (P<0.05)

4.3.3 IVDMD%, IVOMD%, net gas production and methane production in oil cakes and meals incubated for 24 hrs

Feed	IVDMD%	IVOMD%	Gas production (ml/200mg)	Gas production (mmol/200mg)	Methane (g/kg DM)	Methane (g/kg IVDMD)
GNC	85.90 ^b ±0.37	86.68 ^b ±0.48	37.35 ^{cd} ±0.02	1.69 ^b ±0.02	21.56 ^a ±0.79	24.91 ^b ±0.85
Deoiled GNC	78.65 ^d ±0.28	79.29 ^d ±0.24	35.60 ^d ±0.59	1.40 ^c ±0.04	19.45 ^b ±2.58	21.32 ^{cd} ±0.29
Mustard cake	82.23 ^c ±0.73	83.08 ^c ±0.08	38.18 ^c ±0.31	1.49 ^c ±0.01	18.08 ^c ±0.40	22.11 ^{cd} ±0.46
DOMC	74.98 ^e ±0.29	75.31 ^e ±0.23	42.69 ^a ±0.50	1.71 ^a ±0.02	16.13 ^d ±0.50	19.10 ^d ±0.94
SBM	85.59 ^b ±0.45	86.27 ^b ±0.20	40.77 ^b ±0.61	1.04 ^{de} ±0.06	25.59 ^a ±0.95	27.23 ^a ±2.04
CSC	57.18 ^f ±0.37	59.43 ^f ±0.07	29.71 ^f ±0.85	1.19 ^d ±0.03	21.12 ^{ab} ±0.57	21.43 ^c ±0.74
Guar meal	87.60 ^a ±0.56	88.48 ^a ±0.48	32.13 ^e ±0.87	1.18 ^d ±0.03	18.75 ^c ±0.19	19.61 ^d ±0.20

Means bearing different superscripts differ significantly along the column (P<0.05)

Table 4.4.1 Composition of experimental diets (%DM)

Nutrients	Concentrate mixture	Oats green	Wheat straw	TMRs
DM	89.66 ± 0.32	22.09 ± 0.75	90.81 ± 0.17	56.56 ± 0.70
OM	90.94 ± 0.43	90.72 ± 0.06	92.39 ± 0.61	89.81 ± 0.01
CP	20.04 ± 0.54	10.69 ± 0.06	3.15 ± 0.06	11.50 ± 0.06
EE	2.49 ± 0.03	1.99 ± 0.02	0.85 ± 0.01	2.27 ± 0.04
Total ash	9.06 ± 0.43	9.28 ± 0.06	7.61 ± 0.61	10.19 ± 0.01
CHO	68.41 ± 0.99	80.04 ± 0.12	88.39 ± 0.54	76.04 ± 0.12
NFC	38.57 ± 0.94	16.39 ± 0.12	12.23 ± 0.54	23.81 ± 0.02
NDF	31.54 ± 0.05	65.88 ± 0.01	78.66 ± 0.01	54.33 ± 0.20
ADF	11.11 ± 0.56	38.72 ± 0.10	51.28 ± 0.56	32.11 ± 0.19
NDICP	1.69 ± 0.20	2.24 ± 0.01	2.50 ± 0.04	2.10 ± 0.01
ADICP	0.67 ± 0.08	1.83 ± 0.15	1.27 ± 0.01	0.72 ± 0.01
Hemicellulose	20.43 ± 0.50	27.16 ± 0.01	27.38 ± 0.55	22.22 ± 0.19
Cellulose	9.15 ± 0.05	34.82 ± 0.03	46.07 ± 1.40	29.08 ± 0.77
ADL	2.32 ± 0.04	4.48 ± 0.22	8.11 ± 0.95	4.02 ± 0.01
td NFC	37.80 ± 0.92	16.07 ± 0.12	11.99 ± 0.53	23.33 ± 0.02
td CP	19.77 ± 0.54	6.75 ± 0.04	1.94 ± 0.06	11.21 ± 0.06
td FA	1.49 ± 0.03	0.99 ± 0.01	0.52 ± 0.02	1.27 ± 0.04
td NDF	16.88 ± 0.07	36.81 ± 0.39	39.60 ± 1.46	29.62 ± 0.01
TDN	70.80 ± 0.24	54.86 ± 0.32	46.33 ± 0.74	60.02 ± 0.05
DE(MJ/kg)	13.05 ± 0.05	10.11 ± 0.06	8.58 ± 0.38	11.06 ± 0.01
ME(MJ/kg)	11.30 ± 0.05	8.33 ± 0.06	6.78 ± 0.38	9.29 ± 0.01

respectively. The TDN and metabolizable energy (ME) content of the TMR was 60.02 % and 9.29 MJ/kg.

