BINDURA UNIVERSITY OF SCIENCE EDUCATION

FACULTY OF AGRICULTURE AND ENVIRONMENTAL SCIENCE

DEPARTMENT OF ANIMAL SCIENCE

COMPARATIVE *IN VITRO* DIGESTIBILITY VALUES OF PLAIN MAIZE AND INTERCROPPED MAIZE X HIGH PROTEIN FORAGE LEGUMES SILAGES GIVEN TO EARLY LACTATING COWS IN CHITOMBORWIZI.

BY

MAPOLISA TICHAONA

B1645554

SUPERVISOR: MR DHLIWAYO

A dissertation submitted in partial fulfillment of the requirements of the bachelor of agricultural science honors degree in animal science.

December 2021

DECLARATION

I, Tichaona Mapolisa, declare that this project is my own work and all other sources used were cited. I declare that this thesis has never been submitted to any university or an academic institution.

Tichaona Mapolisa………………………. Date ……………………………

Supervisor(s)' declaration:

I have supervised this research project of the above-mentioned student and I am convinced that the research project can be submitted.

…………………………………………… Date………………………….

Mr. M. Dhliwayo (Major Supervisor)

…………………………………………… Date…………………………

Dr. A. Chikwanda (Co- supervisor)

I certify that I have checked this Research Project and I am satisfied that it conforms to The Department of Animal Science Guidelines for Project Preparation and Presentation. I therefore, authorize the student to submit this dissertation for marking.

……………………………….. Date………………………………………

(Quality Controller)

DEDICATION

This piece of work is dedicated to my family for the support they gave me towards the degree programme and for being my inspiration at all times.

ACKNOWLEGMENT

This piece of work is not only the sweat of the author; therefore, I wish to extend my sincere gratitude to the following for their assistance during my internship period and made it to be a success.

My sincere appreciation goes to my supervisor at Zimbabwe Open University: Professor N.T Ngongoni and fellow research student Mr. B Gabi and my supervisor at BUSE (Mr. Dhliwayo) who guided me through this research period and support in great abundance.

My profound gratitude also goes to the leadership of Department of Animal Science in the Faculty of Agriculture and Environmental Science: Chairperson (Mr. Kunaka) and all lecturers and all other staff in the Animal Science department.

Chitomborwizi smallholder dairy farmers are not an exception to get acknowledgements for giving me the opportunity to learn and acquire skills which builds my knowledge base.

I would also want to express my sincere gratitude towards my family for all the sacrifice they made for me to get to this stage and all the support, love you guys.

Above all, l would like to acknowledge the God Almighty who has guided me from the beginning. I wouldn’t be able to do anything without Him. He gave me the opportunity and strength during my industrial attachment period and made it successful.

ABSTRACT

During the winter season, small-scale dairy farmers in Zimbabwe face livestock nutritional challenge due to the decline of the forage nutritive value. This study aimed to evaluate the energy values (DE and ME) for plain maize silage (control) and intercropped maize x forage legume (cowpeas and velvet beans) mixed silages fed to dairy cows in Chitomborwizi. Also, the study analysed the relationship between CP and CF with metabolisable and digestible energy. Laboratory data (proximate analysis) from a total of 29 samples of feed from 7 farmers was used in the analysis. 38% of the samples obtained from the farmers were from commercial sources, while 62% were home-made. Of these samples, 48% were concentrates, 31% were plain maize, 10% were maize mixed with cowpea, and 10% were maize mixed with velvet beans. Mean DE (mj/kg) and ME (mj/kg) values were low for plain maize silage. Intercropped maize x cowpea silage had higher mean DE (11.45) and ME (10.13), intercropped maize x velvet beans had DE (10.30) and ME (9.20) and plain maize silage had DE (8.95) and ME (8.14). The results show that there was a statistically significant difference in energy values between plain maize silage and intercropped maize x cowpea silage ($p < 0.05$) and there was a statistically significant difference between energy values for plain maize silage and intercropped maize x velvet beans mixed silage ($p < 0.05$). There was a correlation between metabolisable energy and crude protein content ($p \leq 0.045$), for a unit increase in crude protein content the metabolisable energy increases by approximately 0.733. There was a correlation between digestible energy levels and crude protein levels ($p \leq 0.05$), for a unit increase in the crude protein levels, the digestible energy increases by approximately 0.27.

Table of Contents

List of Tables

List of acronyms

CF	Crude fibre
CP	Crude protein
CRD	Complete Randomized Design
DE	Digestible energy
ME	Metabolisable energy
AHDB	Agriculture and Horticulture Development Board

Chapter 1

1.1 Introduction

Milk production in Zimbabwe is below the domestic demand. (Gwiriri *et al*., 2016) suggested that an estimate of 65 million litres of milk per annum was being produced which was only 45% of the current domestic demand of 120 litres per annum. The remaining 55% deficit was being imported from countries like South Africa and Zambia using scarce foreign currency in the country. There is a need to increase dairy milk production in Zimbabwe to meet the domestic demand and save the scarce foreign currency in the country. The increase of milk production can be actually attained either by increasing the number of dairy cows which can be milked or increasing milk production per cow (Waite, 1943). In order to increases milk production, the government of Zimbabwe through the Dairy Development Program (DDP) introduced the Small-scale Dairy Farming Schemes in 1983 (Washaya and Chifamba, 2018). Introduction of the Small-scale Dairy Farming Scheme was regarded as a development tool for rural areas as it focused on improving the livelihoods and road networks. In developing countries, smallholder dairy production serves as an important nutrition source and income (FAO, 2019).

However, despite the introduction of Small-scale Dairy Farming in Zimbabwe, milk production is still low. The nutritional value of forage had become the challenge mostly during the dry period that is between May and November when the nutritive value of forage decreases due to the elevation of structural carbohydrate component (Ngongoni *et al*., 2006). Poor forages result in high cost of production through greater reliance on dairy concentrates for both maintenance and production.

