# ASSESSMENT OF THE EFFECTIVENESS OF RUMEN LIQUOR FROM SLAUGHTERED CATTLE AS INOCULUM FOR ESTIMATION OF *IN VITRO* DIGESTIBILITY

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN TROPICAL ANIMAL PRODUCTION OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.**

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## ABSTRACT

A study was conducted to validate the use of rumen liquor from slaughtered cattle as an alternative source of inoculum for estimation of *in vitro* digestibility of ruminant feedstuffs. The experiments were conducted in three (3) different institutions, namely Sokoine University of Agriculture (SUA), Tanzania, National Livestock Resources Research Institute (NALIRRI) Uganda and Rwanda Agricultural Board (RAB) in Rwanda. Each institution evaluated 4 diets of different nutritional quality. The dry matter digestibility (DMD) and organic matter digestibility (OMD) of the diets were determined using *in vivo* total collection method and *in vitro* two stage technique of Tilley and Terry (1963). The *in vivo* experiment was conducted using four rumen fistulated steers which were allocated to the 4 diets for 4 periods in a 4 x 4 Latin square design. The *in vitro* technique followed 2 x 4 factorial arrangement in which the 4 diets were incubated in 2 different sources of rumen liquor; that is rumen liquor collected from fistulated and from slaughtered cattle. The obtained *in vitro* results using rumen liquor from fistulated and slaughtered cattle from all institutions were regressed against those determined by *in vivo* technique to derive prediction equations. The rumen liquor collected from the experimental animals at SUA was assessed for pH and concentrations of ammonia nitrogen ($NH_3$-N) and total volatile fatty acids (VFAs). The pH value (6.59±0.06) of the rumen liquor from fistulated cattle was not different ($P > 0.05$) from that of slaughtered cattle (6.53±0.06). Rumen liquor from slaughtered cattle contained higher ($P < 0.05$) concentration of rumen $NH_3$-N (122.74 ± 1.71 versus 111.34 ± 1.71 mg/l) and total VFAs (151.84 ± 7.75 versus 124.04 ± 7.75 mmoles/l) than that from fistulated cattle. The *in vivo* DMD of the different diets at SUA ranged from 51.6% - 61.8% while *in vivo* OMD ranged 51.4 – 62.2% and were both significantly ($P < 0.05$) different. There was no significant difference ($P > 0.05$) on the *in vivo* DMD and OMD of the diets used at

NALIRRI. The *in vivo* DMD of the diets used at RAB ranged from 84.7% - 90.5% and *in vivo* OMD ranged from 82.1% - 87.9% and they were both significantly ($P < 0.05$) different and relatively higher than the values obtained from the other institutions. The *in vivo* DMD and OMD of the diets in all institutions were significantly ($P < 0.05$) higher than the *in vitro* values obtained using rumen liquor from fistulated and slaughtered cattle. The *in vitro* DMD (42.6%) and OMD (38.8%) obtained using rumen liquor from slaughtered cattle at SUA were significantly ($P < 0.05$) higher than those obtained using rumen liquor from fistulated cattle (DMD = 40.0% and OMD = 36.2%). In the other institutions, there was no significant difference on the values of *in vitro* DMD and OMD obtained using rumen liquor from the two sources. There was also no significant difference ($P > 0.05$) on the prediction equations when rumen liquor from fistulated or slaughtered cattle is used in *in vitro* technique for predicting *in vivo* DMD and OMD. From this study it is concluded that, rumen liquor from slaughtered cattle may be used for estimating *in vitro* digestibility of ruminant feedstuffs.

## DECLARATION

I, Charles Mpemba, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted, nor being concurrently submitted for degree award in any other institution.

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## DEDICATION

This dissertation is dedicated to Almighty God for His abundance blessings and to my parents Henry Mpemba and Hildegarda Mbaule who laid down the foundation of my studies.

# TABLE OF CONTENTS

## LIST OF TABLES

# LIST OF FIGURES

## LIST OF PLATES

## LIST OF APPENDICES

## LIST OF ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| % | Percentage |
| °C | Degree Celsius/Centigrade |
| ADF | Acid detergent fibre |
| ANOVA | Analysis of variance |
| AOAC | Association of Official Analytical Chemists |
| ASARECA | Association for Strengthening Agricultural Research in Eastern and Central Africa |
| ASDP | Agricultural Sector Development Programme |
| $CaCl_2$ | Calcium chloride |
| CF | Crude Fibre |
| $CH_4$ | Methane |
| $CO_2$ | Carbondioxide |
| CP | Crude Protein |
| DASP | Department of Animal Science and Production |
| DF | Degrees of freedom |
| DM | dry matter |
| DMD | Dry Matter Digestibility |
| E.E | Ether extract |
| e.g | For example |
| Fig. | Figure |
| G | Gram |
| GLM | General Linear Model |
| $H_2SO_4$ | Sulphuric acid |
| Hr | Hour |

| | |
|---|---|
| kg | Kilogram |
| l | Litre |
| Lsmeans | Least square means |
| M | Molar |
| Mg | Milligrams |
| $MgSO_4.7H_2O$ | Hydrated magnesium sulphate |
| Ml | Millilitre |
| MLFD | Ministry of Livestock and Fisheries Development |
| mm | millimetre |
| N | Nitrogen |
| NaCl | Sodium chloride |
| $NaHCO_3$ | Sodium Bicarbonate |
| $NaHPO_4$ | Sodium Hydrophosphate |
| NALIRRI | National Livestock Resources Research Institute |
| NaOH | Sodium hydroxide |
| NDF | Neutral detergent fibre |
| $NH_3$-N | Ammonia nitrogen |
| Nm | Normality |
| OMD | Organic Matter Digestibility |
| P – Value | Probability of type I error |
| pH | Hydrogen ion concentration |
| R.L | Rumen liquor |
| r.p.m | Revolution per minute |
| R.S.D | Residual standard deviation |
| $R^2$ | Coefficient of determination |

| | |
|---|---|
| RAB | Rwanda Agricultural Board |
| RMSE | Residual mean square error |
| s.d | Standard deviation |
| S.E | Standard error |
| SAS | Statistical Analysis System |
| SEM | Standard error of the mean |
| SOV | Source of variation |
| *sp* | Specie |
| *Spp* | Species |
| SS | Sum of squares |
| SUA | Sokoine University of Agriculture |
| t | Time |
| V | Volume |
| VFAs | Volatile fatty acids |
| Vs | Versus |
| Wt | Weight |

# CHAPTER ONE

## 1.0 INTRODUCTION

Efficient utilization of feed resources for ruminant animal production relies on the knowledge of the quantity and quality of the available feeds. Feeds for ruminants have been evaluated for their nutritional characteristics for balancing animal nutrients needs and hence improve animal performance. The measure of the amount of nutrients absorbed by the animal is done through determination of their digestibility value, which take into account the losses of nutrients that occur during digestion and absorption. Digestibility is an important measure of the nutritive value of feeds because it provides information on the amount of nutrients that the animal can digest and use. Therefore, digestibility data can offer an insight into proper feeding of animals. The most accurate way of obtaining digestibility of feeds for ruminants is by conducting *in vivo* digestibility experiments. In this technique, live animals are used to evaluate feeds. It is considered to be a standard procedure because the data are obtained inside the animal (McDonald *et al*., 2010). However, the *in vivo* technique has been criticized for being laborious and expensive to carry out.

Based on the practical limitation of the *in vivo* methods, numerous attempts have been made to develop simple techniques of determining digestibility of animal feedstuffs. The two stage *in vitro* technique of Tilley and Terry (1963) is one of such techniques, whereby digestibility of the food is determined by reproducing in the laboratory the reactions that take place in the alimentary tract of the animal (McDonald *et al.,* 2010). The advantage of this technique is that several samples are incubated at a time. It is relatively cheap, less laborious and takes a shorter time to obtain results as compared to *in*

*vivo* method (Stern *et al.,* 1997). The technique is therefore known to be useful in estimating digestibility of ruminant feedstuffs.

Nevertheless, the technique relies on the rumen liquor or inoculum, which is normally obtained from live ruminant animals that have undergone surgery and fitted with a rumen fistula to allow direct access into the rumen. This practice has challenges due to moral and ethical issues related to animal welfare and management cost of surgical operations and expenses of maintaining the fistulated animals particularly in tropical countries (Jones and Barnes, 1996; Kitessa *et al.,* 1999).

Some attempts have been made to search for other sources of rumen liquor. The use of slaughtered cattle as source of rumen fluid for the estimation of the *in vitro* digestibility of feeds has been proposed by various authors (Borba *et al.,* 2001; Chaudhry, 2008; Mohamed and Chaudhry, 2012; Mutimura *et al.,* 2013). The quality of rumen liquor may be affected by the diet of donor animal, sampling time of rumen liquor after feeding and handling of rumen liquor (Mould *et al.,* 2005). However, it is not clear how these factors especially previous diet of donor animal would affect the quality of rumen liquor and hence the value of *in vitro* digestibility.

The diet fed to the donor animal from which the rumen fluid is obtained affects the microbial population and hence the fermentation pattern in *in vitro* cultures (Ottou and Doreau, 1996). It has been found that the rumen bacterial population remains constant with different diets, except in diets with high concentrates (Bryant and Burkey, 1953). Since the composition of diet and nutrient availability for microbes in the rumen are the largest factors affecting microbial growth in the rumen, these may have a major impact on microbial activity of the inocula and hence the digestibility values obtained by *in vitro*

technique. Holden (1999) reported lower digestibility values when the inoculum was taken from donor cow fed grass hay than when it was taken from cow fed total mixed ration. Mould *et al.* (2005) suggested that, if the microbial population is to remain normal in numbers and activity, it is necessary to use a test substrate, which is similar to the diet of the animal from which the rumen liquor is obtained.

The quality of the rumen liquor is assessed by looking into its fermentation products. The fermentation products which are pH, rumen ammonia - nitrogen ($NH_3$-N) and total volatile fatty acids (VFA) are the viability indicators of the quality of rumen liquor. The fermentation products of the rumen liquor provide information about activity of the rumen microbes. There are optimum range of pH and concentrations of total VFA and $NH_3$-N in the rumen at which the activity and growth of microorganisms may increase (Mekasha *et al.,* 2003; Oosting, 1993) and hence increase the intensity of fermentation. McDonald *et al*. (1995) reported a pH range of 5.5 – 6.5 to be optimum for microbial growth and activity, while Van Soest (1994) reported a pH range of 6.2 – 7.2 to be optimal for fibre digestion. For optimal microbial activity rumen $NH_3$-N concentration should be above 50 mg/l (Satter and Slyter, 1974) and total VFA should be within the physiological normal range of 70 -150 mmol/l (McDonald *et al.,* 1995). Therefore the fermentation characteristics of rumen liquor may have influence on the *in vitro* value of digestibility of feed.

The fermentation characteristics have shown to be affected by the diet of the animal. Cattle which are brought to the abattoirs are mostly originated from the traditional sector, which is characterised by grazing on natural pastures and seasonal availability of feeds. Crude protein (CP) and mineral contents of these natural pastures may not meet the minimum requirement for the proper growth and functions of the rumen microbes. It is

known that if energy, protein and mineral contents are low in the feeds then microbial number decline resulting in low digestibility. The CP contents of natural pastures range from 2 – 5% especially during the dry seasons (Njau *et al.,* 2013), which do not meet the minimum CP requirement of 8% for microbial function (Komwihangilo *et al.,* 2005). The animals are also grazing on weeds, legumes and fodder trees which are usually rich in protein and minerals and acts as supplements to available poor quality feeds. Therefore, the feeds of cattle before slaughter may affect the fermentation characteristics of rumen liquor and hence the *in vitro* digestibility value of the feeds.

Rumen liquor from slaughtered cattle may therefore offer an alternative source of inoculum for estimating the *in vitro* digestibility of ruminant feeds. However, information on the fermentation characteristics and effectiveness of rumen liquor from slaughtered cattle in estimating digestibility values of ruminant feedstuffs is lacking. Therefore, the general objective of the study was to establish whether rumen liquor from slaughtered cattle could be an alternative source of inoculum for estimating the *in vitro* digestibility of feedstuffs for ruminants. Specifically the study intended to:-

i. To assess the relative fermentative characteristics of rumen liquor from slaughtered and fistulated cattle.

ii. To evaluate the effectiveness of rumen liquor from slaughtered cattle as source of inoculum in estimating *in vitro* digestibility of feeds for ruminants.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 General overview

The important measure of nutritive value of a feed is its digestibility value. Different techniques are used to determine digestibility of feeds. The *in vitro* techniques that involve incubation of feed samples using rumen liquor in laboratory are the most convenient for obtaining digestibility of feedstuffs. The major source of the rumen liquor for these techniques is fistulated ruminants, which are difficult to acquire in some laboratories. This review intends to provide information on the different techniques used for determining digestibility of feeds, sources of the rumen liquor for *in vitro* techniques and the factors affecting quality of the rumen liquor. The coverage includes the use of rumen liquor from slaughtered cattle as alternative source to that from fistulated cattle, the effects of diet of the donor animal on the quality of rumen liquor. In addition, the review covers the different types of feeds taken by slaughter animals and how they might influence the fermentation characteristics of the rumen liquor. Aspects of prediction of *in vivo* digestibility from *in vitro* values including those obtained by incubating samples using rumen liquor from slaughtered cattle are also reviewed.