4.4.2 Individual variation in maintenance energy requirements and efficiency of Murrah buffalo calves

Data regarding energy requirements and efficiency have been presented in table 4.4.2. The ME intake of animals varied from 3.08 to 7.91 Mcal/d. The recommended ME intake by ICAR standard calculated was between 4.17 and 9.17 Mcal/d. Actual as well as the expected ME requirements, ME requirements for maintenance and ME requirements for growth were showing considerable individual variation between the calves. Maintenance variability coefficient (Mcal ME_m/100kg BW) varied from 2.86 to 4.42 Mcal/d among the animals. Efficiency for utilization of ME for growth (g weight gain/Mcal ME_g) was showing individual differences which varied from 213.06 to 933.67 g/ Mcal ME_g. Individual differences existed in the residual feed intake which was estimated as the difference between actual and expected ME_m of animals. RFI calculated by regression was also showing individual variation and the animals with low RFI (more efficient) had low ME_m compared to the animals with high RFI (less efficient). Variation in ME requirements among buffalo calves indicated that the opportunity to select buffalo calves with lower ME requirements and better efficiency of energy utilization for weight gain is possible within a herd. Similar variations within herd have been reported earlier (Ferrell and Jenkins, 1985). It has been reported that ME requirements in beef cattle is heritable (Hotovy *et al.*, 1991).

4.4.3 Individual variation in feed intake and feed efficiency among buffalo calves

Data regarding feed intake, growth rate and feed efficiency traits for eighteen Murrah buffalo calves during the fifty two days feeding trial are shown in table 4.4.3. Maintenance variability coefficient averaged 3.48 Mcal/100kg BW/day and ranged from 2.09 to 4.47 Mcal/100kg BW/day, representing a difference of 2.38 Mcal/kg BW/day of ME_m between the highest and least ranked animals. Figure 3 summarizes the variation of DMI/100kg BW of buffalo calves with difference in ME_m/100kg BW. Animals which were having low ME_m were consuming less feed than the animals which were having high ME_m. Considerable individual variation existed among the buffalo calves in terms of DMI and feed efficiency traits like RFI and FCR.

4.4.4 Feed intake and feed efficiency in buffalo calves with different maintenance variability coefficients

Out of eighteen buffalo calves nine animals were showing low $ME_m/100\text{kg BW}$ (mean = 3.04 Mcal), they were grouped into low ME_m group. The remaining nine animals which were having high $ME_m/100\text{kg BW}$ values (mean RFI = 3.92 Mcal) were assigned into high ME_m group (table 4.4.4). Mean DMI (g/100kg of BW, g/kg of $BW^{0.75}$) during the feeding trial period were significantly different ($P < 0.05$) among the high and low ME_m groups. Animals which were having low ME_m were showing DMI of 2642.98 g/100kg of BW which was lower than animals having high ME_m (2788.28 g/100kg BW). The DMI (g/kg $BW^{0.75}$) in both groups during the feeding trial period were estimated to be 80.65 and 84.99 g/kg of $BW^{0.75}$, respectively. Initial body weights, final body weight, mid test metabolic body weight and growth rate were not found to be significantly different among the two groups. Feed conversion ratio in low and high ME_m groups was found to be 4.49 and 5.07, respectively which were not statistically different among the two groups.

4.4.5 Nutrients digestibility in buffalo calves

Nutrient digestibility (%) in low and high ME_m groups is presented in table 4.4.5. DM digestibility was almost similar in both groups i.e. 65.58 and 64.17% for low and high ME_m groups, respectively. There was no significant difference in OM digestibility, CP digestibility and EE digestibility values between the low and high RFI groups. Higher NDF digestibility was recorded in low ME_m (63.29%) than high ME_m (60.86%) groups, but the values were not significantly different among both groups. Similar trend was observed in digestibility of ADF.

4.4.6 Nitrogen balance in buffalo calves

Nitrogen balance in low and high ME_m groups during metabolism trial have been presented in table 4.4.6. N intake, N voided in faeces and urine and total N out go did not differ significantly among groups. N balance was 18.32 g/day in low ME_m and 14.40 g/day in high ME_m group. However it was also not statistically different among groups. Absorbed N as % of N intake, retained N as % of N intake and N absorbed did not differ significantly among groups.

Table 4.4.2 Individual variation in energy requirements and efficiency of Murrah buffalo calves (Mcal/d)