1.2 Background to the study.

The research project was carried out in Chitomborwizi small-scale farming area comprising of 447 farmers where 50 of those farmers are into dairy farming. Dairy farming enables farmers to receive regular income throughout the year which helps them in meeting day to day cash demands as compared with cash crops such as tobacco which only allow farmers to receive income only once after harvesting. Chitomborwizi is in Mashonaland West Province of Zimbabwe and its centre is 18km south of Chinhoyi Town along the Chinhoyi-Chegutu highway and about 25 km north of Murombedzi Growth Point. The area is found in agroecological region 2b characterized by average annual temperature of 28 degree Celsius and annual rainfall ranging from 750mm to 1000mm. Due to the later mentioned climatic conditions, Chitomborwizi is subject either rather more severe dry spells during rainy season or to the occurrence of relatively short rainy season.

With the help of Nestle Zimbabwe, farmers in Chitomborwizi acquired Jersey heifers from South Africa. Nestle Zimbabwe provided loans on the security that all beneficiary farmers would be obliged to sell their milk only to Nestle Zimbabwe. Those farmers were trained at Gwebi Agricultural college. Through a Memorandum of Understanding, the Zimbabwe Open University is currently carrying out on farm research in this area and providing advisory support to the farmers. Only 21 farmers received the first batch of heifers in 2015.

The dairy cows were mainly fed on dairy concentrates, plain maize silage and on natural veld. And the milk yield varied from 9 to 11litres per cow per day. In this research study, maize was intercropped with forage legumes [cowpeas (*Vigna unguilculata*) and velvet beans (*Mucuna pruriens*)]. and used to make silage. Unavailability of other forage legume seeds in the country has limited the research on mixed silages of intercropped maize x cowpeas and intercropped maize x velvet beans.

1.3. Problem statement

Chifamba (2018) found that those cows given intercropped maize silage increased their milk production. However, regardless of the capacity of improving performance of dairy cows due to intercropped silages, there was no data on the digestibility of these maize intercropped mixed silages as well as other nutritive determinants such as crude protein content and crude fibre. Also, farmers in Chitomborwizi are facing high cost of production mostly during the period of May to November when the nutritive value of forage deteriorates when they put a greater reliance of dairy concentrates for both maintenance and production.

1.4. Justification

Research in this study aims to reduce cost of production per litre of milk. This is because 76% of cost in dairy production comes from nutrition, hence there is a need of greater reliance for milk production on quality forage legumes instead of buying commercial concentrates from commercial companies which are expensive (Chifamba, 2018). Therefore, knowing the digestibility and nutrient content of the later named silages will be of great importance in the sense that farmers will enable them to properly and adequately feed their cows. Smallholder dairy farmers in Chitomborwizi are not an exception to this challenge. Cost of production is much higher during dry period of May to November; therefore, the use of conserved maize x high protein forage legumes mixed silages will have a nutritive advantage when the natural pastures are nutritionally very poor (Ngongoni *et al*., 2006). Also, high protein legumes improve soil fertility through nitrogen fixation and they are significant by acting as a remedy to internal worms in dairy animals (Ngongoni *et al*., 2007).

Crop farming brings cash incomes only once in a year after harvest, but with dairy, farmers get regular cash incomes throughout the year (Gwiriri *et al*., 2016). Therefore, there is need to reduce the cost of production through making of intercropped maize and forage legume silages. Therefore, the aim of the study was….

1.5. Objectives:

1.5.1 Main objective

1. The main objective of the study was to determine *in vitro* digestibility values (digestible and metabolizable energy) of plain maize silage and intercropped maize x cowpeas mixed silage.

1.5.2 Specific objectives

1. The specific objective was to analyse the relationship between feed crude protein and crude fibre content with digestible and metabolisable energy.

2. To compare ME and DE values of commercial feeds and home- made feeds.

1.6. Hypothesis.

H_0: There is no significant difference on the digestibility between plain maize silage and intercropped maize X cowpeas silage.

H_0: There is no significant difference on the digestibility between plain maize silage and intercropped maize X velvet beans silage.

H_0: There is no significant difference on energy values (DE and ME) between commercial feeds and home-made feeds.

1.7 Delimitation of the study

The study is only limited to Chitomborwizi farming area. Feed digestibility values were determined by *in vitro* experiments because of animal welfare regulations.

1.8. Ethical considerations

Animals will be kept accordance with good agricultural and good veterinary practices and observing all applicable statutory and legal obligations.

Chapter 2

2.0 Literature review

2.1. Introduction

Knowing digestibility of a feed helps farmers to predict animal performance when fed with a particular feed. This is because level of nutrition and type of diet plays a significant role in determining both milk yield and milk composition since nutrition (Moe and Tyrrell, 2010). Moe and Tyrrell (1975) had highlighted that the type of diet influence the nutrients offered to an animal which are available for milk production. Utilization of nutrients of a feed is determined by the level of digestibility of the feed. Digestibility values helps to estimate nutrients available for the animal in the feed hence helps in predicting animal performance. Zewdie (2018) states that: "digestibility usually provides a fairly reliable index of nutritive value of feed/food stuff because more digestible feeds are normally consumed to a greater extent than less digestible feeds in ruminant nutrition." Digestibility of animal feeds is obtained either by *in vitro* or *in vivo* techniques. *In vitro* digestibility gives the value of Metabolizable Energy (ME) which is calculated as Digestible Energy (DE) minus energy in methane and urine. It is an expression of the amount of energy available for metabolism by the animal for maintenance and production (Moe, 1981).

Digestibility is an indicative value of the energy available to an animal as this energy is required by an animal to perform functions such as body tissue construction, synthesis of products like milk and wool and for work done by an animal which can either be mechanical or chemical. Two energy values which are Apparently Digestible Energy (DE) and Metabolisable Energy (ME) are well discussed as they significantly indicate energy available to the animal. Digestible energy is calculated by subtracting the fecal energy (feed and metabolic origin) from the Gross energy of a feed. DE minus gaseous products of digestion and urine energy (feed and endogenous origin) gives metabolizable energy. This metabolisable energy minus heat increment from fermentation and metabolisation gives Net Energy which is then used for maintenance and production. ME is feed energy portion which is available for metabolic processes of an animal. Thus, in this research DE and ME has great impact on determining milk yield of lactating cows.