### 2.2 Methods of estimating digestibility

The ability of feeds to sustain animal performance depends mainly on their digestion efficiency, which is measured by digestibility values. Feed digestibility is affected by its chemical composition and physical characteristics because these properties affect capability of digestive enzymes to colonize and digest the feed particles (Kitessa *et al.,* 1999). Various methods have been used to determine the digestibility of ruminant feeds and these are grouped into *in vivo* and *in vitro* techniques.

### 2.2.1 The *in vivo* techniques

The digestibility of the feed conducted by *in vivo* techniques involves the direct use of the animal, through total collection or using markers. In the total collection method, the animal is given a known quantity of food which is under investigation and the quantitative collection of faeces is made. The chemical composition of the feed consumed and faeces voided is estimated and the digestibility value of its various fractions is computed. This technique is considered the most reliable method of measuring digestibility of feedstuffs because digestion is conducted inside the animal (Stern *et al.,* 1997).

Use of markers is common in animals fed in a group or grazing animals where it is impossible to measure feed intake and faecal output of individual animals. Digestibility is measured by using indicator substances which are naturally occurring components of the food that are completely indigestible. Some of the components of the food that are used as indicators in digestibility trials are acid detergent lignin, acid indigestible fibre or acid insoluble ash (Block *et al.,* 1981). Also the long chain fatty acids and long chain *n* – alkanes found in plant waxy compounds are used in *in vivo* digestibility trial for grazing animals. Other substances such as Chromic Oxide ($Cr_2O_3$) are added to foods and act as indicators because they are insoluble and indigestible (Mayes and Dove, 2000). The digestibility of the feeds can be determined by the ratio of the indicator substances in the feeds and in small samples of the faeces of each animal. However, this method of using indicator has problem in that it is difficult to obtain the representative sample of the amount of food eaten by the animal. The sample taken by hand may not be the proper representation of the sample eaten because animals select different parts of the plants during grazing. Also the digestibility may be affected by the type of the indicator used (McDonald *et al.,* 1995).

During setting the trial, replication is done to allow more opportunity for detection of experimental errors. More than one animal are used, because animals, even of the same species, age and sex, differ slightly in their digestive ability. The animals used in *in vivo* digestibility trial should be docile and in good health (Givens and Moss, 1994). The animals are confined in metabolic cages in order to make it easier for collection of faeces. Larger animals, such as cattle and sheep may also be fitted with harnesses and faeces collection bags made of rubber or a similar impervious material. Male or castrated animals are preferred to females because it is easier to separate the faeces from the urine. For females a bladder catheter can be used to separate the urine from the faeces. The faeces collected into the bags can be emptied once or twice a day (McDonald *et al.,* 2010).

The digestibility trial consists of two periods, the preliminary period (adaptation period) and the collection period. The length of the preliminary period varies due to the nature of the experimental feed. The preliminary period can be as long as 30 days and as short as 6 days (Schneider and Flatt., 1975). Feeds that have wide variation in voluntary intake such as crop residues, requires longer preliminary period in order to standardize the level of intake. With straws the preliminary period can be up to 14 days (Cottyn *et al.,* 1989). Omed *et al.* (1989) reported that the preliminary period should vary from 4 to 12 days, but normally 7 to 8 days is used. Chenost and Demarquilly (1982) reported that a preliminary period of 10 to 14 days is required for the animals to obtain maximum intake level, while McDonald *et al.* (2010) proposed a preliminary period of 14 to 20 days. The length of collection period differs depending on the nature of the diet. Cottyn *et al.* (1989) proposed a collection period of 10 days for straws and a short time for more uniform diets. Generally a longer collection period is required because it provides more accurate results. McDonald *et al.* (2010) proposed a collection period of 7 to 10 days.

The digestibility values for some forage fed to ruminants obtained by *in vivo* technique are presented in Table 1. *In vivo* technique is the most reliable for evaluation of feed digestibility, but it has some limitations. One of the limitations of the technique is that it does not take into account the energy loss in the form of methane and therefore it overestimates digestibility of feeds.

Other limitation is due to the constituents of faeces. Not all the faeces consist of undigested food materials. Some of the faecal constituents are contributed by enzymes and other substances such as minerals secreted into the gut and not re-absorbed, and by cellular materials abraded from the lining of the gut. Therefore some of the nutrients in the faeces are contributed by the substances being secreted into the gut and not originated from feeds. For example some of the ash fraction in faeces is contributed by mineral elements being secreted into the gut because faeces serves as the route of excretion for certain mineral elements particularly calcium. Therefore the excretion in faeces of substances not arising directly from the food leads to underestimation of the digestibility of the food. In addition, *in vivo* techniques appear laborious, time consuming and expensive, and therefore it is not suitable for a routine and large scale feed evaluation (Stern *et al.,* 1997). However, the *in vivo* digestibility values are needed for the validation of the *in vitro* techniques (Rymer, 2000).

**Table 1: *In vivo* digestibility values (%DM) of some tropical forages**

| Forage | DMD | OMD | References |
|---|---|---|---|
| *Cenchrus ciliaris* | 60 - 69 | 49 - 69 | Heuzé *et al.,* 2013 |
| *Lucerne* hay | 61.7 | 62.9 | Yona, 2004 |
| Maize silage | 58.2 | 60.6 | ” |
| *Brachiaria brizantha* | 53.5 | 56.3 | ” |
| *Brachiaria decumbens* | 62.5 | 61.5 | Loch, 1997 |

### 2.2.2 The *in vitro* techniques

The *in vitro* techniques have been developed to overcome the shortcoming of the *in vivo* technique. The advantages of the *in vitro* techniques are that they are less laborious and are more suitable for a large scale evaluation of ruminant feeds. The most important techniques are the *in sacco* (nylon bag) technique using the fistulated ruminants, *in vitro* gas production technique and the two stage *in vitro* technique, which involve the incubation of feed samples in rumen liquor. These techniques have been used to predict the *in vivo* digestibility of the feeds. The *in sacco* technique (nylon bag technique) involves incubation of feed samples into nylon bags which are placed in the rumen of fistulated animals. In this technique the bags are extracted and weighed at fixed times for measuring the disappearance of feed from the bags, providing information about rate and extent of feed digestion (Kitessa *et al.,* 1999). The technique has been largely employed to evaluate rumen degradability of feeds and found to predict well the *in vivo* digestibility of the feed (Damiran *et al.,* 2008). However, the technique is criticized for the need of rumen fistulated ruminants.

The *in vitro* gas production technique measures the appearance of fermentation products (gases, volatile fatty acids, $NH_3$) when feed samples are incubated in rumen liquor. When a feed is incubated with buffered rumen liquor, it is degraded, and the degraded matter is partitioned to yield gases (mainly $CO_2$ and $CH_4$) and microbial biomass. It is assumed that gas production is related to the rate and extent of feed digestion.

The two stage *in vitro* technique for estimation of digestibility of feedstuffs for ruminants was introduced by Tilley and Terry (1963). This technique attempts to approximate digestion in an artificial environment, where rumen conditions are simulated in a test tube (Barnes, 1973). The first stage involves 48 hours incubation of the feed samples at 39°C

in a test tube with buffered rumen fluid under anaerobic condition. In the second stage the residues are incubated for 48 hours at 39°C with pepsin in an acid solution under aerobic condition (Tilley and Terry, 1963). The insoluble residues are filtered off, dried and ignited to obtain ash. The contents of organic matter of the feed and residues are obtained by subtracting ash from the dry matter of the feed and residue respectively.

The two stage *in vitro* technique of Tilley and Terry (1963) provides a quick, in-expensive and precise prediction of *in vivo* or conventionally determined digestibility in ruminants. It produces values that are numerically similar to *in vivo* values for many types of forages. However, there are some technical limitations of using two stage *in vitro* techniques. There are variations in the *in vitro* digestibility values of forages obtained by the technique in different laboratories as shown in Table 2. These variations are mainly caused by the quality of rumen liquor which is due to the diet fed to the donor animal for rumen liquor (Mould *et al.,* 2005).

**Table 2: Values (%DM) of *in vitro* DMD and OMD of some tropical forages obtained using the two stage technique**

| Forage name | DMD | OMD | REFERENCE |
|---|---|---|---|
| *Cenchrus sp.* | 60 - 69 | 53 - 64 | Jacobs *et al.* (2004) |
| ” | 41.1 | 39.5 | Mutimura *et al.,* (2013) |
| *Brachiaria sp.* | 60.2 | 56.3 | Ribeiro *et al.* (2014) |
| ” | 65.3 | 66.5 | Rwechungura (2000) |
| *Cynodon dactylon* | 51.5 | 39.9 | Temu (1977) |
| ” | 79.0 | 69.2 | Rwechungura (2000) |
| *Pennisetum purperum* | 38.7 | 38.8 | Muyekho *et al.*(2000) |
| ” | 52.2 | 38.4 | Temu (1997) |

Other limitation of the technique is the need for fresh rumen fluid, which involves the need for fistulated ruminants, such as cattle, sheep and goats available as donor animals. Surgical operation modifies animals for experimentation, which appears to be unkind,

harshly and cruel to the animal leading to some countries ban the use of rumen liquor from fistulated ruminants (Jones and Barnes, 1996). These concerns raise the need for alternative approach. Using rumen liquor from slaughtered animals is one of such options. Various studies have shown that rumen liquor from slaughtered cattle has a high possibility of being a replacement to fistulated ruminants as source of inoculum for *in vitro* digestibility studies. Chaudhry (2008) reported the possibility of using slaughtered cattle as a source of inoculum to evaluate supplements for *in vitro* forage degradation. In addition, a study conducted by Mutimura *et al.* (2013) found that rumen fluid from slaughtered cattle could be used for feed evaluation using *in vitro* gas production technique.

Although rumen liquor from slaughtered cattle has shown possibility of being the replacement to that from fistulated cattle in *in vitro* techniques, there is still a great challenge on the quality of the rumen liquor from slaughtered cattle, which may affect the results of *in vitro* techniques. The common source of variation of *in vitro* digestibility is the quality of liquor used as inoculum (Mould *et al.,* 2005). Since the dietary history of the animal which is brought to the abattoir for slaughter is not known, information on the quality of rumen liquor from slaughtered cattle coming from different areas with different dietary history should be assessed to know its effect on the digestibility values. The effect of diet of cattle before slaughter on the rumen fermentation characteristics can be assessed by measuring the pH, concentrations of the rumen ammonia nitrogen ($NH_3$-N) and total volatile fatty acids (total VFAs) of rumen liquor from slaughtered cattle reared under different feed sources.

## 2.3 Constituents of rumen liquor

Rumen liquor is the liquid phase found in the rumen of ruminant animals where microbial fermentation takes place. Each millilitre of rumen liquor contain roughly $10^9$ - $10^{11}$ bacterial population, $10^5$ - $10^6$ protozoa population and variable numbers of yeast and fungi (Paul *et al.,* 2004). Bacteria are often classified by their substrate preference or the end products they produce. Many of them utilise multiple substrate, although there are some of the major groups which utilise specific substrate. Each group of bacteria contain multiple genera and species. The groups include cellulolytic bacteria (digest cellulose), hemicellulolytic bacteria (digest hemicellulose), amylolytic bacteria (digest starch) and proteolytic bacteria (digest proteins). Other groups are sugar utilising bacteria (utilising monosaccharides and disaccharides), acid utilising bacteria (utilise lactic, succinic and malic acids), ammonia producers, vitamin synthesizers and methane producers (Odenyo *et al.*, 1999).

The population of protozoa is far less than bacterial population, but they are so much larger than bacteria that they may occupy a volume nearly equal to that occupied by bacteria. In general protozoa utilise the same set of substrate as bacteria in which different populations of protozoa show distinctive substrate preference as bacteria. Many utilise sugars and some store ingested carbohydrates as glycogen. Many species of protozoa have been found to consume bacteria, which are thought to perhaps play a role in limiting bacteria overgrowth (Hungate, 1966). The fungi are considered important in the rumen as they have unique ability to break and penetrate the fibrous feed particles and provide more surface area for the action of other microbes. They produce highly active enzymes for lignocellulose degradation. Thus rumen fungi play an important catalytic role in the digestion of poor quality fibrous feeds (Paul *et al.,* 2004). Almost all rumen microbes are anaerobes although a few facultative microbes exist, performing a key role in removing

oxygen quickly from the rumen (Hungate, 1966). These microorganisms interact and support one another in a complex ecosystem with products of some species serving as nutrients for other species. Through fermentation they can convert plant materials that could not otherwise be digested to volatile fatty acids (VFAs), methane, carbon dioxide, ammonia and microbial cells. Ammonia is used as a nitrogen source for microbial growth and VFAs are absorbed from the rumen and used as a key energy source for the ruminants.

The quality of the rumen liquor is assessed by measuring the by-products of the rumen fermentation, such as pH, $NH_3$-N and total VFAs. The fermentation characteristics of the rumen liquor provide information about the microbial population and activity of rumen microbes (Mekasha *et al.,* 2003). The quantity of $NH_3$-N and total VFAs present in the rumen liquor is a reflection of microbial activity and their absorption or passage out of the rumen (Habib and Akbar, 2005). There are optimal concentrations of these fermentation characteristics in which rumen microbes function well. The optimal environmental conditions of the rumen have been found to be at the pH of around 6. The pH values for normal microbial activities in the rumen have been proposed by different authors. McDonald *et al.* (1995) proposed the pH range of 5.5 to 6.5 as the optimum for microbial fermentation. Van Soest (1994) reported the pH of 6.2 – 7.2 being optimal for fibre digestion. Fibre digesting bacteria perform best at pH 6.0 – 6.8 and starch digesting bacteria at pH 5.5 – 6.0 (Russell and Wilson., 1996). The change in the ruminal pH is always caused by the type of feed consumed by the animal (Mekasha *et al.,* 2003). Saliva which is produced by animal during chewing activity acts as the buffer for pH. High feed intake is associated with high pH because of increased chewing activities and saliva production (Nørgaard, 1993). The buffering ions in the saliva elevate the pH value. If large amounts of soluble carbohydrates are consumed, then the pH may fall. If pH drops

to about 5.5, protozoal populations become markedly depressed because of acid intolerance. In the study by Vargas *et al.* (2009) it was found that the type of diet fed to donor animals had a marked effect on the inoculum pH, which was noticeably lower with diet having high concentrate to forages ratio.