Animal No	MEI std	ME _m std	ME _g std	MEI actual	ME _m actual	ME _g actual	RFI (actual ME _m - expected ME _m)	ME _m /100kg BW
6566	6.44 ^f ±0.30	4.29 ^{fg} ±0.10	2.15 ^{ef} ±0.21	6.12 ^{cde} ±0.42	3.97 ^a ±0.45	1.83 ^{de} ±0.38	-0.32±0.45	4.25 ^a ±0.49
6567	9.49 ^a ±0.73	5.21 ^a ±0.15	4.28 ^a ±0.68	7.73 ^a ±0.33	3.46 ^{cde} ±0.71	2.53 ^b ±0.24	-1.75±0.71	2.86 ^{ab} ±0.56
6568	6.90 ^d ±0.33	4.12 ^{fg} ±0.11	2.78 ^{bc} ±0.22	6.10 ^{cde} ±0.18	3.31 ^{cdef} ±0.12	1.98 ^d ±0.09	-0.80±0.19	3.79 ^{ab} ±0.22
6570	6.20 ^g ±0.32	3.97 ^{hi} ±0.09	2.23 ^{de} ±0.27	4.94 ^{efg} ±0.19	2.71 ^{cdef} ±0.25	0.98 ^{fg} ±0.19	-1.25±0.30	3.25 ^{ab} ±0.35
6571	6.70 ^{gh} ±0.41	4.49 ^{cd} ±0.10	2.21 ^e ±0.44	6.66 ^{bcd} ±0.42	4.44 ^a ±0.72	2.17 ^{cd} ±0.38	-0.05±0.67	4.42 ^a ±0.37
6572	7.97 ^c ±0.49	4.82 ^{bc} ±0.15	3.14 ^b ±0.43	7.10 ^{abc} ±0.43	3.96 ^a ±0.55	2.28 ^c ±0.36	-0.87±0.53	3.62 ^{ab} ±0.47
6573	9.17 ^b ±0.30	5.04 ^{ab} ±0.15	4.13 ^{ab} ±0.18	7.91 ^a ±0.38	3.78 ^{ab} ±0.33	2.87 ^{ab} ±0.33	-1.26±0.33	3.29 ^{ab} ±0.29
6574	5.26 ^k ±0.36	3.71 ^{ij} ±0.07	1.55 ^g ±0.37	4.14 ^{hi} ±0.19	2.58 ^{cdefg} ±0.28	0.43 ^{hi} ±0.19	-1.12±0.25	3.33 ^{ab} ±0.32
6575	6.63 ^e ±0.28	4.10 ^{fg} ±0.13	2.53 ^d ±0.23	6.30 ^{cde} ±0.28	3.77 ^{ab} ±0.42	2.20 ^c ±0.18	-0.33±0.37	4.30 ^a ±0.39
6576	5.83 ^h ±0.37	3.62 ^{ij} ±0.10	2.21 ^e ±0.32	4.41 ^h ±0.27	2.20 ^{efg} ±0.32	0.80 ^g ±0.21	-1.41±0.31	2.97 ^{ab} ±0.39
6577	5.11 ^l ±0.43	3.10 ^k ±0.10	2.00 ^f ±0.35	3.26 ^{ij} ±0.29	1.26 ^g ±0.43	0.16 ⁱ ±0.26	-1.84±0.48	2.14 ^c ±0.73
6578	5.52 ⁱ ±0.18	3.7 ^{ij} ±0.11	1.81 ^{gh} ±0.12	4.26 ^h ±0.17	2.45 ^{defg} ±0.19	0.54 ^h ±0.13	-1.26±0.18	3.18 ^{ab} ±0.23
6579	8.83 ^{bc} ±0.38	4.62 ^{cd} ±0.17	4.21 ^a ±0.33	7.54 ^{ab} ±0.45	3.34 ^{cdef} ±0.59	2.92 ^a ±0.31	-1.28±0.50	3.21 ^{ab} ±0.49
6580	5.48 ⁱ ±0.19	3.56 ^j ±0.08	1.92 ^g ±0.13	4.61 ^{fg} ±0.37	2.69 ^{cdef} ±0.40	1.05 ^{fg} ±0.35	-0.87±0.40	3.69 ^{ab} ±0.56
6583	7.09 ^d ±0.40	4.39 ^e ±0.12	2.70 ^c ±0.34	5.78 ^{de} ±0.35	3.08 ^{cdef} ±0.23	1.38 ^e ±0.33	-1.32±0.25	3.22 ^{ab} ±0.26
6587	4.17 ^k ±0.18	3.00 ^k ±0.05	1.16 ⁱ ±0.13	3.08 ^k ±0.41	1.92 ^{fg} ±0.42	0.08 ^j ±0.42	-1.09±0.45	3.33 ^{ab} ±0.75
6588	5.98 ^{gh} ±0.30	3.75 ^{ij} ±0.11	2.23 ^{ed} ±0.30	5.55 ^{ef} ±0.29	3.32 ^{cdef} ±0.581	1.80 ^d ±0.22	-0.43±0.46	4.21 ^a ±0.57
6589	5.71 ^{hi} ±0.29	3.59 ^j ±0.10	2.12±0.26	4.73 ^{fg} ±0.18	2.61 ^{cdefg} ±0.40	1.14 ^f ±0.15	-0.98±0.39	3.54 ^{ab} ±0.54

Means bearing superscripts differ significantly within a column (P < 0.05)

Table 4.4.3 Individual variation in feed intake and feed efficiency in buffalo calves