There are two types of digestibility, which are: apparent and true digestibility.

1) Apparent digestibility

Apparent digestibility refers to the value in which endogenous materials of compounds (from the erosion of epithelial cells, products of bacterial fermentation and enzymes) are included in the faecal component. Simply there is no correction for the endogenous compounds. (Boisen and Moughan, 1996).

$$Apparent\ digestibility = \frac{Nutrient\ consumed - Nutrient\ excreted\ in\ faeces}{Nutrient\ consumed}$$

2) True digestibility

It takes into account endogenous losses which comprises of epithelial cells of intestine, enzymes and products of bacterial fermentation. (Boisen and Moughan, 1996)

$$True\ digestibility = \frac{Nutrient\ consumed - (Nutrient\ excreted\ in\ faeces - Endogenous\ losses)}{Nutrient\ consumed}$$

In vivo experiments (using animals) give better values for digestibility of a feed than *in vitro* experiments. It gives results which represents actual animal response to a dietary treatment. However, *in vivo* experiments are being neglected in current research due to the fact that they are laborious, time consuming, expensive and they are not applicable when the quantities of feedstuffs are not in large amount. Also *in vivo* experiments are being deserted because they cause pain on animals since they require animals to be surgically modified. In addition, *in vivo* methods used to determine intestinal digestibility or rumen degradability are subject to greater variability and additional errors due to measurements of digesta flows and of microbial and endogenous contributions. Therefore, because of the later indicated reasons, *in vitro* experiments were used to predict feed digestibility or energy values. *In vitro* techniques have been developed and adapted to represent biological models that simulate the *in vivo* digestion. These techniques are less time consuming, have a high repeatability and are less expensive (Kitessa *et al.*, 1999). *In vitro* techniques are used to estimate the extent of ruminal degradation and digestibility of feeds and variation in response to changes in rumen conditions. Furthermore, in vitro techniques have been used also to investigate microbial fermentation mechanisms and in studying mode of action of additives, feed supplements Such techniques include: Van Soest method, The Menke gas technique and Tilley and Terry method (Lopez, 2005).

2.2. *In vivo* methods to determine digestibility

2.2.1. Marker technique (Indicator method)

This is an *in vivo* technique which does not require total collection of faeces and it can be carried out in the absence of special balance cages. In addition to the chemical analysis of the usual proximate nutrients, the method has the capacity to determine content in the feed and faeces of an indigestible reference substance (Khan *et al*., 2003). The method uses two types of markers that is internal and external markers(indicators). Internal markers refer to the indigestible natural constituent of the feed and they include lignin, indigestible acid detergent fibre and acid insoluble ash (silica) (Huhtanen *et al*., 1994). External markers are substances which are added to the feed and they include: ferric oxide, chromic oxide (Cr_2O_3) for ruminants, chromogen and TiO_2 (for non-ruminants) (Van Keulen and Young, 1977; Waller *et al*., 1980).

With the indicator method, it is presumed that the reference substance passes through the digestive tract at a constant rate (Khan *et al*., 2003). If there is a variation in the rate of excretion during the day due to variations with time and intake, special sampling plans should be followed to adjust for diurnal variation (Khan *et al*., 2003). Also, if the indicator and the nutrients ratio is the same throughout the 24hour period, a small amount of dung is sufficient for digestibility calculations. However, it is advisable to collect samples more than once for sample for digestibility calculation and the faecal samples should be collected for several consecutive days. (Khan *et al*., 2003).

Characteristics of good indicators are:

1. should be non-absorbable
2. Should have no effect to the degesta flow
3. Should have a steady transit time
4. Its analysis should be accurate
5. Could be incorporated into the test diet homogenously

Calculation of digestibility when using the marker technique:

$$digestibility = \frac{gIndicator/kg\ faeces - gIndicator/kgfeed}{gIndcator/kg\ feed}$$

Advantages of the marker technique

1. Total collection of faeces is not necessary
2. Total intake determination is not necessary
3. simple and not laborious

Disadvantages of the marker technique are:

1. Representative sampling essential
2. Accurate estimation of nutrient or marker concentration is required.
3. The method assumes that there is complete excretion of marker hence recovery of marker determines accuracy of digestibility
4. There is difficulty on mixing marker with feed
5. External markers may contaminate forage

2.2.2. Nylon bag technique

It is an *in-situ* method, in which the samples of feeds are put into nylon bags and incubated into the rumen of a cannulated animal for periods of from 6 to 120 hours (Khan *et al*., 2003). There must be free movement of incubated feed within the bags to avoid poor replication due to the formation of micro-environments in the bags. The method is based on measuring the degradation or disappearance of dry matter and/or other nutrients (Dong *et al*., 2017). It assumes that the disappearance of substrate from bags represents the actual degradation of substrate by the rumen microbes and enzymes in the rumen. It provides a means of ranking feeds according to the rate and extent of degradation of dry matter, nitrogen, organic matter and other nutritional parameters and subsequent determination of the disappearance of the different feed components (Zewdie, 2019). The nylon-bag degradability of the feed is determined by the weight loss during the incubation periods. The nylon-bags are designed in a such a way that pore sizes (50μm or less) allow the entry of rumen microbes and escape of accumulated gases and having the ability to keep solid particles losses to a minimum. The bags are made up of nylon mesh of size (30 - 50 μm) and each nylon bag is of size 6.5 x 14 cm. The leakage of feed particles out of the bag without breaking down is corrected through the use of zero-hour bags (empty bags). Zero-hour bags are filled with the substrate but are not incubated in the rumen (Zewdie, 2019). During the process, a sample of known mass is tightly sealed in the nylon bags and placed in the rumen of a fistulated animal (Zewdie, 2019). After the required period of time, it is washed, dried and weighed. The difference between the initial weight of the feed sample and of the residue gives the amount of nutrient digested in the feed (Zewdie, 2019). To prevent surfacing on the rumen bags are secured to a weighted cord and this will also ensure adequate exposure of feed sample to microbial digestion (Khan. *et al.,* 2003).