Variations in ruminal pH have direct effects on rumen microbial composition, population and their fermentation activity (Hungate, 1966). The pH below or above the optimal range recommended may affect the microbial growth and activity. Greater pH decline means decreased population and activity of fibre digesters (fibrolytic bacteria) and protozoa population. More drastic lowering of rumen pH, as can occur with higher feeding of concentrate can destroy many species and have serious consequences to the animal. If diets of the ruminants contain 50 to 60% of concentrate then there is high possibility of ruminal protozoa to drop from $10^6$ to$10^3$ and hence reduction in digestibility of the feeds (Calsamiglia *et al.,* 2008).

The increase in the amount of total VFA indicates the increased microbial activity in the rumen (Oosting, 1993). The total VFA concentration in the rumen liquor for normal function of the rumen in cattle should be in the range of 70 – 150 mmol/l of rumen liquor (McDonald *et al.,* 1995).

The rumen $NH_3$-N concentration is a limiting factor for rumen microorganisms that affect the digestion of fibrous feeds (Ørskov, 1995). The increase in the concentration of rumen $NH_3$-N may show the reduced utilisation of ammonia by rumen microbes, which indicates the decrease in intensity of fermentation due to decrease in microbial growth and activity (Mekasha *et al.,* 2003). It may also be due to inability of rumen microbes to effectively utilise ammonia (Males and Purser, 1970). Thus, higher $NH_3$-N concentration resulted in

a reduction in rumen microbial populations (Mekasha *et al.,* 2003; Males and Purser, 1970). Lower $NH_3$-N concentration could be due to higher uptake of ammonia, due to increased activity and growth of the rumen microbes or due to low dietary N - intake (Hristov *et al.,* 2001). The physiological normal range of the rumen ammonia nitrogen described by McDonald *et al.* (1995) is 85 – 300 mg/l. Satter and Slyter (1974) suggested a concentration of rumen $NH_3$-N above 50mg/l rumen liquor to be optimal for microbial growth. In addition, the concentration of 60 – 100 mg/l rumen liquor is considered appropriate level for maximum *in vitro* digestibility of low quality feeds (Oosting *et al.,* 1989).

Given that the constituents of rumen liquor are affected by the diet of animal, then in utilising rumen liquor from slaughtered cattle for estimating *in vitro* digestibility there is a need to understand if the concentration of the constituents in the rumen liquor to be utilised is in the required level. This will be important since the cattle brought for slaughter in the abattoir are coming from different areas and are feeding on different types and quantities of feeds, which may affect the quality of rumen liquor and hence the results.

### 2.4 Factors affecting the quantity and quality of constituents of the rumen liquor

Diet composition and the level at which it is offered are the largest factors affecting microbial growth in the rumen, which may have a major impact on microbial activity of inoculum (Belanche *et al.,* 2012). Rumen microbial population and activity depend on the nutrients available in the diet for their body maintenance, cell growth and multiplication and for effective fermentation activity (Tejido *et al.,* 2002; Baker and Dijkstra, 1999). The bacterial numbers may remain constant with different diets, but may change in high concentrate diets. The population of bacterial in the rumen contents generally increase

with addition of concentrate to roughage diets (Hoover and Stokes., 1991). Changing the concentrate to forage ratio will provide more energy for microbial growth. Concentrates provide a source of rapidly fermentable carbohydrates for rumen microbial growth, multiplication and increase in their total number and fermentation activity. In animals fed high concentrate diets the total bacteria can count up to 90% of the total microbial population (Bryant and Burkey, 1953; Makir and Foster, 1957). But higher inclusion rates of concentrate can most likely result in decreased population of fibre fermenting bacteria and hence reduced activity of fibre fermentation. Protozoa numbers have been found to be higher when concentrate diets are offered, but long term feeding with concentrate has a negative effect on protozoa numbers due to decrease in rumen pH (Ivan *et al.,* 2001). Increasing feeding frequency to the donor animal tend to maintain a higher rumen pH and a higher protozoa population. Rumen fungal populations also vary with diets. Lowest fungal concentrations have been reported when ruminants are grazed in high quality pastures, or fed with silage diets and free lipids. Feeding of poor quality pastures or feeding of hay tends to increase fungal numbers (Paul *et al.*, 2004).

Studies by Vanzant *et al.* (1996) showed that rumen microbes become more complex when mixed diets are offered. Feeding total mixed diet provides an optimum balance of nutrients to the microbes, thereby stabilizing their population, composition and fermentation activity. Feeding the same quality mixed diet more frequently, say 2 - 4 times instead of once a day results in doubling the microbial concentrations and activity (Paul *et al.,* 2004). If the same quantity and quality diet is fed twice daily the rumen bacterial concentration is still higher than those fed once daily. This is because multiple feeding reduces diurnal variations and prevents drastic fluctuations in rumen pH. Rumen environments are modified greatly when restricted feeding is practiced than when the animal is provided with adlib access to feed. Since diet is a major determinant of the

quality of rumen liquor, then the inoculum used for incubating the feeds *in vitro* should be obtained from animals fed the same feed as substrate, so as to reduce the diet effect on the quality of inoculum (Kittesa *et al.,* 1999).

The use of rumen liquor from slaughtered cattle as inoculum for *in vitro* determination of digestibility of feeds may have a major problem associated to the quality of inoculum as these animals may be of different origin accessing different types of feeds. Many cattle brought for slaughter in Tanzania originate from the traditional sector, which is characterised by grazing on natural pastures and seasonal availability of feeds. The animals are raised on natural pastures in the rangelands which are known to have low productivity and nutritive value. The quality and quantity of these natural pastures for animals remains high for a short period of rainy season and low in long dry season (Njau *et al.,* 2013).

The CP and mineral contents in the natural pastures may decline during dry season and hence may not meet the minimum requirement for microbial function. Microbial numbers depend on adequate supplies of nutrients. If feed is low in protein contents and minerals, microbial numbers decline, hence results in lower forage digestibility (Ravhuhali *et al.,* 2010). It has been reported that during dry season the natural pastures may contain 2 – 5 % CP which do not meet the minimum CP requirements of 8% for minimum microbial function (Komwihangilo *et al.,* 2005). However, Leng (1993) reported that the average CP of 5% in pasture is enough to meet the minimum nutrient requirements for grazing animals. Due to scarcity of feeds during the dry season, smallholder livestock keepers use crop residues as alternative feeds to their livestock. These crop residues are also low in nutritive value and in other places they are not enough for large number of livestock. Also during these period animals depend mostly on weeds, legumes and fodder trees for their

diet which are usually rich in protein and minerals (Mtui *et al.,* 2008). Fodder trees and legumes provide green forage as supplement to available poor quality feeds. The common tree legumes in rangelands which are used by many smallholder livestock keepers are *Acacia sp., Cassia sp., Croton sp*. and their pods (Selemani *et al.,* 2012). The concentration of CP in the leaves and pods of majority of fodder and shrubs is above 10% even in dry season. This CP content is above the range of the CP required for proper functioning of rumen microbes.

In addition, grazing areas have weeds which dominate during the dry season and they form part of the feeds for livestock. Weeds such as *Solanum sp. Sida sp*. and *Tephrosia incana* are the most perennial weeds which are mostly dominating grazing land of most part of the country during the dry season. Therefore, the feeds grazed to ruminants by smallholder livestock keepers in many parts of the country, may contain energy and protein needed for proper growth and activity of rumen microorganisms. Changing the diet of the animal every day then the required microbes may not be present in sufficient numbers; therefore the diet should be as consistent as possible (Mould *et al.,* 2005). Therefore, the rumen liquor from slaughtered animals is expected to have sufficient number of microbes as these animals are grazed to the same types of forages for a prolonged period.

Cattle brought to the abattoirs for slaughter are transported a long distances without feed and water. This may have an effect on the growth and activity of rumen microbes, since the microbes need energy, protein and water for their proper growth and activity, which are obtained from the feeds consumed and drinking water by the animal. However, these animals are always grazed to mature forages, containing higher fibre and lower soluble nutrients which move more slowly through the digestive system and hence building

slowly the fibre digesting microbes (Leng, 1993). Therefore, slaughtered animals may have sufficient number of microbes in the rumen liquor, as a result of the prior type of feeds consumed. Limited information is documented on whether the required population and activity of microbes in the rumen exist without access to water for prolonged times. Water is very important for maintaining the rumen liquid phase and diluting the acids in the rumen. In addition, water supports metabolism of microbes and contains some minerals for the microbes.

Sampling time of rumen liquor after feeding has effect on its quality. The population of rumen microbes for the inocula sampled 4 hours after feeding is always low due to dilution with feed, water and saliva (Mould *et al.,* 2005). Samples collected 4 to 8 hrs after feeding is dominated by microbes with different fermentative activities (Blummel and Ørskov, 1993; Pell and Schofield, 1993; Theodorou *et al.,* 1994; Williams *et al.,* 1995). Menke and Steingass (1988) recommended the use of rumen fluid collected before feeding, which is the routine for many laboratories. However, rumen fluid taken after overnight fasting is less active than that taken after feeding (Hvelplund *et al.,* 1999) but is more consistent in its composition and activities (Yona, 2004). In order to have rumen liquor of good quality, for inoculating feed samples Hvelplund *et al.* (1999) recommended sampling after overnight fasting from at least 3 animals, all being fed the same diet of medium quality hay with small amounts of concentrate. Mould *et al.* (2005) on the other hand recommended that the donor animal for rumen liquor to be fed frequently on an *ad libitum* basis in order to have a stable population of rumen microbes.

Handling of rumen liquor was also found to contribute considerably to the variations in the quality of the rumen fluid (Tilley and Terry, 1963). During handling of rumen liquor there must be assurance of exclusion of atmospheric air in the liquor so that the anaerobic

environment is maintained. Poor handling of the rumen liquor may lead to the disturbance of anaerobic environment and cause microbial population and activity to decrease leading into partial digestibility.

## 2.5 Effects of diets and frequency of feeding donor animal on values of *in vitro* digestibility

The values of digestibility obtained by *in vitro* techniques are affected by quality of rumen liquor used for incubating feed samples. Quality of rumen liquor is mainly affected by the diet of donor animal. The diet composition and feeding frequency of donor animal have major effect on the quality of rumen liquor and hence results from *in vitro* techniques (Makir and Foster, 1957; Thorley *et al.,* 1968). It has been reported that the *in vitro* digestibility of forages is higher when rumen liquor is obtained from animals fed forage based diets compared to that of animals receiving high concentrate diets (Vargas *et al.,* 2009; Cronje, 1992). In the study by Vargas *et al.* (2009) the type of diet fed to donor animals had a marked effect on the forage digestibility. The *in vitro* digestibility of Lucerne hay, grass hay, straw and beet pulp dropped by 3, 11, 13 and 14 percent, respectively when rumen liquor was collected from donor animal fed diets with varied forage to concentrate ratio, decreasing as concentrate increased. Tejido *et al.* (2002) also found that the use of rumen liquor from animals fed on a diet with 80% concentrate resulted in lower *in vitro* DM digestibility for six forages (oat straw and five hays) compared to rumen liquor from animals fed on a diet with only 20% concentrate. But in other study it was found that the *in vitro* digestibility was higher when rumen liquor was obtained from a cow supplemented with small amount of concentrate than when it was obtained from a cow fed on hay alone (Stern *et al.,* 1997). Similarly, Holden (1999) reported lower digestibility values when the inoculum was taken from donor cow fed grass hay than when it was taken from a cow fed on total mixed rations. These findings

show that diets have great effect on the quality of rumen liquor and hence on the digestibility values obtained by *in vitro* techniques.

## 2.6 Calibrating the estimates of *in vitro* digestibility

High costs associated with *in vivo* procedures led to the development of the *in vitro* techniques (Stern *et al.,* 1997). These techniques are constantly being modified, adapted and ultimately replaced by faster, cheaper and more accurate methods. Since the *in vivo* technique and other *in vitro* techniques, such as *in sacco*, are used to predict nutritive values of feedstuffs, therefore there is need of ensuring that the *in vitro* techniques are precise, reproducible and repeatable. To achieve this, it is important to develop prediction equations relating the *in vitro* values with the *in vivo* values, so that correction factors are determined and used.

Various authors have conducted experiments using the two stage *in vitro* techniques with different inoculums to predict the *in vivo* digestibility values. Pace *et al.* (1984) utilised rumen liquor and the enzyme methods in predicting the *in vivo* OMD values of three feed samples, namely maize silage, grass hay and grass silage. The rumen liquor by Tilley and Terry (1963) showed high ability in predicting *in vivo* OMD of all the three feed samples (Table 3).