Animal no	BW (kg)	BW ^{0.75} (kg)	DMI(g)	DMI/100 kg BW (g)	Growth rate(g/d)	ME _m per 100kg BW	FCR	RFI calculated by regression
6566	94.17	30.23	2718.61	2887.04	446	4.24	6.1	0.23
6567	120.99	36.48	3229.92	2669.58	825	2.86	3.92	-0.2
6568	88.9	28.95	2576.2	2897.99	506	3.75	5.09	0.14
6570	84.74	27.93	2175.46	2567.15	442	3.21	4.92	-0.11
6571	99.48	31.5	2519.81	2533.08	506	4.47	4.98	-0.15
6572	109.76	33.91	3063.84	2791.4	660	3.63	4.64	0.03
6573	115.54	35.24	3206.31	2774.96	739	3.28	4.34	-0.02
6574	77.37	26.09	2010.09	2598.16	321	3.35	6.26	0.01
6575	88.19	28.78	2564.97	2908.32	589	4.3	4.35	0.06
6576	74.27	25.3	2070.27	2787.57	413	2.99	5.01	0.15
6577	60.76	21.76	1579.14	2598.98	405	2.09	3.9	-0.12
6578	77.56	26.14	2053.61	2647.7	492	3.17	4.17	-0.12
6579	102.49	32.21	2820.45	2752.03	856	3.27	3.29	0.13
6580	73.1	25.00	2055.78	2812.45	381	3.69	5.4	0.09
6583	97.11	30.94	2612.32	2690	542	3.19	4.82	-0.04
6587	58.08	21.04	1335.21	2298.84	222	3.29	6.01	-0.12
6588	78.26	26.31	2282.21	2916.33	511	4.26	4.47	0.07
6589	73.37	25.07	2017.36	2749.73	461	3.55	4.38	-0.03

Table 4.4.4 Feed intake, and feed efficiency in buffalo calves with different maintenance variability coefficients

Parameters	Low ME _m	High ME _m
BW (kg)	87.95 ± 7.53	86.95 ± 4.21
BW ^{0.75} (kg)	28.56 ± 1.85	28.43 ± 1.03
DMI (kg)	2.34 ± 0.22	2.42 ± 0.12
DMI (g)	2342.52 ± 224.16	2423.21 ± 120.23
Growth rate (g/d)	548.44 ± 71.44	486.78 ± 33.91
DMI/100 kg BW (kg)	2.64 ± 0.05	2.79 ± 0.05
DMI / 100 kg BW (g)	2642.98 ^b ± 49.90	2788.28 ^a ± 46.54
DMI/ kg BW ^{0.75} (g)	80.65 ± 2.90	84.99 ± 1.69
ME _m /100 kg BW	3.04 ^b ± 0.13	3.92 ^a ± 0.13
FCR	4.49 ± 0.27	5.07 ± 0.24
RFI	-0.05 ± 0.04	0.05 ± 0.03

Means bearing different superscripts differ significantly in a row (P<0.05)

Figure 3. Relationship between DMI (kg/100kg BW) and ME for maintenance (Mcal/100kg BW)