Factors which affect nylon-bag degradation (Zewdie, 2019).

1. How the bags are placed in the rumen,
2. Particle size of sample,
3. Loss of feed particles through the bag cloth (a function of fineness of grinding, cloth pore size and feed material), (Playne *et al*., 1978).
4. Method of washing,
5. The period length that the samples are incubated in the rumen,
6. The rumen
7. Environment in which degradability is determined.

Advantages of the nylon bag technique

The nylon-bag technique is a very simple and useful biological tool for *in vivo (in sacco)* animal

2.3. Laboratory methods (*in vitro* methods)

In vitro methods are an alternative to *in vivo* methods in determining feed digestibility due to the fact that animal trials are expensive to carryout. For example, the cost of the animals up to 8 animals per trial is quite high. Also, *in vivo* experiments are laborious and take a long time that is 21 days of the adaptation period and 14 days of total collection period. Furthermore, *in vivo* trials are being disregarded as there are animal welfare groups that are advocating for no use of animals in the experiments and even arguing that animals should be treated as equals to humans.

It has been argued that these experiments put stress on animals as they are kept under unnatural conditions. The use of *in vitro* methods is being promoted as they are cheap, less- time consuming, repeatable robust, broad-based, biological meaningful and are suited for routine feed evaluations by commercial laboratories who provide such information to feed manufacturers. Moreso, *in vitro* methods provide the opportunity of analysing both the residue and the metabolites of microbial degradation (Mohamed and Chaudhry, 2008). *In vitro* methods allow uniform description of feeds for DM and protein degradation as they may allow for the control of various factors (microbial, animal, environment) that have much effect on altering the feed degradation (Mohamed and Chaudhry, 2008). Such methods involve the use of buffers, rumen fluid, chemical solvents and enzymes that are either extracted from rumen contents or commercially produced (Galyean, 2010). There are numerous *in vitro* techniques which are being widely used for feed digestibility that include the following: Tilley and Terry two-stage method, The Menke gas production method, Neutral cellulase method and Rusitec method.

2.3.1. Tilley and Terry IVDMD

In vitro digestibility techniques provide a quick, inexpensive, and precise prediction of *in vivo* or conventionally determined digestibility in ruminants (Khan *et al*., 2003). The method measures apparent digestibility in rumen fluid in two stages. The two- stage Tilley and Terry method is an *in vitro* technique which is used as to determine the plant quality index for animal feed (Zewdie, 2019). The method was developed to estimate the apparent DM digestibility of feeds for ruminants in the laboratory (Tilley and Terry, 1963). The method has two stages: during the first stage, a feed sample (~0.5g in a 50 mL centrifuge tube) is incubated under anaerobic conditions at 39°C in rumen fluid containing microorganisms for 48 hours (Zewdie, 2019). The rumen fluid is diluted with a buffer solution to maintain pH. Examples of commonly used buffer are Kansas buffer, Ohio buffer and Van Soest buffer. After 48 hours, the incubation process is stopped and the incubation mixture filtered and in the second stage the feed residues are consequently incubated for another 48 hours with acid-pepsin at 39°C (to simulate abomasum digestion). Lastly, the contents are filtered and the residue is oven dried at 105°C for 2 hours (Zewdie, 2019). (Zewdie, 2019).

The Tilley and Terry method is simple and inexpensive. However, because of its reliance on rumen fluid from fistulated amides (which may not be available), long incubation periods and the method is not accurate for poor quality roughages (tropical forages).

Drawbacks of the Tilley &Terry (VDMI) technique.

1. It does not account for the endogenous secretions that form an integral part of the digesta in the real practical system.
2. The method excludes the post abomasal digestion
3. The use of the rumen fluid-neutral detergent system has been noted that it gives higher digestibility values as the technique digests Maillard products which are not digested *in vivo.*
4. It has the problem of still requiring the rumen liquor from cannulated animals.
5. There is need for standardisation to correct the variability in rumen fluid composition and activity.
6. This technique disregards/ or inappropriately represents ruminal outflow (uses a batch process).
7. The method does not account for the associative effects between feeds as it is rare that an animal is fed on one type of feed in real life.

2.3.2. Gas production technique

This is an *in vitro* technique developed by Menke and Steingass to estimate the rate and extent of dry matter degradation (Murillo *et al*., 2011; Kamalak *et al*., 2005) and to evaluate the nutritive value of forages indirectly (Murillo *et al*., 2011). The technique and its variants are superior to other digestibility and degradability technique as they account for contributions from insoluble and soluble feed fractions while providing information on the dynamics of forage fermentation in postulated animal and under laboratory conditions (Khan *et al*., 2003; Zewdie, 2019). Also, *in vitro* gas method is more efficient as it evaluates the effects of tannins or other anti-nutritional factors in feeds than the *in sacco* method (Vercoe *et al*., 2010). This technique works by imitating the digestive processes of animals generated by microbial activity and it provides a way to understand better feed degradability and fermentation as a function of nutrient availability for the bacteria and nutritional quality (Murillo *et al*., 2011). The method does not require the use of expensive and complicated equipment or a large number of animals. Also, feeds evaluation is not limited only to feed constituents based on the dry matter digestibility but also on the effectiveness of microbial protein synthesis (Vercoe *et al*., 2010).

The Menke and Steingass method is done using 100 mL capacity calibrated glass syringes containing feedstuff buffered with rumen liquor (Vercoe *et al*., 2010). 200mg of feed dry matter is incubated for 24hrs. After incubation the levels of other chemical constituents are used to predict digestibility of organic matter determined *in vivo* and metabolizable energy (Vercoe *et al*., 2010). The amount of gas released when a feed is incubated *in vitro* buffered with rumen fluid is highly correlated to the digestibility of that feed (Isah *et al*., 2010). The gas produced is read either at a fixed incubation time, 24 hours, or at a series of incubation times (sequential incubation), mainly 6, 12, 24, 48, 72 and 96 hours. (Murillo et al., 2011).