**Table 3: Prediction of *in vivo* digestibility by *in vitro* methods**

| Method used | Maize silage | | Grass silage | | Grass hay | |
|---|---|---|---|---|---|---|
| | OMD | $R^2$ | OMD | $R^2$ | OMD | $R^2$ |
| *In vivo* method | 73.5 | - | 69.5 | - | 68.4 | - |
| Tilley and Terry (1963) | 72.3 | 0.84 | 66 | 0.90 | 66.9 | 0.96 |
| Enzymes method by Kellner and Kirchgessner (1977) | 74.9 | 0.89 | 65 | 0.45 | 66.1 | 0.91 |

$R^2$ = coefficient of determination. - = missing values.

In addition, Terry *et al.* (1978) compared the pepsin-cellulase technique with the two-stage inoculum method (Tilley and Terry, 1963) for predicting *in vivo* digestibility for grasses, lucerne, red clover and sanfoin (Table 4). The coefficient of determinations ($R^2$) showed that there is little difference in the predictive value between the two methods.

**Table 4: Coefficients of determination ($R^2$) for prediction of *in vivo* digestibility by *in vitro* techniques**

| Method | Grass | Lucerne | Red clover | Sanfoin |
|---|---|---|---|---|
| Tilley and Terry (1963) | 0.97 | 0.99 | 0.89 | 0.99 |
| Pepsin-cellulase (Terry *et al.*, 1978) | 0.96 | 0.98 | 0.86 | 0.99 |

Borba and Ribeiro (1996) made a comparison of 3 sources of inocula (rumen liquor from fistulated sheep and slaughtered cattle, and sheep faeces) for an *in vitro* digestibility method in prediction of *in vivo* digestibility. In their experiment, 24 graminaceae forage samples (oat, Italian ryegrass, perennial ryegrass and maize) were used in three stages of growth, fresh and ensiled, with a known chemical composition and *in vivo* digestibility. The equation of regression between the *in vitro* digestibility of dry matter and the *in vivo* determinations for all the forages are presented in Table 5. From the study by Borba and Ribeiro (1996) it was found that, best results for the prediction of the *in vivo* digestibility were obtained using the traditional method of rumen liquor from fistulated sheep. However, it was recommended to use the rumen liquor from slaughtered cattle as the alternative source to rumen liquor from fistulated ruminant as the technique showed high correlation to *in vivo* values ($r = 0.97$). But it was observed that the variations in the composition of rumen liquor from slaughtered cattle were due to management and diets of the cattle before slaughter.

Palić and Leeuw (2009) determined the OMD of six complete diets for ruminants using *in vivo* trials with sheep and *in vitro* using two stages Tilley and Terry (1963) method, gas production techniques and multi-enzymes incubation procedures. The obtained *in vitro* results were regressed against determined *in vivo* values to derive prediction equations as shown in Table 5. From the study it was shown that the *in vivo* OMD was predicted successfully by all methods. The multi enzymes incubation procedures gave a better predictive value than other techniques.

**Table 5: Relationship between the dry matter digestibility estimated *in vitro* to predict *in vivo* digestibility**

| Method | equations | n | $R^2$ | RSD | Source |
|---|---|---|---|---|---|
| Tilley and Terry (1963) technique Sheep rumen liquor | $0.55X_1 + 25.48$ | 24 | 0.76* | 3.35 | Borba and Ribeiro (1996) |
| Tilley and Terry (1963) technique Cattle rumen liquor | $0.35X_2 + 42.01$ | 24 | 0.42* | 5.14 | ‘’ |
| Tilley and Terry (1963) technique Sheep faeces | $0.33X_3 + 35.38$ | 24 | 0.33* | 5.52 | ‘’ |
| Tilley and Terry (1963) rumen liquor from fistulated | $0.98X - 17.36$ | - | 0.75 | - | Palić and Leeuw (2009) |
| Gas production technique | $0.71 X_1 + 198.98$ | - | 0.21 | - | ‘’ |
| Multi enzyme incubation method | $0.82 X_2 + 102$ | - | 0.86 | - | ‘’ |

n = number of samples; $R^2$ = coefficient of determination; RSD = Residual standard deviation; $X_1$, $X_2$ and $X_3$ are *in vitro* dry matter digestibility determined using rumen liquor from fistulated sheep, slaughtered cattle and sheep faeces respectively; * ≤0.05; - = missing values.

## 2.7 Conclusions from the review

Feed evaluation schemes based on the digestibility experiments with live animals are limited by the high cost of management of the animals. Also the methods are time consuming and labour intensive. The *in vitro* techniques specifically the two stage Tilley and Terry (1963) technique, have a wider application and produce results which are

accurate in predicting digestibility of ruminant feeds. However, its application is limited by the animal welfare concerns and cost of maintaining surgically modified animals as source of rumen liquor. Recently, the use of rumen liquor from slaughtered cattle has been proposed as an alternative source of inoculum for *in vitro* techniques. The use of rumen liquor from slaughtered cattle as alternative to that from fistulated ruminants would be prospective if its use gives results comparable to the *in vivo* values. The use of rumen liquor from slaughtered cattle will eliminate the need for fistulated animals, which are limited by the animal welfare issues and cost of maintaining them especially in developing countries. However, utilizing rumen liquor from slaughtered cattle as inoculum for *in vitro* digestibility studies may have some variations in the quality of rumen liquor due to dietary history of the cattle before slaughter. Since it is not easy to know the dietary history of the slaughtered cattle from the traditional sector, it is important to understand the potential variability in quality and effectiveness of rumen liquor from array of slaughtered animals, on estimating *in vitro* digestibility of ruminant feeds.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Overview

Two digestibility experiments, *in vivo* and two stage *in vitro* techniques were carried out with the aim of evaluating the effectiveness of rumen liquor from slaughtered cattle as source of inoculum for estimating digestibility of ruminant feedstuffs. The two stage *in vitro* Tilley and Terry (1963) technique utilized two sources of rumen liquor that is from fistulated and slaughtered cattle as inoculum. The experiments were conducted in three different institutions namely Sokoine University of Agriculture (SUA), National Livestock Resources Research Institute (NALIRRI) and Rwanda Agricultural Board (RAB), each evaluating four different diets.

### 3.2 Description of the study area

SUA is located in Morogoro Tanzania between Latitudes 6˚49' and longitudes 37˚39'. The university is situated at the foot of the Uluguru Mountains on the northern slopes at an elevation of approximately 600 metres above sea level. The climate is tropical semi-arid with a bimodal rainfall pattern. Average rainfall is 600 to 800 mm. Short rains fall during November to January and long rains during March to May. Peak rainfall is normally received in April. The temperature ranges from 15 to 30˚C with the hottest periods being October to January, while June and July are the coolest months. Average monthly relative humidity is about 46 % during the dry season and 66 % during the wet season. Day lengths range from 11 to 13 hours.

NALIRRI is located in Tororo district Eastern Uganda at Latitude 0˚41' North and longitude 34˚10' East. The area receives average annual rainfall of 1494 mm with highest rainfall during March to May. The temperature of the area range 16 to 29˚ C.

Rubona Institute of RAB is located in Southern Rwanda at Latitude 2˚40' South and Longitude 29˚45' East. The climate of the area is tropical with average annual rainfall of about 1200 mm, having high rainfall during April. Mean temperature of the area is between 14 and 28˚ C. Average monthly relative humidity is about 59% during the dry season and 83% during the wet season.

## 3.3 The *in vivo* digestibility trial

### 3.3.1 Experimental design

In each institution, four rumen fistulated steers were randomly allocated to four diets with four runs in a 4 x 4 Latin square design.

### 3.3.2 Source of animals and their management

In each institution, four castrated steers (crossbred *Bos indicus x Bos Taurus*) weighing 250 – 370 kg, age 2 – 2.5 years were identified by numbered ear-tags and housed in individual pens, which allowed individual animal feeding. The animals were fistulated four weeks before the start of the experiment. They were fed with hay and had free access to water during the whole period before the start of the experiment. They were dewormed and acaricide was applied to control ectoparasites twice a week.

### 3.3.3 Feeds and feeding

The species of hay used at SUA was *Cenchrus ciliaris*. It was harvested after flowering stage and used to make hay. The hay was treated with urea in the ratio of 50 g of urea in 600 ml of water for 1 kg DM of hay. The formulated concentrate mixture contained 58.5% hominy meal, 39% cotton seed cake, 1.95% mineral mix and 0.5% salt. This concentrate mixture substituted hay at 0, 10, 20 and 30 percent to make diets 1, 2, 3 and 4, respectively. The forage used to make hay in NALIRRI and RAB was *Brachiaria*

*brizantha*. It was harvested at flowering stage. The concentrate mixture used for the experiment at NALIRRI contained 60% maize bran and 40% cotton seed cake, substituted hay at 0, 10, 20 and 30 percent to make diets 5, 6, 7 and 8, respectively. The concentrate formulated for the experiment at RAB contained 66% maize bran, 30% cotton seed cake, 2% mineral mix and 1% salt and substituted hay at 10, 15, 20 and 25 percent to make diets 9, 10, 11 and 12, respectively (Table 6).

**Table 6: Diets used at the different institutions**

| Institution | Diets | % constituent | |
|---|---|---|---|
| | | Hay | Concentrate |
| **SUA** | 1 | 100 | 0 |
| | 2 | 90 | 10 |
| | 3 | 80 | 20 |
| | 4 | 70 | 30 |
| **NALIRRI** | 5 | 100 | 0 |
| | 6 | 90 | 10 |
| | 7 | 80 | 20 |
| | 8 | 70 | 30 |
| **RAB** | 9 | 90 | 10 |
| | 10 | 85 | 15 |
| | 11 | 80 | 20 |
| | 12 | 75 | 25 |

A preliminary period of 10 days was followed in each institution. On the day of setting the trial the animals were weighed and confined in individual pens. Animals were fed in the morning at 0800 h and evening at 1600 h each day. During this period the quantity of feed given to the animals was determined as 3% of their body weights. The amounts of hay and concentrate were calculated and offered according to the respective diet to be fed to the animal. The refusals were collected and weighed each morning, and the amount of feed fed on each day was reduced from the amount fed the previous day by subtracting the weight of refusals. This was done every day during the preliminary period until the constant weight of feed eaten by each animal was established. All animals had free access to fresh drinking water through automatic drinkers.

The animals were harnessed with bags to prepare for the faecal collection. The harnessed bags were made of polythene/hessian lined with polythene sheet. The bags contained a small hole to allow the tail of the animal to move freely. Five straps were attached to the bags, while one free strap was wrapped on the animal either around the chest or in between the fore limbs. Three of the straps were passed over the back and attached to the strap around the animal, whereas two were passed in between the hind limbs and attached to the two of the 3 straps (Plate 1).

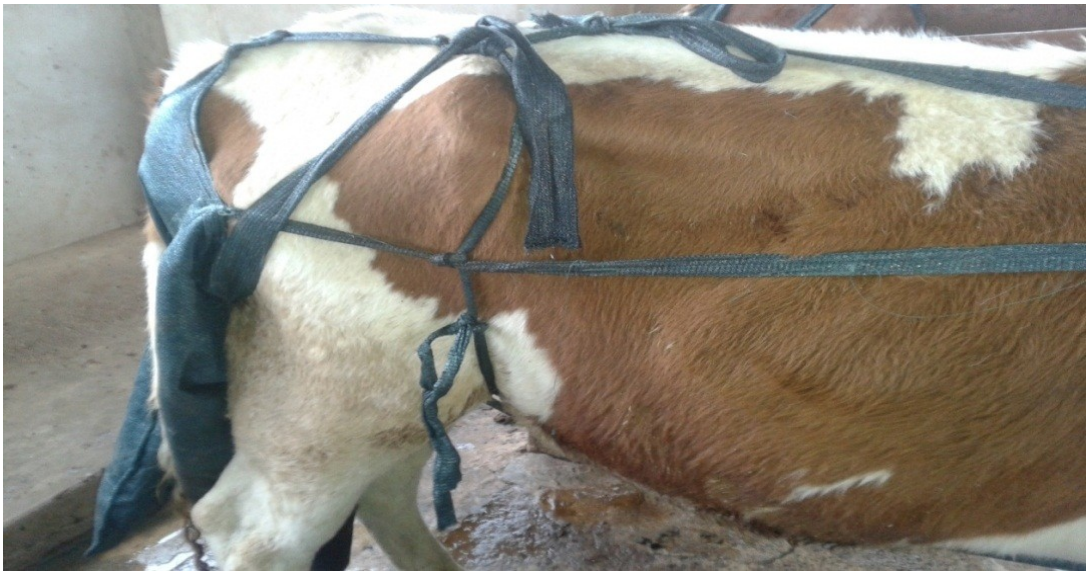

**Plate 1: Faecal collection bag tied to the animal**

The preliminary period was followed by 7 days of collection period. On the day of setting the collection period, animals were weighed and returned to their respective pens. The amount of feed established during the preliminary period was weighed and divided into two portions, which were fed in the morning at 0800 h and evening at 1600 h each day. Refusals were collected from each animal daily in the morning before next feeding, weighed and oven dried at 60°C to constant weight for determining dry matter. Faecal collection bag from each animal was emptied in a weighed bucket each morning and the

bucket with faeces was reweighed to obtain the weight of faeces voided by difference. After thorough mixing, approximately 10% of the daily faecal collection from each animal was sampled into polythene bags and stored in a deep freezer at -20°C. At the end of collection period the sampled portions of the faeces from each animal were thawed and thoroughly mixed and samples were taken in duplicate for subsequent analysis.