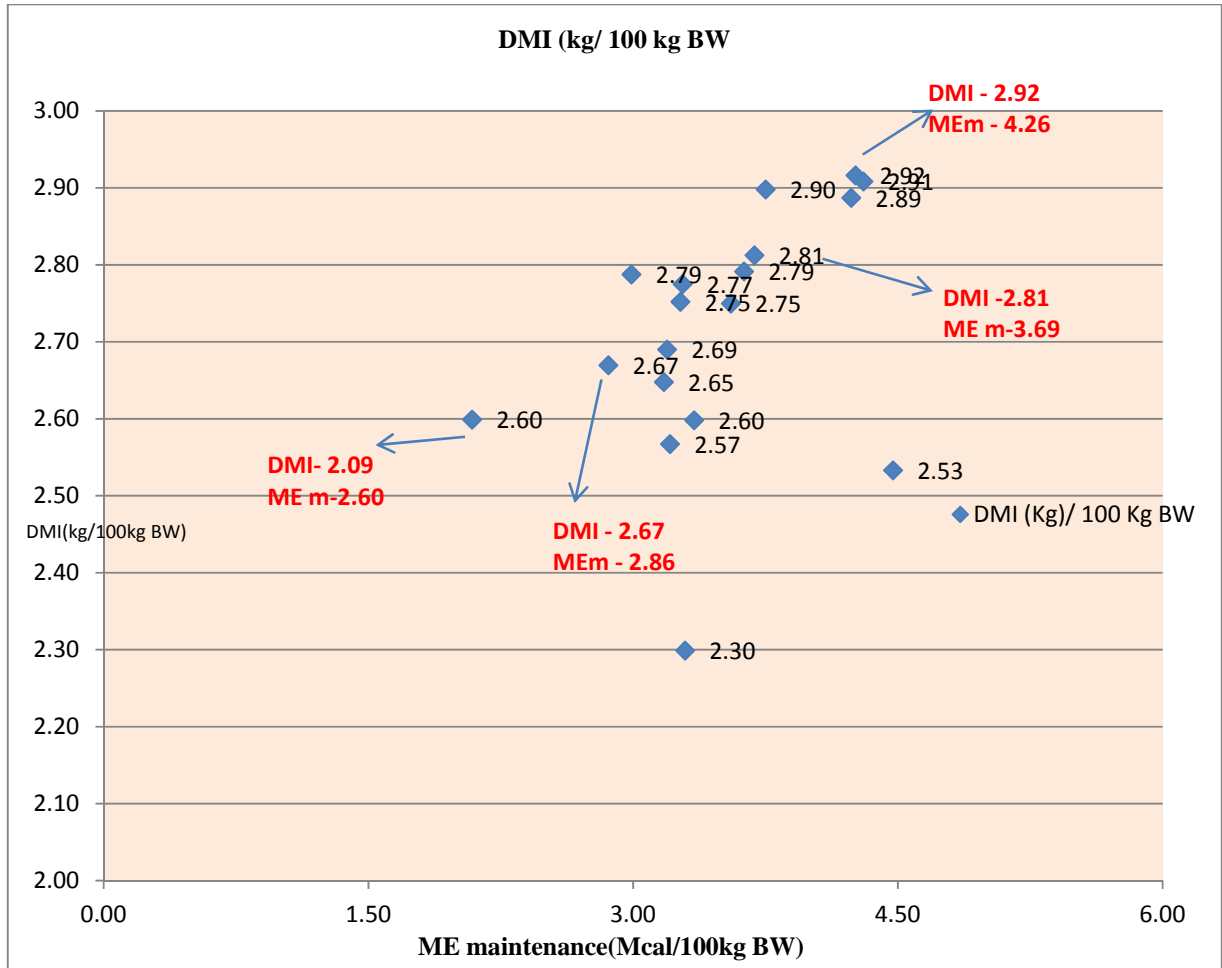


Table 4.4.5 Nutrients digestibility in buffalo calves

Parameters	Low ME _m coefficient	High ME _m coefficient
Dry matter	65.58 ± 2.19	64.17 ± 1.78
Organic matter	66.87 ± 2.25	67.04 ± 1.89
Crude protein	66.00 ± 1.96	66.24 ± 2.61
EE	62.14 ± 2.98	64.73 ± 3.09
NDF	63.29 ± 2.22	60.86 ± 1.87
ADF	57.24 ± 2.57	53.68 ± 2.55
NFC	84.56 ± 1.40	85.95 ± 1.08

Table 4.4.6 Nitrogen balance in buffalo calves

Parameters	Low ME _m coefficient	High ME _m coefficient
N intake (g)	56.50 ± 5.12	65.66 ± 3.33
N voided in feces (g)	18.80 ± 1.65	18.02 ± 1.71
N voided in urine (g)	19.42 ± 1.88	20.74 ± 1.18
Total N outgo (g)	38.22 ± 3.15	38.76 ± 2.40
N balance (g)	18.32 ± 2.84	14.40 ± 2.63
Absorbed N as % of N intake	66.00 ± 1.96	66.24 ± 2.61
Retained N as % of intake	31.03 ± 2.73	26.29 ± 4.33
Retained N as % of absorbed	46.68 ± 3.53	38.68 ± 5.53

Table 4.4.7 Nutrient intake in buffalo calves during metabolism trial

Parameters	Low ME _m	High ME _n
BW at starting of trial (kg)	99.21 ± 8.33	91.14 ± 4.25
BW ^{0.75} (kg)	31.26 ± 2.00	29.45 ± 1.02
DMI (g/day)	3085.58 ± 287.96	2824.95 ± 210.13
DMI/100kg BW (g/d)	3108.58 ± 193.93	3075.67 ± 125.39
DMI/kg W ^{0.75} (g/d)	97.60 ± 6.10	95.07 ± 4.46
OM intake (g/d)	2703.66 ± 290.17	2646.95 ± 165.42
OM intake/100kg BW (g/d)	2681.30 ± 145.94	2886.03 ± 68.91
Water intake by feed (L/d)	8.87 ± 0.42	8.59 ± 0.47
Water intake by drinking (L/d)	7.87 ± 0.84	7.54 ± 0.39
Total water intake (L/d)	16.73 ± 1.07	16.13 ± 0.76
Water intake/100kg BW (L/d)	19.03 ± 1.00	19.45 ± 1.23
CP intake (g/d)	363.05 ± 33.94	322.41 ± 22.98
CP intake/100kg BW (g/d)	403.44 ± 26.56	389.93 ± 14.61
EE intake (g/d)	63.71 ± 6.42	55.10 ± 4.18
EE intake/100kg BW (g/d)	70.21 ± 4.68	65.17 ± 3.42
CHO intake (g/d)	2405.23 ± 221.91	2149.96 ± 152.64
CHO intake/100kg BW (g/d)	2672.32 ± 177.47	2543.74 ± 116.01
NFC intake (g/d)	1290.44 ± 108.55	1180.80 ± 56.89
NFC/100kg BW (g/d)	1436.94 ± 65.20	1408.86 ± 50.65
NDF intake (g/d)	1666.31 ± 155.84	1482.02 ± 116.42
NDF intake/100kg BW (g/d)	1850.85 ± 129.80	1749.23 ± 90.23
ADF intake (g/d)	935.34 ± 85.75	835.13 ± 64.94
ADF intake/100kg BW (g/d)	1040.63 ± 73.80	986.37 ± 52.03