However, Menke gas technique was revised and improved in that feeds are now incubated in a thermostatically controlled water bath instead of a rotor in an incubator (Blummel and Orskov, 1993; Getachew *et al*., 1998). The method was also further modified by increasing the amount of feed sample from 200 to 500 mg and increasing the amount of buffer by two-fold (Vercoe *et al*., 2010; Getachew *et al*., 1998).

Advantages of gas method.

1. It is less animal dependent.
2. Large number of samples are handled at a time.
3. Less expensive and less time-consuming.
4. More appropriate for characterizing soluble or small particulate feeds (Adesogan, 2002).
5. The method can be used to evaluate the effects of tannins or other anti-nutritional factors.
6. The method can monitor nutrient-anti-nutrient and anti-nutrient-anti-nutrient interactions.

2.3.3. Van Soest method

It is a method of solubility which was developed in in the 1960s by P.J. Van Soest. for evaluating the fibre fraction of feeds. The method separate feeds into soluble and insoluble components and explain that there is a significant correlation between digestibility and solubility. This method was advanced because it was seen that Crude Fibre did not accurately estimate the roughage content of forages for ruminants. As a result, the Van Soest technique measures the neutral detergent fibre (NDF) which is the fraction of cell wall contents (cellulose, hemicellulose, silica, lignin and any heat damaged proteins) and acid detergent fibre (ADF) fractions in forages (Mould, 2003). Neutral detergent fibre is determined by boiling the sample in a detergent solution with a pH of 7.0. in this stage the soluble portion is removed and it comprises of sugars, starch, lipids, pectins, soluble carbohydrates, non-protein nitrogen and protein) and what left is the insoluble NDF fraction. NDF fraction represent the undigested portion of the feed although much of the hemicellulose and part of cellulose is digested. High content of NDF indicates that the forage is of lower quality. Acid detergent fibre is determined by boiling the sample in an acid detergent solution (Asi.k-state.edu, 2021). The soluble portion is removed, and the insoluble ADF fraction remains. The ADF contains cellulose and lignin. Most laboratories use ADF to estimate the digestibility and energy value of forages. High levels of ADF cause forages to be less digestible, and have a lower energy value (Asi.k-state.edu, 2021). (Orskov, 1991, 1994).

2.3.4. Proximate analysis

The proximate analysis is an in vitro technique used to evaluate animal feeds. The method classifies feed components into six parameters: moisture, ash, ether extract, crude protein, crude fibre, and nitrogen free extract (Shang et al., 2015). (Saha *et al*., 2010).

Crude Protein

Crude protein is determined by measuring the nitrogen content of the feed and multiplying it by a factor of 6.25 (Kennedy *et al*., 1999). This factor is based on the fact that most protein contains 16% nitrogen (Vijverberg and TH FRANK, 1976). Crude protein is determined by Kjeldahl method**.**

Ash

Ash is the inorganic residue obtained by burning off the organic matter of feedstuff at 400-600^{o}C in muffle furnace for 4hrs (Saha *et al*., 2010). It measures the mineral content of the feed.

Nitrogen Free Extract (NFE)

NFE is determined by mathematical calculation. It is obtained by subtracting the sum of percentages of all the nutrients already determined from 100 (Seghosime *et al*., 2017). NFE represents soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in feed (Habibu et al., 2016).

Crude Fibre

It measures the cell wall component of the feed that is cellulose, hemicellulose and pectins

2.4. Nutritive composition and energy values of silages

2.4.1. Maize silages

Maize silages are widely used to feed livestock. The quality and digestibility of the silage being affected by stage at harvest (maturity) (Di Marco *et al*., 2002). The CP content being decreasing as the corn matures whilst the CF value increases. The digestibility of the silage declines as the crude fibre content decreases. This because as the crop matures, stem and leaf quality declines, but this is counterbalanced by the rise in grain in the cob, which is very digestible and high in starch content (Feed value of maize silage and maize grain | AHDB, 2021).

From the research study by Wei *et al* (2018) the digestibility of maize silage as obtained by direct method were 8.86 and 5.15 MJ/kg dry matter (DM) metabolisable energy and net energy respectively and by regression method were 8.96, 5.34 MJ/kg dry matter (DM) metabolisable and net energy respectively. According to Feed value of maize silage and maize grain | AHDB (2021) ME and CP values for maize silage were 10.8-11.5 and 8-9 respectively.

2.4.2. Intercropped maize-legumes silages

A major limitation of maize is its low protein content as compared with legumes (Mut *et al*., 2017). Therefore, efforts to intercrop maize with legumes such as cowpea and velvet beans aim to improve its protein content hence increasing nutritive value of silages for better animal performance (Riday and Albrecht, 2008). Uher *et al* (2019) highlighted that the crude protein for plain maize silage and intercropped maize-cowpea silages were 76g/kg DM and 96g/kg DM respectively.

Table 1. Chemical composition of maize and maize cowpea intercropped fodder (% DM)

Parameter	**Maize**	**Maize: cowpea**	
		85: 15	**70:30**
Dry matter	35.86	34.50	33.00
Crude protein	8.32[b]	9.75[b]	14.43[a]
Crude fibre	23.29[a]	22.90[a]	20.95[b]
Ether extract	2.36	2.23	2.18
Ash	5.95	6.21	6.32
Water soluble carbohydrate (WSC)	10.58[a]	9.30[ab]	8.91[b]
TDN	65.00	64.39	66.84

Means within same row with different superscripts differ ($p<0.05$). * Calculated value. (Azim *et al*., 2000).

2.5. Feed factors which affect digestibility.

Feed digestibility is chiefly affected by its chemical composition that is CF, CP, fat and oils of feed and age and stage of maturity of forages as well as animal factors such as breed, physiological status and age. However, in this discussion on factors to do with the nature type of feed were discussed.