### 3.3.4 Sample preparation and chemical analysis

The duplicate faecal samples were dried at 100°C to constant weight for dry matter (DM) determination. The dried refusals for each animal were mixed thoroughly and sub sampled. Thereafter the dried faecal samples, feed and refusals were ground to pass through a 1 mm sieve, labelled and stored in airtight containers for DM and ash determination according to the standard procedures described by AOAC (2000).

### 3.3.5 Derived parameters

**Dry matter intake**

$DM_{consumed} = (FEED_{offered} * \%DM_{feed} - REFUSAL * \%DM_{refusal})$……(i)

**Faecal dry matter**

$DM_{faeces} = Wt_{faeces} * \%\ DM_{faeces}$ ……(ii)

**Dry matter digestibility**

$$DMD\ (\%) = \left\{\frac{DMconsumed - DMfaeces}{DMconsumed}\right\} * 100$$ ……(iii)

**Organic matter digestibility**

In calculating organic matter digestibility (OMD) of the feed the following formula was used:-

$$OMD\ (\%) = \left\{\frac{(DM\ consumed - Ash\ food) - (DM\ faeces - Ash\ faeces)}{DM\ consumed - Ash\ food}\right\} * 100$$ ……...(iv)

## 3.4 Two Stage *in vitro* digestibility

### 3.4.1 Experimental design and treatments

In each institution the experiment was carried out using a 2 x 4 factorial arrangement. Samples from the four diets used in the *in vivo* trial were incubated using two sources of inoculum in four runs.

### 3.4.2 Preparation of the feed samples

The hay was milled to pass through 1 mm sieve. It was then mixed with formulated concentrate according to specific proportion of hay to concentrate in each of the four diets. Subsamples were then drawn from each diet and kept into airtight bottles.

### 3.4.3 Collection and preparation of rumen liquor

In each run, rumen liquor was obtained from the four fistulated steers, which were used during the *in vivo* experiment and from four slaughtered cattle. Rumen liquor from fistulated and slaughtered cattle were collected concurrently. Rumen liquor from fistulated cattle was collected before the morning feeding at 0500 Hr. The liquor was collected from Magadu Farm at SUA, NALIRRI farm and Rubona station at RAB. It was drawn through the fistulae of steers by hand. Both the solid and liquid contents were collected into large warmed thermos flasks. Rumen liquor from the slaughtered cattle was collected from four animals which were selected randomly from herds of cattle brought for slaughter in the Morogoro, Tororo and Huye abattoirs. After slaughter and evisceration the rumen was opened and the rumen contents were taken from the central part of the rumen. Both the solid and the liquid contents were collected into large warmed thermos flask (Plate 2) until full when it was closed and taken to the laboratory within one hour.

**Plate 2: Thermos flasks used for collection of rumen liquor from fistulated and slaughtered cattle**

At laboratory the rumen contents from each animal was measured for pH using a portable pH meter. Then the rumen contents was divided into two parts, one part was put into two plastic bottles, each containing 250 mls, for determining the rumen $NH_3$-N and total VFAs. Twelve (12) ml of concentrated (12N) $H_2SO_4$ was added to the rumen contents to be used for determination of $NH_3$-N to stabilize the nitrogen. The bottles containing rumen liquor were stored in a deep freezer at -20°C. The other part of the rumen contents were thoroughly mixed separately and thereafter filtered into warm flasks using double layer of cheese cloth. The flasks were put in a water bath maintained at 39°C and bubbled with $CO_2$ gas for 5 minutes to maintain an anaerobic environment.

### 3.4.4 Preparation of buffer solution and pepsin-HCL

Buffer solution was prepared by dissolving 3.71g of $NaHPO_4$, 9.8g of $NaHCO_3$, 0.47g of NaCl, 0.57g of KCl, 0.04g of $CaCl_2$ and 0.12g of $MgSO_4.7H_2O$ in 1000 ml of distilled

water. Carbondioxide ($CO_2$) gas was bubbled into the solution to adjust the pH to 6.8 – 7.0. The solution was then kept in a water bath maintained at 39°C. Pepsin-hydrochloric acid solution was prepared by dissolving 2g crystalline pepsin (1:10 000) in 800 ml distilled water. Then, 100ml 1N HCL was added and the solution filled up to 1 liter with distilled water.

### 3.4.5 Procedure for Tilley and Terry (1963) technique

The feed samples were incubated according to the procedure of Tilley and Terry (1963). Half a gram of each sample was weighed into centrifuge test tubes in triplicates. Forty (40) mls of saturated buffer solution maintained at 39°C were added to each tube, followed by 10 mls of rumen liquor collected from the two sources. Three blank test tubes without a sample but with all other reagents and rumen liquor were added. The test tubes with rubber stopper and valves plugged on were placed in a water bath maintained at 39°C and incubated for 48 hrs. The tubes were frequently shaken carefully to make sure that no particles stuck onto the rubber stoppers or onto the upper parts of the test tubes. After 48 hrs of incubation the test tubes were taken out of the water bath and top stoppers were removed. They were then centrifuged at a speed of 3000 r.p.m for 10 minutes. The supernatant was discarded carefully and 50 mls of pepsin-HCL solution were added into each test tube containing the residue. The test tubes were placed in a water bath maintained at 39°C and incubated for 48 hrs without top rubber stoppers on it. The test tubes were covered with wire mesh to prevent contamination. After 48 hrs the test tubes were removed from the water bath and centrifuged for 10 minutes at a speed of 3000 r.p.m. Thereafter the residue was carefully transferred into weighed crucibles and dried at 105°C for 24 hours cooled in desiccator and weighed to obtain residue dry matter. Then the crucibles were heated in a muffle furnance at 550°C for 4 hours cooled and weighed to obtain residue ash.

### 3.4.6 Derived parameters

***In vitro* DM digestibility**

$$\%DMD = \frac{DM\ sample - (DM\ sample\ residue - DM\ blank\ residue)}{DM\ sample} * 100\% \quad \text{.....(v)}$$

***In vitro* OM digestibility**

$$\%OMD = \frac{OM\ sample - (OM\ sample\ residue - OM\ blank\ residue)}{OM\ sample} * 100\% \quad \text{.....(vi)}$$

**Where:**

$OM_{sample}$ = DM sample – Ash sample

$OM_{sample\ residue}$ = DM sample residue – Ash sample residue

$OM_{blank}$ = DM blank – Ash blank.

## 3.5 Chemical and Data Analysis

### 3.5.1 Chemical analysis

Dry matter (DM), ash, crude protein (CP), Crude Fibre (CF) and Ether Extract (EE) were determined according to proximate analysis of the Weende method (A.O.A.C., 2000). The Neutral Detergent Fibre (NDF) and Acid Detergent Fiber (ADF) were determined according to procedures described by Van Soest *et al.* (1991).

Samples of rumen liquor at SUA were analysed for concentration of rumen ammonia nitrogen (Rumen $NH_3$-N) and total volatile fatty acids (total VFAs). $NH_3$-N was determined as described by Abdulrazak and Fujihara (1999). Three (3) mls of 20% Trichloroacetic acid and 3 mls of rumen liquor were put in a test tube and centrifuged at 3000 r.p.m for 10 minutes. Two (2) mls of the supernatant were mixed with 3 mls of sodium hydroxide (NAOH) and distilled into boric acid using Kjeltec apparatus. The

distillate was titrated using 0.05 N $H_2SO_4$. Rumen ammonia nitrogen was calculated as follows:-

$$\text{Rumen } NH_3\text{-N (mg/l)} = \frac{\text{Titre volume (ml)x Nm (N)}}{\text{Volume of supernatant (2mls)}} \text{ x } 1000\text{ml} \quad \text{.....(vii)}$$

**Where:** Nm = Normality of $H_2SO_4$ acid used during titration.

The total VFAs in the rumen liquor was determined by steam distillation as described by Abdulrazak and Fujihara (1999). In semi macro Kjeldahl flask, 5 ml of rumen liquor were pipetted and 3 ml of distilled water and 10 ml of $MgSO_4$ and $H_2SO_4$ were added. 18 ml of distillate were collected in a flask and 1-2 drops of Phenolphthalein indicator was added and titrated using 0.05N NaOH solutions. Total VFAs was calculated as follows:-

$$\text{Total VFAs (mmol/100ml)} = \frac{\text{Titrate (ml) x Nm (N)}}{\text{Volume of rumen liquor}} \text{ x } 100\text{ml} \quad \text{.....(viii)}$$

Where: Nm = Normality of NaOH used during titration.

### 3.5.2 Data analysis

The data on the *in vivo* DMD and OMD, rumen pH, $NH_3$ – N, total VFAs and effect of method were analysed using the general linear model (GLM) procedure of SAS program (SAS 2003) according to the model;

$$Y_{ij} = \mu + A_i + E_i \quad \text{.....(ix)}$$

Where;

$Y_{ij}$= the *in vivo* digestibility value of the $i^{th}$ diet or rumen pH, $NH_3$ – N, total VFA concentrations of rumen liquor from $i^{th}$ liquor source or digestibility of diet in $i^{th}$ method.

$\mu$ = the mean effect

$A_i$ = the effect of $i^{th}$ diet or $i^{th}$ liquor source or effect of method.

$E_{ij}$ = the random error.

The data from *in vitro* Tilley and Terry (1963) experiment in each institution were analysed using the procedure of SAS program (SAS, 2003) according to the model;

$Y_{ij} = \mu + A_i + B_j + AB_{ij} + E_{ij}$ .....(x)

Where;

$Y_{ij}$ = the in vitro digestibility value of the $i^{th}$ diet assigned to the $j^{th}$ source of inoculum

$\mu$ = the mean effect

$A_i$ = the effect of $i^{th}$ diet

$B_j$ = the effect of $j^{th}$ source of inoculum

$AB_{ij}$ = the effect associated with interaction of $i^{th}$ diet and $j^{th}$ source of inoculum

$E_{ij}$ = the random error.

The values of digestibility obtained using *in vitro* Tilley and Terry (1963) techniques were regressed against those of *in vivo* digestibility values to derive prediction equations. A simple linear regression model was used:

$Y_i = Ax_i + C$……..……...……..(xi)

Where;

$Y_i$ = *in vivo* value (predicted value/dependent parameter)

A = The regression coefficient

$x_i$ = *in vitro* value (independent value)

C = Constant.

$E_i$ = Random error.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Chemical composition of the experimental diets

The chemical composition of the diets used in the study from the different institutions is shown in Table 7. Substitution of hay with concentrate in the diets improved the CP contents while decreasing the fibre contents of the diets.

**Table 7: Chemical composition (% DM) of the diets and hay used in the experiments**

| INSTITUTIONS | DIETS | DM | CP | Ash | CF | EE | NDF | ADF |
|---|---|---|---|---|---|---|---|---|
| **SUA** | **1** | 92.2 | 9.89 | 6.32 | 36.7 | 1.48 | 76.6 | 46.5 |
| | **2** | 92.4 | 13.0 | 7.09 | 34.9 | 2.16 | 71.6 | 43.2 |
| | **3** | 92.5 | 14.3 | 6.79 | 32.5 | 2.58 | 67.0 | 41.1 |
| | **4** | 92.6 | 15.4 | 7.20 | 31.6 | 3.52 | 64.6 | 38.4 |
| **NALIRRI** | **5** | 92.6 | 9.23 | 7.42 | - | - | 80.0 | 44.1 |
| | **6** | 91.9 | 9.82 | 6.95 | - | - | 72.4 | 40.0 |
| | **7** | 91.6 | 10.58 | 6.59 | - | - | 66.3 | 36.1 |
| | **8** | 91.3 | 11.36 | 6.37 | - | - | 59.1 | 32.3 |
| **RAB** | **9** | 91.3 | 8.70 | 10.8 | - | - | - | - |
| | **10** | 91.9 | 8.78 | 10.9 | - | - | - | - |
| | **11** | 92.6 | 11.4 | 5.98 | - | - | - | - |
| | **12** | 93.7 | 12.6 | 6.19 | - | - | - | - |
| | ***Cenchrus* hay (untreated hay)** | 89.1 | 4.80 | - | - | - | - | - |
| | ***Brachiaria* hay** | 88.2 | 4.2 | - | - | - | 78.7 | 43.2 |

**Note:** 1 = $H_{100}C_{0,}$ 2 = $H_{90}C_{10,}$ 3 = $H_{80}C_{20}$, 4 = $H_{70}C_{30}$, 5 = $H_{100}C_{0}$, 6 = $H_{90}C_{10}$, 7 = $H_{80}C_{20}$, 8 = $H_{70}C_{30}$, 9 = $H_{90}C_{10}$, 10 = $H_{85}C_{15}$, 11 = $H_{80}C_{20,}$ 12 = $H_{75}C_{25}$; - = missing values.

## 4.2 Fermentation characteristics of rumen liquor

Least square means for the rumen pH and concentrations of $NH_3$-N and total VFAs are shown in Table 8 (and in Appendices 1, 2 and 3 respectively). There was no significant difference ($P > 0.05$) in the pH of rumen liquor from fistulated and slaughtered cattle. Rumen liquor from slaughtered cattle had significantly higher ($P < 0.05$) concentrations of $NH_3$-N and total VFAs than that from fistulated cattle.