4.4.7 Nutrient intake and plane of nutrition in buffalo calves during metabolism trial

Data regarding nutrient intake in low and high ME_m groups have been presented in table 4.4.7 and 4.4.8. None of these nutrient intake parameters were found to be significantly different between groups. Water intake (L/kg DMI) value was numerically higher in low ME_m group compared to high ME_m group animals despite the difference was not statistically significant. Plane of nutrition in low and high ME_m buffalo calves in terms of DMI, OMI, EEI, CPI, NDFI ADFI and TDNI has been given in table 4.5.8. None of these parameters differ significantly between the low and high ME_m buffalo calves.

4.4.8 Relationship of ME_m with Hydroxyproline and Creatinine in urine parameters

Hydroxyproline and creatinine in urine of low and high ME_m are presented in table 4.4.9. The data showed no difference between low and high ME_m groups (81.09, 80.55 and 7.42, 7.64). However, creatinine was strongly correlated with ME_m ($r = -0.75$ for low ME_m and $r = +0.81$ for high ME_m) while hydroxy proline was very weakly correlated with ME_m ($r = +0.052$ and $r = -0.102$ in low and high ME_m, respectively). George *et al.* (2011) gave similar reports to present study.

4.4.9 Variation in feed intake and feed cost in buffalo calves

Data regarding variation in feed intake during the 52 days feeding period are presented in table 4.4.10 and 4.4.11. Total DMI of calves varied between 138.71 and 146.19 kg/d and the total feed cost per animal ranged from Rs. 1743.08 to Rs. 1800.07. The animals having low ME_m/100kg BW were having lesser DMI/100kg BW compared to the animals with high ME_m/100kg BW, consequently the feed cost was found to be lower in the low ME_m animals in comparison with high ME_m animals. Over the course of the 52 day feeding period, animals with low ME_m consumed 5.34 % less feed than high ME_m group of calves, yet performed exactly the same in terms of growth rate. Arthur *et al* (2001) reported that lines of cattle selected for low RFI had similar weights and performance after two generations, yet consumed 11% less feed.

Table 4.4.8 Plane of nutrition in buffalo calves

Parameters	Low ME _m	High ME _m
DDMI (g/d)	2035.48±218.21	1824.57±159.66
DDMI/100kg BW (g/d)	2018.82±99.61	1978.52±107.51
DOMI (g/d)	1791.28±242.53	1777.51±128.61
DOMI /100kg BW (g/d)	1735.98±121.93	1937.51±78.72
DEEI (g/d)	40.52±5.14	36.28±4.02
DEEI/100kg BW (g/d)	39.71±3.01	39.12±2.99
DCPI (g/d)	242.24±26.54	212.25±15.42
DCPI/100kg BW (g/d)	240.88±14.39	231.73±9.63
DNDFI (g/d)	1057.84±112.30	911.58±87.82
DNDFI/100kg BW (g/d)	1050.48±53.10	988.27±65.77
DADFI (g/d)	537.39±58.70	454.19±47.39
DADFI/100kg BW (g/d)	531.87±25.65	491.96±38.59
TDNI (g/d)	1978.94±202.17	1788.55±150.40
TDNI/100kg BW (g/d)	1971.46±100.87	1941.45±98.10

Table 4.4.9 Relationship of ME_m with Hydroxy-proline and Creatinine in urine

Parameters	Low ME _m	Correlation coefficient (r)	High ME _m	Correlation coefficient (r)
Creatinine (mg/dl)	7.42	- 0.75	7.64	+ 0.81
Hydroxy proline (ng/ml)	81.09	+ 0.052	80.55	- 0.102
Hydroxy proline/creatinine	10.93	-	10.54	-