2.5.1 Age and stage at maturity.

Age and stage at maturity play a significant role on determining the digestibility of forages. This is because when plant matures the proportion of cell wall components of the forages (cellulose, hemicellulose and lignin) increases, whereas the proportion of cell contents decreases. The increase of CF decreases the digestibility. Also, with increase of age, the ratio of stem to leaf increases (Terry and Tilley, 1964). The digestibility of the stem is already lower than the digestibility of the leaf before the plant reaches an advanced stage of maturity, but also declines faster over time, and with increasing maturity the ratio of stem to leaf increases). Due to the changes in cell wall content and the stem: leaf ratio with increasing maturity, the digestibility of grasses is highest in the vegetative stage (Terry and Tilley, 1964). Generally, forage high in crude fibre has low digestibility and with maturity lignin contents of plant/forage increases which reduces digestibility. (Bruinenberg *et al*., 2000).

2.5.2 Crude Protein content and protein solubility.

Digestibility of forages and feeds is closely related to CP content. Givens et al (2000) had highlighted that DM digestibility is increased with increase of CP content. The increase can be attributed to the high digestibility of protein or a positive effect on microbial fermentation and digestion in the rumen (Givens *et al.*, 2000). Also, the solubility of proteins is positively correlated with digestibility.

2.5.3 Associative Effect of Feeds

Digestibility of feeds is also affected by the associative effects of nutrients that is one nutrient component might influence the digestibility of other, for example; protein facilitate rumen micro-organisms that break down complex carbohydrate in the rumen. The addition of a protein or Non-Protein Nitrogen compound to a low protein ration enhances the microbial digestion of the crude fibre by stimulating the growth of microorganisms in the rumen. Therefore, an increase dietary protein level will increase the digestibility of all other nutrients and decreasing the dietary protein level, lower the digestibility of all the nutrients.

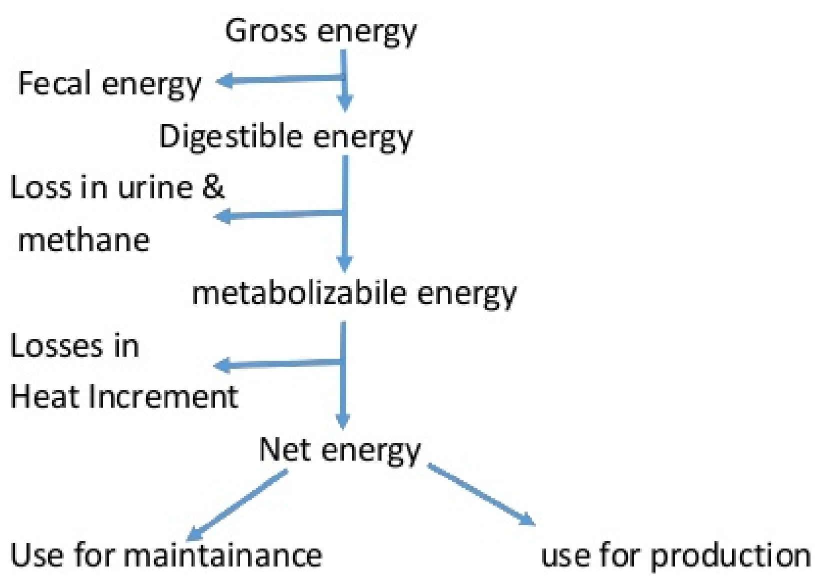
Also, nature and level of dietary carbohydrates has an effect on the digestibility of all nutrients present in the diet. Generally, the digestibility of all nutrients is decreased if crude fibre content of mixed diets is high. Therefore, the higher the percentage of crude fibre in a ration, the lower is the digestibility of dry matter and all other nutrients. Moreover, adequate amount of salt and water have positive effect on digestibility.

2.5.4 Processing of feeds.

Processing method of feeds also has an impact on digestibility. Such methods include: boiling, steam processing, micronization, pelleting, extrusion cooking. Generally, these methods improve their digestibility however, some may result in heat damaged proteins that are comparatively less digestible.

2.6. Calculation of Digestible and Metabolizable energies from proximate analysis values.

In vitro techniques results are used to calculate digestible energy and Metabolizable energy. Below is a flow chart diagram which show different energy classes.



The calculation of gross energy from the proximate nutrient values is as follows;

Gross Energy = CP_{feed}× 0.056 + CPE_{NPN} × 0.0089 + Fat × 0.094 + [100 – CP_{feed} – (CPE_{NPN} / 288) – Fat – ash) × 0.042]. (Weiss and Tebbe, 2019).

Where nutrients are expressed as percentage of dry matter and CPfeed is the CP in feeds other than supplemental NPN and CPENPN is the CP equivalent (i.e., N × 6.25) provided by NPN (assumed to be urea). This formula does not overestimate GE for diets with urea or any source of NPN. (Weiss and Tebbe, 2019).

DE which is GE minus faecal energy. From this point DE is used to obtain ME. Calculation of ME varies with the system used as shown below.

Beef System

ME = 0.96 X DE − 0.3 (units are Mcal/kg).

Conversion of DE to ME is decreased with increasing CP and increased with increasing ether extract. However, feeding CP above requirement results in amino acids oxidation for energy with the nitrogen excreted in urine, hence lowering DE to ME efficiency. (Galyean et al., 2016).

Dairy system

The Dairy System calculate ME from DE as follows:

$ME = 1.01 \times DE - 0.45$ (units are Mcal/kg) plus an adjustment for dietary fat concentration. Accuracy of ME estimation can be improved by incorporating directly dietary factors such as CP, NDF and fat.

CHAPTER 3: METHODOLOGY

3.1 Location of the study

The research project was carried out in Chitomborwizi small-scale farming area comprising of 447 farmers. Chitomborwizi is in Mashonaland West Province of Zimbabwe and its centre is 18km south of Chinhoyi Town along the Chinhoyi-Chegutu highway and about 25 km north of Murombedzi Growth Point. The area is in agroecological region II (b) characterised by an average annual temperature of 28 degree Celsius and annual rainfall ranging from 750mm to 1000mm. Chitomborwizi is subject to severe dry spells during the rainy season and to the occurrence of a relatively short rainy season.