**Table 8: Least square means of pH and concentrations of $NH_3$-N and total VFAs in the rumen liquor from fistulated and slaughtered cattle at SUA.**

| | pH [$H^+$ ions] | $NH_3$-N (mg/l) | Total VFA (mmoles/l) |
|---|---|---|---|
| **Number of observations** | 16 | 16 | 16 |
| **Fistulated** | 6.59 | 111.3[a] | 124.0[a] |
| **Slaughtered** | 6.53 | 122.7[b] | 151.8[b] |
| **SEM** | 0.06 | 1.71 | 7.75 |
| **P Value** | NS | 0.0001 | 0.0166 |

[ab] least square means within columns with different superscripts are statistically different
SEM = Standard error of the mean
P-value = Probability of Type III error.
N.S = Not significant.

## 4.3 The *in vivo* digestibility of the diets

The Least Square Means for *in vivo* dry matter (DMD) and organic matter (OMD) digestibility of the diets used in the experiment are shown in Table 9 (and in appendices 4 – 9). Diets 3 and 4 had significantly higher ($P < 0.05$) values for *in vivo* DMD and OMD than Diets 1 and 2. There was no significant difference on the values for DMD and OMD of diets 5 – 8 used at NALIRRI. Diets 10, 11 and 12 used at RAB had significantly higher values of DMD than Diet 9; Diet 12 had significantly higher ($P < 0.05$) value of OMD than other diets.

**Table 9: Least Square Means for *in vivo* digestibility (%DM) of the diets used in the study**

| Institution | Parameter | Diets | | | | SEM | P - value |
|---|---|---|---|---|---|---|---|
| **SUA (Diets)** | | **1** | **2** | **3** | **4** | | |
| | **DMD** | 51.6[a] | 53.7[a] | 59.0[b] | 61.8[b] | 1.29 | 0.0001 |
| | **OMD** | 51.4[a] | 54.0[a] | 59.1[b] | 62.2[b] | 1.27 | 0.0001 |
| **NALIRRI (Diets)** | | **5** | **6** | **7** | **8** | | |
| | **DMD** | 53.1 | 52.7 | 52.9 | 53.4 | 1.45 | 0.9866 |
| | **OMD** | 51.0 | 53.9 | 55.2 | 55.6 | 1.38 | 0.0884 |
| **RAB (Diets)** | | **9** | **10** | **11** | **12** | | |
| | **DMD** | 84.7[a] | 90.9[b] | 90.7[b] | 90.5[b] | 1.79 | 0.0439 |
| | **OMD** | 82.1[a] | 84.3[a] | 83.8[a] | 87.9[b] | 0.83 | 0.0001 |

SEM = Standard error of the mean.
[abcd]Least square means within rows with different superscripts are statistically different (P <0.05).
P-Value = Probability of Type III error.
DMD = Dry Matter Digestibility, OMD = Organic Matter Digestibility.
**Note:** 1 = $H_{100}C_{0,}$ 2 = $H_{90}C_{10,}$ 3 = $H_{80}C_{20}$, 4 = $H_{70}C_{30}$, 5 = $H_{100}C_{0}$, 6 = $H_{90}C_{10}$, 7 = $H_{80}C_{20}$, 8 = $H_{70}C_{30}$, 9 = $H_{90}C_{10}$, 10 = $H_{85}C_{15}$, 11 = $H_{80}C_{20,}$ 12 = $H_{75}C_{25}$.

## 4.4 Least Square means for *in vitro* digestibility of the diets

Table 10 shows the least square means for *in vitro* DMD obtained by *in vitro* technique of Tilley and Terry (1963), utilising rumen liquor from fistulated and slaughtered cattle in the different institutions. Diet 4 had significantly higher ($P < 0.05$) least square means for *in vitro* DMD than other diets followed by diets 3 and 2 for diets used at SUA. The least square means for *in vitro* DMD obtained using rumen liquor from slaughtered cattle was significantly ($P < 0.05$) higher than those obtained using rumen liquor from fistulated cattle. Diets 8 and 7 had significantly ($P < 0.05$) higher mean values for *in vitro* DMD, followed by Diet 6 and least with Diet 5. Using rumen liquor from either fistulated or slaughtered cattle for incubating samples at NALIRRI and RAB did not influence the *in*

*vitro* DMD of the diets. The interaction effect of rumen liquor source and the *in vitro* DMD of the diet was not significant.

**Table 10: Least square means for i*n vitro* DMD (%) of the diets used in the experiments in different institutions**

| Institution | Diets | | | | SEM | P value | RL source | | SEM | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| **SUA (diets)** | **1** | **2** | **3** | **4** | | | **Fistu.** | **Slau.** | | |
| | 37.2[a] | 40.6[b] | 41.9[b] | 45.4[c] | 0.97 | 0.0001 | 40.0[a] | 42.6[b] | 0.70 | 0.0089 |
| **NALIRRI (Diets)** | **5** | **6** | **7** | **8** | | | | | | |
| | 22.2[a] | 26.9[b] | 33.0[c] | 34.6[c] | 1.18 | 0.0001 | 29.0 | 29.3 | 0.84 | 0.7680 |
| **RAB (Diets)** | **9** | **10** | **11** | **12** | | | | | | |
| | 46.4 | 46.5 | 46.8 | 46.6 | 3.48 | 0.9965 | 47.5 | 45.7 | 2.46 | 0.6218 |

SEM = Standard error of the mean.

[abcd]Least square means within rows with different superscripts are statistically different (P <0.05).

P-Value = Probability of Type III error.

NS = not significant

Fistu = Fistulated

Slau =Slaughtered

Table 11 shows the mean values for *in vitro* OMD obtained by *in vitro* technique of Tilley and Terry (1963), utilising rumen liquor from fistulated and slaughtered cattle in the different institutions. Among the diets used at SUA, Diet 4 had significantly higher ($P < 0.05$) mean *in vitro* OMD followed by Diet 3 and 2, and least for Diet 1.

The mean value for *in vitro* OMD obtained using rumen liquor from slaughtered cattle was significantly ($P < 0.05$) higher than those obtained using rumen liquor from fistulated cattle. Among the diets used at NALIRRI, diets 8 and 7 had significantly ($P < 0.05$) higher mean values for *in vitro* OMD, followed by Diet 6 and least with Diet 5. Using rumen liquor from either fistulated or slaughtered cattle for incubating samples at NALIRRI and RAB did not influence the *in vitro* OMD of the diets. There was no

significant difference (P > 0.05) on the values for the *in vitro* OMD between the diets used at RAB. The interaction effect of rumen liquor source and *in vitro* OMD of the diet was not significant.

**Table 11: Least square means for i*n vitro* OMD (%) of the diets used in the experiments in different institutions**

| Institution | Diets | | | | SEM | P value | RL source | | SEM | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| **SUA (Diets)** | **1** | **2** | **3** | **4** | | | Fistu. | Slau. | | |
| | 33.6[a] | 37.1[b] | 37.4[b] | 41.9[c] | 0.88 | 0.0001 | 36.2[a] | 38.8[b] | 0.63 | 0.0052 |
| **NALIRRI (Diets)** | **5** | **6** | **7** | **8** | | | | | | |
| | 15.6[a] | 20.9[b] | 27.8[c] | 29.7[c] | 0.28 | 0.0001 | 23.3 | 23.7 | 0.90 | 0.7853 |
| **RAB (Diets)** | **9** | **10** | **11** | **12** | | | | | | |
| | 46.4 | 45.8 | 48.5 | 45.3 | 3.95 | 0.9361 | 47.7 | 45.3 | 2.79 | 0.5743 |

SEM = Standard error of the mean.

[abcd]Least square means within rows with different superscripts are statistically different (P <0.05).

P-Value = Probability of Type III error.

NS = not significant

Fistu = Fistulated

Slau =Slaughtered

Figure 1 presents the relationships between the mean values for *in vitro* DMD obtained by the two stage technique using rumen liquor from fistulated and slaughtered cattle for all institutions. There was a weak linear relationship between the values of *in vitro* DMD obtained by the two stage technique using rumen liquor from fistulated and slaughtered cattle ($R^2$ = 0.3417 and S.E = 8.3682).

Figure 2 presents the relationships between the values for *in vitro* OMD obtained by two stage technique using rumen liquor from fistulated and slaughtered cattle for all institutions. There was also weak linear relationship between the values for *in vitro* OMD

obtained by the two stage technique using rumen liquor from fistulated and slaughtered cattle ($R^2$ = 0.362 and S.E = 10.34804).

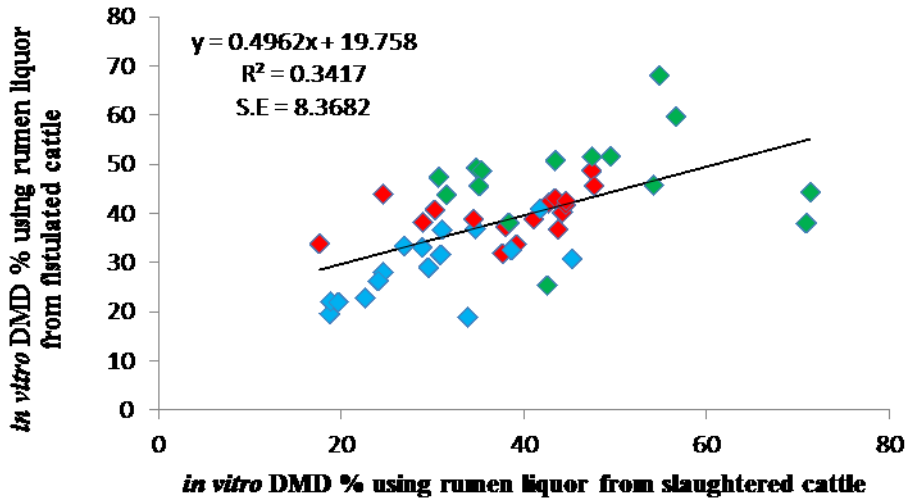


**Figure 1: Relationship between percent DMD obtained using rumen liquor from fistulated and slaughtered cattle**

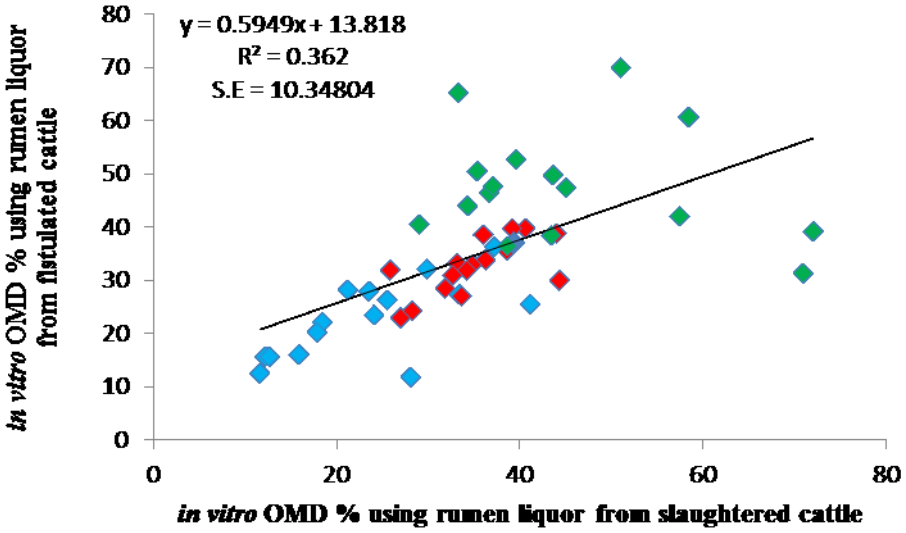


**Figure 2: Relationship between percent OMD obtained using rumen liquor from fistulated and slaughtered cattle**

### 4.5 Relationship between *in vivo* and *in vitro* digestibility

Table 12 shows the relationship between the *in vivo* and *in vitro* digestibility values obtained using rumen liquor from fistulated and slaughtered cattle. The digestibility values obtained by *in vivo* method were significantly higher ($P < 0.05$) than those obtained by *in vitro* methods.

**Table 12: Lsmeans on *in vivo*, *in vitro* using rumen liquor from fistulated cattle and *in vitro* using rumen liquor from slaughtered cattle from different institutions**

| Institution | Parameter | *In vivo* | *In vitro* using rumen liquor from fistulated cattle | *In vitro* using rumen liquor from slaughtered cattle | SEM | P Value |
|---|---|---|---|---|---|---|
| **SUA** | **DMD** | 56.0[b] | 40.0[a] | 42.6[a] | 2.10 | 0.0001 |
| | **OMD** | 56.2[b] | 36.2[a] | 38.8[a] | 1.97 | 0.0001 |
| **NALIRRI** | **DMD** | 54.1[b] | 29.0[a] | 29.3[a] | 2.10 | 0.0001 |
| | **OMD** | 52.0[b] | 23.3[a] | 23.7[a] | 1.97 | 0.0001 |
| **RAB** | **DMD** | 88.1[b] | 47.5[a] | 45.7[a] | 2.10 | 0.0001 |
| | **OMD** | 84.2[b] | 47.7[a] | 45.3[a] | 1.97 | 0.0001 |

SEM = Standard error of the mean.
[ab] Least square means within rows with different superscripts are statistically different ($P < 0.05$).
P-Value = Probability of Type III error.
DMD = Dry Matter Digestibility
OMD = Organic Matter Digestibility.

Table 13 shows the prediction equations relating the values of *in vivo* and *in vitro* digestibility estimated by Tilley and Terry (1963) technique using rumen liquor from fistulated and slaughtered cattle from different institutions. The coefficients of determination ($R^2$) for predicting *in vivo* DMD using rumen liquor from fistulated was

lower than when rumen liquor from slaughtered cattle was used. There was no significant difference (P > 0.05) between prediction equations obtained when rumen liquor from fistulated cattle and slaughtered cattle was used in estimating the *in vivo* DMD.