Table 4.4.10 Variation in feed intake and feed cost in buffalo calves

Animal No	Total DMI(kg) in 52 days	Total feed intake (fresh basis)			Total feed cost(Rs)			Total feed cost concentrate(kg/d)	Feed cost per day green oats fodder intake(kg/d)
		Concentrate(kg/d)	Green oats fodder intake(kg/d)	Wheat straw(kg/d)	Conc @ Rs 1927.00/qtl	Green oats fodder @Rs 150.00/qtl	Wheat straw @ Rs. 300.00/qtl		
6566	152.45	68.52	304.90	33.14	1320.34	457.36	99.43	1877.12	36.10
6567	139.92	62.89	279.85	30.42	1211.82	419.77	91.25	1722.85	33.13
6568	152.46	68.52	304.92	33.14	1320.41	457.38	99.43	1877.22	36.10
6570	135.13	60.73	270.25	29.38	1170.28	405.38	88.13	1663.78	32.00
6571	131.80	59.23	263.60	28.65	1141.46	395.39	85.95	1622.80	31.21
6572	147.23	66.17	294.46	32.01	1275.10	441.69	96.02	1812.81	34.86
6573	145.39	65.34	290.78	31.61	1259.20	436.18	94.82	1790.19	34.43
6574	135.11	60.72	270.22	29.37	1170.16	405.34	88.12	1663.61	31.99
6575	152.99	68.76	305.98	33.26	1324.98	458.97	99.78	1883.72	36.23
6576	146.05	68.74	305.91	31.75	1324.70	458.87	95.25	1878.82	36.13
6577	137.67	61.87	275.34	29.93	1192.32	413.01	89.79	1695.12	32.60
6578	138.61	62.30	277.22	30.13	1200.47	415.83	90.40	1706.70	32.82
6579	143.54	73.59	327.48	31.20	1418.11	491.22	93.61	2002.95	38.52
6580	148.67	66.82	297.34	32.32	1287.59	446.01	96.96	1830.57	35.20
6583	143.38	64.44	286.76	31.17	1241.75	430.13	93.51	1765.39	33.95
6587	118.73	53.36	237.46	25.81	1028.29	356.19	77.43	1461.92	28.11
6588	152.16	68.39	304.33	33.08	1317.85	456.49	99.24	1873.58	36.03
6589	142.87	64.21	285.74	31.06	1237.36	428.61	93.18	1759.15	33.83

Table 4.4.11 Variation in feed intake and feed cost between low and high ME_m groups

Parameters		Low ME _m	High ME _m
Total DMI (kg) in 52 days		138.71	146.19
Total feed intake (fresh basis)	Concentrate (kg/d)	63.70	65.71
	Green oats fodder intake (kg/d)	283.45	292.39
	Wheat straw (kg/d)	30.16	31.78
Total feed cost (Rs)	Conc @ Rs 1927.00/qtl	1227.44	1266.14
	Green oats fodder @Rs 150.00/qtl	425.18	438.58
	Wheat straw @ Rs. 300.00/qtl	90.47	95.34
Total feed cost (Rs)		1743.08	1800.07
Feed cost per day (Rs)		33.52	34.62
Total weight gain (kg) during 52 days		26.54	Total weight gain (kg) during 52 days
Feed cost per wt unit gain (Rs)		76.75	78.40

5. SUMMARY AND CONCLUSIONS

The present study was divided into two phases. In the first phase commonly used feeds and fodders evaluated for their proximate fractions, cell wall fractions, fiber bound protein and rumen fermentation pattern under *in vitro* condition. In the second phase residual feed intake in growing Murrah buffalo calves was determined by difference between ME intake and ME required for maintenance and growth.

Forty feed ingredients (8 grains, 5 green fodders, 7 grasses, 7 oil cakes and meal and 13 agro industrial by-products and straws) were collected and analyzed for their chemical constituents, fiber fractions, *in vitro* gas production, *in vitro* digestibility and *in vitro* methane production. Among the grains, wheat comprised highest CP content (14.50%) and lowest of EE (1.59%). In contrast, CP and TA content in white maize was 8.03% and 3.49%, respectively. In the green fodders, ash % ranged from 8.98 (sorghum) to 13.83 (berseem) which was higher comparable to grains and oil cakes. The CP content in this group was similar to grains, except berseem fodder (19%). Hybrid Napier and Bermuda grass had more CP, EE, TA content (10.24, 1.56, 12.33 and 10.21, 2.09, 12.17%, respectively) than others, among the grasses. Oil cakes and meals are good sources of protein supplemental and CP (%) ranged from 25.15 (CSC) to 50.17 (SBM). In agro industrial by-products and straws, DM was about 90 % except beet pulp (10.39%). The CP content had varied significantly ($P<0.01$) between them. Highest was in MGM (64.26%) and lowest was in gram husk (3.51%). Agro industrial by-products and straws had less EE (%) while rice polish had higher EE (12.31%) than other feedstuffs.

Cell wall constituents and fiber bound protein (%DM) of grains varied significantly ($P<0.01$). Barley had highest content of NDF, ADF, lignin, cellulose and NDICP (25.90, 13.30, 3.92, 9.37 and 2.07%, respectively), whereas yellow maize had the lowest content (13.67, 4.58, 0.87, 3.54 and 1.22%, respectively). Sorghum fodder comprised highest NDF, ADF and cellulose (64.32, 41.04, and 32.22%, respectively) but had lowest NDICP and ADICP (3.10 and 0.98%). In other hand, maize fodder had highest NDICP and ADF (6.03 and 39.36%). The fiber fractions of grasses were significantly ($P<0.01$) higher than grains, oil cakes/meal and agro industrial by-products. Although oil cakes and meals had lower cell wall constituents and fiber bound protein than fodders and grasses but comparable to grains. While straw and gram husk had highest NDF, ADF, lignin and cellulose content among agro

industrial by products, the bran had fiber fractions similar to green fodders but lower than husk and straws. The DDGS had NDICP and ADICP highest in this group (9.69 and 8.70%).