3.2 Intercropping

Intercropping of maize and forage legumes (cowpeas and velvet beans) was done on a mass-by-mass basis using the ratio of 70:30% respectively. For each hectare 17.5kg of maize was mixed 7.5kg of the forage legume to make it 25kg per hectare. Intercropping was done for maize to benefit from the nitrogen fixing of forage legumes and for ease of silage preparation. There is a symbiotic relationship between maize and forage legumes. Cowpeas enrich the soil through nitrogen fixing and maize uses that nitrogen. Intercropping has an advantage of making balanced silages in terms of both proteins and fibre. This is because maize is rich in fibre whilst forage legumes are rich in proteins and oils. Also, high protein legumes act as a remedy to internal worms in dairy cows (Ngongoni *et al.,* 2007).

Farmers were identified as Farmer A, Farmer B and Farmer C for ethical considerations. Those farmers who intercropped maize with velvet beans used Syngenta MRI624 as maize variety and velvet beans from previous harvest (2019). Those who intercropped maize with cowpeas used ARDA seeds (CBC2 cowpeas and ZS 265 maize).

The lead farmers were allocated to the research team by the Chitomborwizi Network Board of Trustees. Intercropping was done on their own farms in terms of their usual farming practices but on mutually agreed between each lead farmers and the research team as described above.

3.3 Treatments and experimental design.

There were two treatments during the study. Treatment1- plain maize silage (control), Treatment2- intercropped maize-cowpeas silage at 70:30 ratio, Treatment 3- intercropped maize x velvet beans silage planted at 70:30 ratio. Complete randomized design (CRD) was used with 7 replicates for Treatment1 and 6 replicates for Treatment2 and Treatment3.

3.4 Silage making

Named lead farmers had grown the maize and the inter-cropped maize-forage legume (cowpeas) crops in the 2019/20 agricultural season. The maize crop was harvested at the milk dough stage, also when the seedpods of the forage crops were still succulent. The maize and the cowpea crops were chopped into smaller pieces about 1-3 cm using a mechanical forage chopper. The chopped forage was left to wilt to avoid excess water that may affect the fermentation process of the silage. The shorter the chops length, the better the compaction, hence less air trapped in the forage, resulting in better quality silage. The polyethylene plastic was spread on the ground and chopped forages placed on top of the plastic. The forages were compacted by using a tractor trampling and at the top by a tractor run, it was compacted until it was difficult to insert a finger into the stack. The top of the silage pit was sealed by a black polythene plastic and sand from the ring pit was placed on top of the polyethylene such that there would be no water run-off through the heap and also to produce an excellent air tight seal (anaerobic condition) and also to prevent air entry during feed out. The forage silage was allowed to ferment for a period of 60 days.

3.5 Data collection.

At every visit, the feed samples were randomly collected from the silos at each farm for a nutritive composition analysis using the proximate analysis method in which Crude Protein content was analysed with Kjeldahl method at Aglabs laboratory, Birmingham Road, Southerton, Harare, Zimbabwe. Digestible Energy (DE) and Metabolizable Energy (ME) values of each feed were calculated by Aglabs personals.

DE and ME values were captured and stored into the Microsoft excel spreadsheet and stored for analysis.

3.6 Statistical analysis.

Data were electronically captured and cleaned using Microsoft excel package. Proportions were used to summarise qualitative data. Medians and ranges were used to summarise quantitative data. The Kruskal-Wallis test was used to determine if there was a statistically significant differences in the energy levels between the different types of feels or source of feed. R (version 4.0.2) was used to compare means by an independent t-test and measure linear regression for the correlation between ME and DE with CP.

A complete randomized design model was used;

$Yij = \mu + T_i + Eij$

Where, Yij= jth observation under treatment i,

μ= Overall mean

T_i=Effect due to treatment i

E_{ij}= random error

Chapter 4: Results

Laboratory data from a total of 29 samples of feed from 7 farmers was used in the analysis. 38% of the samples obtained from the farmers were from commercial sources, while 62% were home-made. Of these samples, 48% were concentrates, 31% were plain maize, 10% were maize mixed with cowpea, and 10% were maize mixed with velvet beans.

Table 2: Description of study data

Variable	**Outcome**
Source of feed, n (%)	
Commercial	11 (38)
Home-made	18 (62)
Type of feed, n (%)	
Concentrate	14 (48)
Plain maize	9 (31)
Maize and cowpea	3 (10)
Maize and velvet beans	3 (10)
DM, median (range)	85.1 (25.6 – 95.8)
CP, median (range)	9 (4.6 – 23.2)
CF, median (range)	12.7 (4.1 – 35.1)
NDF, median (range)	60.4 (16.2 – 38.7)
Fat, median (range)	2.3 (0.9 – 6.5)
Ash, median (range)	5.6 (3.9 – 15.1)
Ca, median (range)	0.45 (0.17 – 2.29)
Phos, median (range)	0.32 (0.05 – 3.25)
DE, median (range)	8.4 (0.05 – 12.8)
ME, median (range)	9.5 (6.0 – 12.21)

There was a statistically significant differences in the median levels of the digestible energy between feed sourced from commercial sources and that which is home-made ($p = 0.010$), the median digestible energy for feed sourced from commercial sources was 10.9 while that which is home-made was 7.7. There was a statistically significant differences in the median levels of the metabolizable energy between feed sourced from commercial sources and that which is home-made ($p = 0.046$), the median digestible energy for feed sourced from commercial sources was 11.1 while that which is home-made was 9.3. There was a statistically significant differences in the median levels of the digestible energy between the different types of feed ($p = 0.042$), the median digestible energy obtained from concentrates was 10.9 while that from plain maize and maize mixed with either cowpea or beans was 7.7 and 8.1, respectively. There was a statistically significant differences in the median levels of the metabolizable energy between the different types of feed ($p = 0.034$), the median metabolizable energy obtained from concentrates was 11.1 while that from plain maize and maize mixed with either cowpea or beans was 7.6 and 10.1, respectively.