When rumen liquor from fistulated cattle was used in estimating OMD, higher mean coefficient of determination ($R^2$ = 0.51 and S.E = 10.92) was observed than utilising rumen liquor from slaughtered cattle ($R^2$ = 0.36 and S.E = 12.47). There was no significant difference (P > 0.05) between the prediction equations obtained when rumen liquor from fistulated cattle and slaughtered cattle was used in estimating the *in vivo* OMD.

**Table 13: Prediction equations relating *in vivo* digestibility (%) and *in vitro* digestibility (%X) estimated by two stage technique using rumen liquor from slaughtered and fistulated cattle**

| Parameter | Source of Rumen Liquor | Prediction equation | S.E | $R^2$ | n |
|---|---|---|---|---|---|
| **DMD** | Fistulated cattle | 0.9525X +29.336 | 14.56 | 0.31 | 48 |
| | Slaughtered cattle | 0.8258X + 34.788 | 14.42 | 0.33 | 48 |
| **OMD** | Fistulated cattle | 0.8595X + 34.371 | 10.92 | 0.51 | 48 |
| | Slaughtered cattle | 0.7142X + 39.157 | 12.47 | 0.36 | 48 |

$R^2$ = Coefficient of determination
n = Number of observations
S.E = standard error

# CHAPTER FIVE

## 5.0 DISCUSSION

### 5.1 Chemical composition of experimental diets

The chemical composition of the diets used in the present study differed from one another and this was intended to bring variability on the digestibility values. The CP content of Diet 1 was within the range of the values reported in the literature for *Cenchrus ciliaris* hay. Ramírez *et al.* (2004) reported the CP content for *C. ciliaris* to vary from 7 – 13% and Manzoor *et al.* (2013) reported the CP content to be 11.3%. However the CP content was higher than 5.7% reported by Komwihangilo *et al.* (2005) and 4.4% reported by Ramírez *et al.* (2007). This difference was due to the urea treatment which might have increased the amount of N in the hay in the current study. It has been reported that treatment of hay with urea may generally increase the CP contents up to 10% of the DM (Ramírez *et al.* 2007). In the current study, the CP of hay was improved from 4.80% to 9.89% after treatment with urea. The CP content of urea treated *C. ciliaris* hay was within the range of the values (7.63 – 16.2%) reported for the hay treated with urea (Ramírez *et al.*, 2007).

The values of CP in diets 2 – 4 used at SUA were higher as compared to Diet 1 because the CP content in the concentrate used to formulate the different diets was higher than that in hay, hence increased the CP values of the diets. It is widely known that supplementation of hay with concentrate increases the nutritive values of the forages (Ramírez *et al.* 2007). The hay (*C. ciliaris*) contained NDF and ADF contents higher than values (67.5% and 46.3% respectively), reported by Manzoor *et al.* (2013) for the same forage. The differences in the NDF and ADF contents may be due to differences on the stage of maturity of the grasses. The observed reduction in NDF and ADF concentration

in the diets with addition of concentrate levels in the diets could be due to the relatively low fibre in the concentrate which tends to decrease the overall fibre content of the whole diets (Tafaj *et al.,* 2005).

The CP content (9.23) of *Brachiaria brizantha* obtained in the present study was within the range of values reported in the literature (Rueda *et al.,* 2003). The values of CP obtained for diet 5 which was composed of *Brachiaria* sp was higher as compared to those (5.0 to 6.8% CP) reported by Fredricksen and Kategile (1980) for Brachiaria sp. The differences in the CP contents is mainly attributed to the stage of maturity of the grasses and location where they have been harvested, in which the soil fertility may have affect the amount of CP in forages.

The CP of the diets provided the good source of nutrients for the experimental animals, as the CP contents were within the recommended range of above 8% for proper functioning of rumen microbes.

## 5.2 Fermentative Characteristics of the Rumen Liquor

The observed pH values for rumen liquor collected from slaughtered and fistulated cattle (6.53 and 6.59 respectively) were slightly higher than the values (5.5 – 6.5) reported by McDonald *et al.* (1995) for optimum microbial fermentation. These values were within the range 6.2 – 7.2 reported by Van Soest (1994) as being optimal for fibre digestion, and also within the range (6.2 - 7.0) considered to be optimum for the functioning of rumen microorganisms (Ndlovu, 1992). The values of pH were within the normal range due to high feed intake which is usually associated with increased chewing activities and saliva production (Oosting, 1993). The ions in the saliva act as buffer for pH in the rumen of the animals (Nørgaard, 1993). In addition, the type of feed fed to the animal may induce

salivation and hence enhance buffering capacity (Mekasha *et al.,* 2003). It is known that when ruminants are fed with hay and feeds high in cell wall contents is expected to increase the rate of saliva production that act as buffer for pH. Since it was during the dry season the slaughtered cattle were possibly feeding on dry forages, trees and shrubs. These types of plants stimulate rumination and high saliva production. The fistulated cattle were fed with hay and concentrate, which could have increased the rate of salivation and hence buffer the pH.

The mean value of pH for rumen liquor from slaughtered cattle was slightly lower than the range of 6.8 to 7.3 reported by Chaudhry (2008). These deviations could have resulted from differences in the management of the animals. Animals used in these two different studies were coming from different locations and fed on different diets. Collection and preservation method for the rumen liquor may have affected its pH. The pH for the rumen liquor from slaughtered animals was measured in the laboratory one hour after collection from the abattoir, which might have led to slightly low pH value of the liquor. Nevertheless, the mean pH value for rumen liquor from slaughtered cattle was within the acceptable levels for rumen fermentation (Rezaaian *et al.,* 2006).

Thus, rumen liquor from slaughtered cattle could be used as inoculum for *in vitro* digestibility of the feed, as the pH was shown to be within the range required for normal functioning of the rumen microbes. The observed higher concentration of $NH_3$-N in the rumen liquor from slaughtered cattle than fistulated cattle (Table 8) could be an indication of reduced ruminal uptake that occur upon termination of life. Males and Purser (1970) reported that high $NH_3$-N concentration in the rumen liquor is attributed to inability of microorganisms to effectively utilise ammonia and could lead to decreased intensity of fermentation (Mekasha *et al.,* 2003).

The slightly lower $NH_3$-N concentration observed in the rumen liquor from the fistulated cattle relative to that from slaughtered cattle could also be an indication of a slightly higher uptake of $NH_3$-N by rumen microorganisms (Hristov *et al.,* 2001). This could be due to increased activity and growth of the microbial population in the response to optimal pH conditions observed in the rumen liquor from fistulated cattle.

The concentration of $NH_3$-N in the rumen liquor from both sources appeared to be sufficient to meet the N requirements for the rumen microbial population since the concentrations were above the critical level of 50 mg/l for optimal microbial growth (Krebs and Leng, 1984) and above the recommended range of 60 – 100 mg/l for maximum *in vitro* digestibility of low quality feeds (Oosting *et al.*, 1989). The findings on the concentration of $NH_3$-N in the rumen liquor from slaughtered cattle are consistent with the earlier work by Chaudhry (2008), who obtained a range between 63.1 to 122.5 mg/l of rumen liquor.

The observed mean values of concentration of total VFAs in the rumen liquor of slaughtered and fistulated cattle were within the physiological normal range (70 – 150 mmol/l) reported by McDonald *et al.* (1995). The observed slightly higher concentration of total VFAs in the rumen liquor from slaughtered cattle than that from the fistulated cattle might indicate larger and more active microbial population in the rumen liquor from slaughtered cattle (Tejido *et al.,* 2002). High concentration of total VFAs may indicate increased microbial activity, which is associated with increased utilisation of $NH_3$-N (Askar *et al.,* 2006). Nevertheless, the concurrently high level of NH3-N in the rumen of slaughtered animals does not support increased microbial activity. The high concentration of total VFAs in the rumen liquor from slaughtered cattle could also indicate reduced absorption of VFAs by the host animal as is well known that the concentration of total

VFAs in the rumen is governed by the rates of fermentation and absorption (Oosting, 1993). The higher concentration of total VFAs in the rumen of the slaughtered cattle relative to fistulated cattle indicates that the animal after slaughter has little ability of absorbing the VFAs produced by the rumen microbes.

Since the fermentation characteristics of rumen liquor from the slaughtered cattle were within the recommended range for proper growth and function of rumen microbes, then rumen liquor from slaughtered cattle could be suitable as inoculum to be used for determination *of in vitro* digestibility.

### 5.3 Effectiveness of rumen liquor from slaughtered cattle in *in vitro* digestibility

The *in vitro* DMD of Diet 1 used for studies at SUA was lower than some of the values reported in the literature for Buffel grass (*Cenchrus ciliaris)*. Jacobs *et al.* (2004) reported values of *in vitro* DMD to be in the range 60 – 69% while Mutimura *et al.* (2013) obtained a value of 41.1%, much higher value of DMD (70.1%) was reported by Minson and Hacker (1995), for the same grass specie. The value of DMD of Buffel grass (37.2%) obtained in the present study was also below a value (71.4 %) obtained by Heuzé *et al.* (2013).

The values obtained were also below 64.0 - 73.4% for leaf part and 47.4 – 61.7% for stem part of *C. ciliaris* (Wilson *et al.,* 1989). Also the value of *in vitro* OMD (33.6%) obtained for Diet 1 was below some of value obtained in different studies for *C. ciliaris*. The values 53 – 64% (Jacobs *et al.,* 2004) and 39.5% (Mutimura *et al.,* 2013) for *in vitro* OMD of *C. ciliaris* have been obtained. These variations in the values of *in vitro* DMD and OMD could be due to the differences in the stages of growth of the forage evaluated,

which tends to decline when the forage matures. The digestibility of Diet 1 was lower because the forage used for making hay may be was too mature and hence the nutritive value declined.

However, the value of *in vitro* DMD and OMD for Diet 1 was within the range of 20 – 50% for *in vitro* DMD and OMD of tropical grasses (Komwihangilo *et al.,* 2005). The digestibility in other diets was increasing because the diets were made purposely to have different digestibility values with inclusion of concentrates feed.

The differences in the digestibility values of the diets 5 - 8 used at NALIRRI were expected as they were made to contain different digestibility values. The diets with higher proportion of concentrate had higher digestibility values. Addition of the concentrates to the feed increased the digestibility of the diets (Dung *et al.,* 2014). The digestibility of diets at NALIRRI was lower than those reported in the literature for hay (*Brachiaria spp*.) which was used to make the diets. The *in vitro* digestibility for *Brachiaria* hay has been reported to be in the range 51.5 – 56.5% DM (Fredricksen *et al.,* 1980). However, the value were within the range 20 – 50% reported by Fisher *et al.* (1995) as the *in vitro* digestibility for some of the tropical forages. The values of *in vitro* DMD and OMD of the diets used at RAB were within the range of the digestibility of *Brachiaria brizantha* which was used for making the diets (Ribeiro *et al.,* 2014).

The observed higher values of *in vitro* DMD and OMD obtained using rumen liquor from slaughtered cattle than from fistulated cattle at SUA, showed that there may be large and active microbial population in the rumen liquor from slaughtered cattle as evidenced by the higher concentrations of total VFA and $NH_3$-N in the rumen liquor from slaughtered cattle than that from fistulated cattle (Table 8). However, the values for *in vitro* DMD

and OMD obtained by utilising rumen liquor from both sources were within the range of the values reported for forages and *Cenchrus ciliaris* based diets (Fisher *et al.,* 1995; Aregheore *et al.,* 2006). The values of *in vitro* digestibility using rumen liquor from fistulated and slaughtered cattle at NALLIRI and RAB were also within the range of the values for forages.

The observed relationship ($R^2$ = 0.3417 for *in vitro* DMD and $R^2$ = 0.362 for *in vitro* OMD) between the values of *in vitro* digestibility obtained using rumen liquor from fistulated and slaughtered cattle, suggests that the two liquor sources have insignificant correlation. This could be due to variations in the fermentation characteristics of the rumen liquor observed. The observed higher total VFAs and $NH_3$ – N in rumen liquor from slaughtered cattle than fistulated cattle, suggest the two sources to have different fermentation ability. There were variations in the digestibility values of the diets within the institutions obtained using rumen liquor from fistulated and slaughtered cattle, which may be due to errors in the laboratory during the experiment. This led to have lower relationship between the values of *in vitro* digestibility obtained using rumen liquor from fistulated and slaughtered cattle. These results are consistent with those reported by Borba and Ribeiro (1996) that used the two stage technique to make comparison between rumen liquor from fistulated sheep, slaughtered cattle and sheep faeces suspension. The authors suggested the use of rumen liquor from slaughtered cattle as a valid alternative to the traditional method of using rumen liquor from fistulated ruminants, although there was a lower relationship ($R^2$ = 0.33) between rumen liquor from slaughtered cattle and fistulated sheep. Therefore, from the findings of this study, rumen liquor from slaughtered cattle can be used as alternative source of inoculum for evaluating feeds by two stage *in vitro* techniques. This is due to the fact that, rumen liquor from slaughtered cattle was as effective as that from fistulated cattle in estimating digestibility of the feeds in all

institutions. This finding supports previous work done using rumen liquor from slaughtered cattle for *in vitro* digestibility determination (Denek *et al.,* 2006; Parand and Taghizadeh, 2010).