Energy value of grains was higher than others as evident from this study. TDN, DE and ME of maize is highest (86.27%, 15.90 and 14.18 MJ/kg, respectively) and comparable among grains. Green fodders and grasses had TDN from 55-60 % and DE and ME from 10.5 to 9.0 (MJ/kg). Oil cakes and meal also had high energy content and had highest in GNC (88.46% of TDN, 16.30MJ/kg of DE and 14.59MJ/kg of ME). Agro industrial by-products had slightly lower DE and ME than grains and cakes but TDN of gram husk and straw were lowest (about 45-48%) among feedstuffs. However, rice polish is good source of energy (TDN, DE and ME) between the groups (89.12%, 16.42 MJ/kg and 14.71MJ/kg).

In vitro dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) of green fodders differed significantly ($P < 0.05$). Highest was recorded in pearl millet and lowest in sorghum (71.40, 72.41 and 56.54, 57.34% respectively). In other hand, methane production is highest in sorghum (28.11g/kg incubated and 37.41g/kg IVDMD). IVDMD, IVOMD, net gas production and methane were slightly lower than green fodders and highest was hybrid Napier comparable in grasses. Rumen fermentation rate of oil cakes and meals were higher compared with others. IVDMD and IVODM of oil cakes were about 80% except CSC (57.18 and 59.43%). But gas production and methane production were slightly lower than green fodders.

Residual feed intake (RFI) or net feed efficiency (NFE) is different from the metabolizable energy intake (MEI) and metabolizable energy required for maintenance and gain (MER). The MEI of buffalo calves varied from 3.08 to 7.91 (Mcal/d). ME requirements for maintenance and ME requirements for growth varied between the calves. ME requirement for maintenance varied between 2.86 and 4.42 (Mcal/d), while growth rate of calves were from 213.06 to 933.67 g/Mcal of ME_g . The DMI of calf with 4.26 (Mcal/100kg BW) ME_m was highest (2.92 kg/100kg BW) while DMI with 2.60 (Mcal/100kg BW) ME_m was lowest (2.09 kg/100kg BW). However, the DMI in the present study varied between low and high ME_m groups (2642.98 and 2788.28 g/100kg BW). ADG, BW and mid test metabolic BW were similar among the groups. Nutrients digestibility coefficient (%) and nitrogen balance in buffalo calves did not differ between low and high ME_m groups. During the metabolism trial, plane of nutrition and nutrient intake by buffalo calves were comparable in low and high ME_m groups. Although hydroxy proline ($r = + 0.052$, $r = - 0.102$) and creatinine ($r = - 0.75$, $r = + 0.81$) in urine were correlated with low and high ME_m but they were similar among the

groups. During 52 days feeding period, the total feed cost ranged from Rs 1461.92 to 2002.95 per calf. In the low ME_m groups of calves, 5.34% feed cost was lower than in high ME_m groups.

However it is concluded from the study “Assessment of feed utilization in buffalo calves to investigate the variation in residual feed intake” as follows

1. Grains are sources energy for livestock; they had higher TDN, DE, ME content more and lower cell wall content than others feedstuffs. Among of grains wheat had highest CP (%) while yellow maize had highest energy values content.
2. Conventional feedstuffs which were consumed the most by ruminants are fodders and grasses. The study showed that fodders and grasses had content high of fiber (cell wall constituents) while the energy values were lower than grains and by products. Furthermore, gases and methane productions of fodders and grasses were lower than that comparable to the cakes. However, CP of grasses was lower than cakes.
3. Agro industrial by products and straws were nutritionally lower than grains and cakes. Among them rice polish had energy values higher than bran and straw while MGM had highest CP (%) content.
4. Assessment of feed utilization in buffalo calves to investigate the variation in residual feed intake (RFI) which were found out by metabolizable energy for maintenance (ME_m). Energy required for maintenance and growth were different between the animals. DMI is less in low RFI or low ME_m (more efficiency) group while DMI were high in high ME_m (less efficiency) group. In present study, all most parameters as digestibility, ADG, FCR, BW hydroxy proline and creatinine are similar between the groups. Nevertheless, creatinine and hydroxy proline correlated with ME_m which was used prediction energy and protein require for animals. A cost-benefit study was done to determine the direct costs of measuring feed intake which in the low ME_m groups of calves had 5.34% feed cost consume lower than in high ME_m groups. For that reason, the opportunity to select buffalo calves with lower maintenance energy requirements and better utilization of energy for weight gain is possible within a herd of similar buffaloes.

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