### 5.5 Relationship between *in vivo* and *in vitro* digestibility

The obtained values of *in vivo* DMD of diets used at SUA were in agreement with previous work by Avilés-Nieto *et al.* (2013) for diets made with *C.ciliaris* hay with different levels of protein contents. In their study the values of *in vivo* DMD ranged from 49 – 54% for the diets made with *C. ciliaris* hay with different levels of protein supplementation. However, the values of *in vivo* OMD were lower than values (76 – 79%) reported in the study by Avilés-Nieto *et al.* (2013). This difference could be due to variation on stage of maturity of the grass used to make hay, and the type of the material used to make diets. In the current study, diets were made with *C. ciliaris* hay and concentrates at different levels while in the study by Avilés-Nieto *et al.* (2013) diets were made with *C. ciliaris* hay and *Gliricidia sepium* at the same levels as in the current study. On the other hand, the values for *in vivo* OMD are within the range reported by Jacobs *et al*. (2004). The values for *in vivo* digestibility for *Brachiaria* hay were also within the range reported elsewhere (Ribeiro *et al.* 2014). The *in vivo* OM digestibility of *Brachiaria* hay has been reported to range from 40 – 77 (Fredricksen and Kategile, 1980). The values of *in vivo* DMD and OMD at RAB were higher than those reported in the literature. This could be due to stage of maturity of the forages used to make the hay.

The mean values of *in vivo* digestibility were higher than *in vitro* digestibility values in all institutions. This is due to the fact that *in vivo* digestibility is often expected to be high because the digestion is a function of physical and biochemical activities involved in

mastication, rumination and contraction of digestion tract and the influence of multiple enzymes in the digestion tract (Ru *et al.,* 2002). Low mean values of *in vitro* digestibility as compared to *in vivo* values could also be due to frequent electric failures in the laboratories that create temperature fluctuations, hence effect on fermentation and lower digestibility values. Poor handling of rumen liquor has also been reported to affect the *in vitro* digestibility values because this can cause some microbes to die and hence partial digestibility resulting into underestimation of digestibility (Yona, 2004). The same trend was found for *in vitro* OMD by Geisert *et al*. (2007) who obtained higher *in vivo* OMD than *in vitro* OMD for five different forages.

The coefficient of determination ($R^2$ = 0.31 and S.E = 14.56) obtained between *in vivo* and *in vitro* DMD values using rumen liquor from fistulated cattle was lower than those reported in the literature for predicting *in vivo* DMD using Tilley and Terry (1963) technique. De Boever *et al*. (1997) reported $R^2$ = 0.81, 0.95 and 0.92 for different feeds for ruminants. Furthermore, the value of coefficient of determination ($R^2$ = 0.51 with S.E = 10.92) for predicting *in vivo* OMD was lower than the value of $R^2$ = 0.81 reported by Hvelplund *et al. (*1999). The differences in the present study and those reported in the literature could be due to the variations in the results obtained by *in vitro* techniques, which have shown to have large variation from *in vivo* values in some institutions. This may be caused by some errors in the laboratory, which suggest that the method that used rumen liquor from fistulated cattle was not able to simulate accurately the condition that exists in the host animal.

The coefficient of determination ($R^2$ = 0.33 with S.E = 14.42) obtained when using rumen liquor from slaughtered cattle in prediction of *in vivo* DMD in the current study was lower than that ($R^2$ = 0.42) reported by Borba and Ribeiro (1996) utilising rumen liquor from

slaughtered cattle. Same authors obtained higher coefficient of determination ($R^2$ = 0.63) than the value from the current study for rumen liquor from slaughtered cattle in determination of *in vivo* dry matter digestibility of fresh forages. But the coefficient of determination for the current study were higher than coefficient of determination $R^2$ = 0.02 obtained for ensiled forages (Borba and Ribeiro, 1996). The current $R^2$ values were also lower than those reported by Denek *et al.* (2010) of $R^2$ = 0.80 in the study to investigate the precision of rumen fluid of slaughtered cows and fistulated sheep as inoculum in the *in vitro* digestibility techniques. The coefficient of determination ($R^2$ = 0.36 with S.E = 12.47) for prediction of *in vivo* OMD by *in vitro* technique using rumen liquor from slaughtered cattle was lower than $R^2$ = 0.83 reported by Hvelplund *et al*. (1999) and 0.92 reported by De Boever *et al.* (1997). The low coefficients of determination obtained in the current study could be due to variability in the digestibility values of the diets in different institutions.

Rumen liquor from fistulated cattle and slaughtered cattle has shown low ability in predicting *in vivo* DMD showing that the rumen liquor were not able to reproduce accurately the condition that exists in the host animal, and hence the equations are not good to rely on prediction of *in vivo* DMD. The method that used rumen liquor from fistulated cattle showed higher ability of predicting *in vivo* OMD than that which used rumen liquor from slaughtered cattle. The low coefficient of determinations for prediction of *in vivo* digestibility using *in vitro* two stage techniques utilising rumen liquor from fistulated and slaughtered cattle, suggest that there are great margin of error and hence would not be adequate for predictive model. The models were weak and need to be used with caution. However further study need to be conducted to include different forages in prediction of *in vivo* digestibility by *in vitro* digestibility values using rumen liquor from

slaughtered cattle so that prediction equation of different forages can be made to support the findings of the current study.

# CHAPTER SIX

## 6.0 CONCLUSIONS AND RECOMMENDATIONS

### 6.1 Conclusions

i. Results from this study have demonstrated that the fermentation characteristics of rumen liquor from slaughtered cattle were slightly different from that of fistulated cattle. However the obtained values were within the range recommended for optimum performance of rumen microbes.

ii. Rumen liquor from slaughtered cattle could be used as inoculum for estimating the *in vitro* digestibility of ruminant feeds as similar digestibility values to those obtained using rumen liquor from fistulated cattle were observed. This will reduce the need for fistulated animals hence reduce the cost of management of animals and responding to issues of animal welfare.

**iii.** *In vitro* values obtained using two stage technique of Tilley and Terry (1963) utilising rumen liquor from slaughtered cattle can be used to predict *in vivo* digestibility. However the low coefficients of determination, suggest that there are great margin of errors and hence would not be adequate for predictive model. The models were weak and need to be used with caution.

### 6.2 Recommendations

Rumen liquor from slaughtered cattle could be used for *in vitro* determination of feeds for ruminants. However further studies need to be conducted to have more convincing results and in order to reduce the variations in the quality of rumen liquor there is need of collecting the rumen contents from several animals.

Further work need to be done to increase the number of observations in order to develop more reliable prediction equations.

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# APPENDICES

## Appendix 1: Analysis of variance for pH

Dependent Variable: pH

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 4 | 0.16326250 | 0.04081562 | 0.75 | 0.5697 |
| Error | 27 | 1.47883438 | 0.05477164 | | |
| Corrected Total | 31 | 1.64209688 | | | |

| R-Square | Coeff Var | Root MSE | PH Mean |
|---|---|---|---|
| 0.099423 | 3.567413 | 0.234033 | 6.560313 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| INOCSRC | 1 | 0.03712812 | 0.03712812 | 0.68 | 0.4175 |
| PERIOD | 3 | 0.12613437 | 0.04204479 | 0.77 | 0.5222 |

## Appendix 2: Analysis of variance for $NH_3$-N

Dependent Variable: NH3-N

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 4 | 1466.633750 | 366.658438 | 10.20 | <.0001 |
| Error | 27 | 970.383437 | 35.940127 | | |
| Corrected Total | 31 | 2437.017188 | | | |

| R-Square | Coeff Var | Root MSE | NH3 Mean |
|---|---|---|---|
| 0.601815 | 5.122160 | 5.995009 | 117.0406 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| INOCSRC | 1 | 1038.540312 | 1038.540312 | 28.90 | <.0001 |
| PERIOD | 3 | 428.093438 | 142.697813 | 3.97 | 0.0182 |

## Appendix 3: Analysis of variance for VFA

Dependent Variable: VFA

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 4 | 10536.30750 | 2634.07688 | 2.91 | 0.0401 |
| Error | 27 | 24447.00750 | 905.44472 | | |
| Corrected Total | 31 | 34983.31500 | | | |

| R-Square | Coeff Var | Root MSE | VFA Mean |
|---|---|---|---|
| 0.301181 | 21.81467 | 30.09061 | 137.9375 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| INOCSRC | 1 | 6182.720000 | 6182.720000 | 6.83 | 0.0145 |
| PERIOD | 3 | 4353.587500 | 1451.195833 | 1.60 | 0.2119 |

## Appendix 4: Analysis of variance for *in vivo* dry matter digestibility at NALIRRI

The GLM Procedure

| Class | Levels | Values |
|---|---|---|
| AnimID | 4 | 632 636 955 957 |
| DIETS | 4 | 5 6 7 8 |

Anova Tables

Dependent Variable: DMD

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 15 | 2768.135714 | 184.542381 | 4.93 | <.0001 |
| Error | 96 | 3590.082857 | 37.396696 | | |
| Corrected Total | 111 | 6358.218571 | | | |

| R-Square | Coeff Var | Root MSE | DMD Mean |
|---|---|---|---|
| 0.435363 | 11.53749 | 6.115284 | 53.00357 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| AnimID | 3 | 771.047857 | 257.015952 | 6.87 | 0.0003 |
| DIETS | 3 | 8.222143 | 2.740714 | 0.07 | 0.9742 |
| AnimID*DIETS | 9 | 1988.865714 | 220.985079 | 5.91 | <.0001 |

## Appendix 5: Analysis of variance for *in vivo* organic matter digestibility at NALIRRI

Dependent Variable: OMD

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 15 | 2577.093482 | 171.806232 | 4.63 | <.0001 |
| Error | 96 | 3561.977143 | 37.103929 | | |
| Corrected Total | 111 | 6139.070625 | | | |

| R-Square | Coeff Var | Root MSE | OMD Mean |
|---|---|---|---|
| 0.419786 | 11.29718 | 6.091299 | 53.91875 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| AnimID | 3 | 548.454554 | 182.818185 | 4.93 | 0.0032 |
| DIETS | 3 | 358.665982 | 119.555327 | 3.22 | 0.0261 |
| AnimID*DIETS | 9 | 1669.972946 | 185.552550 | 5.00 | <.0001 |

## Appendix 6: Analysis of variance for *in vivo* dry matter digestibility at SUA

The GLM Procedure

| Class | Levels | Values |
|---|---|---|
| AnimID | 4 | 9 10 16 1241 |
| DIETS | 4 | 1 2 3 4 |

Anova Tables

Dependent Variable: DMD

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 15 | 2952.771429 | 196.851429 | 4.85 | <.0001 |
| Error | 96 | 3895.457143 | 40.577679 | | |
| Corrected Total | 111 | 6848.228571 | | | |

| R-Square | Coeff Var | Root MSE | DMD Mean |
|---|---|---|---|
| 0.431173 | 11.26875 | 6.370061 | 56.52857 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| AnimID | 3 | 412.967143 | 137.655714 | 3.39 | 0.0211 |
| DIETS | 3 | 1848.742143 | 616.247381 | 15.19 | <.0001 |
| AnimID*DIETS | 9 | 691.062143 | 76.784683 | 1.89 | 0.0621 |

## Appendix 7: Analysis of variance for *in vivo* organic matter digestibility at SUA

Dependent Variable: OMD

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 15 | 3006.985357 | 200.465690 | 4.97 | <.0001 |
| Error | 96 | 3874.894286 | 40.363482 | | |
| Corrected Total | 111 | 6881.879643 | | | |

| R-Square | Coeff Var | Root MSE | OMD Mean |
|---|---|---|---|
| 0.436942 | 11.21028 | 6.353226 | 56.67321 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| AnimID | 3 | 376.566786 | 125.522262 | 3.11 | 0.0300 |
| DIETS | 3 | 1981.778214 | 660.592738 | 16.37 | <.0001 |
| AnimID*DIETS | 9 | 648.640357 | 72.071151 | 1.79 | 0.0808 |

## Appendix 8: Analysis of variance for *in vivo* dry matter digestibility at RAB

The GLM Procedure

| Class | Levels | Values |
|---|---|---|
| AnimID | 4 | AF268 ASF488 RD019 RD026 |
| DIETS | 4 | 9 10 11 12 |

Anova Tables

Dependent Variable: DMD

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 15 | 8768.041223 | 584.536082 | 195.81 | <.0001 |
| Error | 88 | 262.697143 | 2.985195 | | |
| Corrected Total | 103 | 9030.738365 | | | |

| R-Square | Coeff Var | Root MSE | DMD Mean |
|---|---|---|---|
| 0.970911 | 1.937193 | 1.727772 | 89.18942 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| AnimID | 3 | 408.071231 | 136.023744 | 45.57 | <.0001 |
| DIETS | 3 | 620.713308 | 206.904436 | 69.31 | <.0001 |
| AnimID*DIETS | 9 | 7604.430062 | 844.936674 | 283.04 | <.0001 |

**Appendix 9: Analysis of variance for *in vivo* organic matter digestibility at RAB**

Dependent Variable: OMD

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 15 | 2243.959615 | 149.597308 | Infty | <.0001 |
| Error | 88 | 0.000000 | 0.000000 | | |
| Corrected Total | 103 | 2243.959615 | | | |

| R-Square | Coeff Var | Root MSE | OMD Mean |
|---|---|---|---|
| 1.000000 | 0 | 0 | 84.50192 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| AnimID | 3 | 1051.177273 | 350.392424 | Infty | <.0001 |
| DIETS | 3 | 415.831818 | 138.610606 | Infty | <.0001 |
| AnimID*DIETS | 9 | 620.798365 | 68.977596 | Infty | <.0001 |