Mean energy values

Mean DE and ME values were low for plain maize silage. Intercropped maize x cowpea silage had higher mean DE (11.45) and ME (10.13), intercropped maize x velvet beans had DE (10.30) and ME (9.20) and plain maize silage had DE (8.95) and ME (8.14). The results show that there was a statistically significance difference in energy values between plain maize silage and intercropped maize x cowpea silage ($p < 0.05$) and there was a statistically significance difference between energy values for plain maize silage and intercropped maize x velvet beans mixed silage ($p < 0.05$). Below is a table showing the results.

Table 3: DE and ME values for plain maize silage and intercropped maize x cowpea mixed silage.

	DE (mj/kg)	**ME (mj/kg)**
Plain maize	8.95	8.14
Maize x cowpea	11.45	10.13

Maize x velvet beans	10.30	9.20

Relationship between metabolisable energy and crude protein.

There was a correlation between metabolisable energy and crude protein content ($p \leq 0.045$), for a unit increase in crude protein content the metabolisable energy increases by approximately 0.733.

$ME = 3.723 + 0.733CP$; $R^2 = 0.537$

Relationship between digestible energy and crude protein content.

There was a correlation between digestible energy levels and crude protein levels ($p \leq 0.05$), for a unit increase in the crude protein levels, the digestible energy increases by approximately 0.27.

Chapter 5

Discussions

Higher DE and ME in intercropped maize x forage legume (cowpeas and velvet beans) mixed silage than in plain maize silage could be due to increase in CP content. Ngongoni *et al* (2010) highlighted that cereal x legume silages have higher protein content than the plain cereal silage and this have implication on digestibility. Givens *et al* (2000) had highlighted that DM digestibility is increased with increase of CP content. The increase can be attributed to the high digestibility of protein or a positive effect on microbial fermentation and digestion in the rumen (Givens *et al*., 2000). High protein content in the diet promotes microbial population and growth (Kang *et al*., 2015), which in turn has positive impact on microbial rumen degradation. Also, statistics had showed that ME and DE are positively correlated with CP content, therefore, increasing CP content increases ME and DE.

According to Feed value of maize silage and maize grain | AHDB (2021) ME and CP values for maize silage were 10.8-11.5 and 8-9 respectively. However, in this study, the average ME and CP values were 8.14 and 9 respectively. ME value is slightly lower than that by Weiss *et al* (2018) (8.86mj/kg) and that posted by AHDB (2021). This difference could be due to difference of stage at harvest. The quality and digestibility of the silage being affected by stage at harvest (maturity) (Di Marco *et al*., 2002). The CP content being decreasing as the corn matures whilst the CF value increases. This because as the crop matures, stem and leaf quality declines, but this is counterbalanced by the rise in grain in the cob, which is very digestible and high in starch content (Feed value of maize silage and maize grain | AHDB, 2021). Therefore, there may be late harvest on which then decrease ME.

This study explains the results obtained by Chifamba (2018). According to Chifamba (2018), lactating cows fed with intercropped maize x cowpea mixed silage had higher milk yield than those given plain maize silage. Therefore, increased in milk yield basis on the fact that, intercropped maize x cowpea silage has higher CP content and high digestibility which positively increases voluntary feed intake hence animal performance.

Chapter 6.

6.1 Conclusions.

In conclusion, intercropped maize-legume silages have high digestibility than plain maize silage. This increase VFI and performance of animal hence improves production. Also, through the inclusion of high protein content (cowpea) improves the nutritive value of the silages which in turn reduce greater reliance on commercial feeds mostly during the dry period for supplementation when the nutritive value of the forage declines. Therefore, minimising cost of production.

6.2 Recommendations

Adoption of intercropped maize x forage legumes by smallholder dairy farmers is key in their production as this will reduces cost of production by not putting greater reliance on commercial dairy feeds. Intercropping enhances growth of cereals as legumes enriches the soil through nitrogen fixation and also the silage has higher nutritive value. Also, other areas of livestock such as beef sector should adopt this for fattening.

More research has to be done on intercropped cereals x forage legume mixed silage with other cereals such as rapoko**.**

References

Ahdb.org.uk. 2021. *Feed value of maize silage and maize grain | AHDB*. [online] Available at: <https://ahdb.org.uk/knowledge-library/feed-value-maize-silage-maize-grain> [Accessed 15 December 2021].

Habibu, T., Enoch, J.B., Agiteh, I.A., Suliat, A.I. and Susinya, H., 2016. Proximate, minerals and antinutrient assessment of the leaves and tenderstem of Leptadenia hastate (pers.) decne. *Bayero Journal of Pure and Applied Sciences*, *9*(2), pp.43-50.

Mould, F.L., 2003. Predicting feed quality—chemical analysis and in vitro evaluation. *Field Crops Research*, *84*(1-2), pp.31-44.

Playne, M.J., Khumnualthong, W. and Echevarria, M.G., 1978. Factors affecting the digestion of oesophageal fistula samples and hay samples in nylon bags in the rumen of cattle. *The Journal of Agricultural Science*, *90*(1), pp.193-204.

Seghosime, A., Awudza, J.A.M., Richard, B. and Kwarteng, S.O., 2017. Comparative studies on proximate composition and phytochemical screening of mango, key lime, African star apple and African pear seeds as possible coagulant aids for water treatment. *American Journal of Environmental Sciences*, *13*(4), pp.325-333.

DONG, S.Z., Azarfar, A., Yang, Z.O.U., LI, S.L., WANG, Y.J. and CAO, Z.J., 2017. Effects of sequence of nylon bags rumen incubation on kinetics of degradation in some commonly used feedstuffs in dairy rations. *Journal of integrative agriculture*, *16*(1), pp.162-168.

Moe, P.W. and Tyrrell, H.F., 1975. Efficiency of conversion of digested energy to milk. *Journal of Dairy Science*, *58*(4), pp.602-610.

Azim, A., Khan, A.G., Nadeem, M.A. and Muhammad, D., 2000. Influence of maize and cowpea intercropping on fodder production and characteristics of silage. *Asian-Australasian Journal of Animal Sciences*, *13*(6), pp.781-784.

Vercoe, P.E., Makkar, H.P. and Schlink, A.C. eds., 2010. *In vitro screening of plant resources for extra-nutritional attributes in ruminants: nuclear and related methodologies*. Heidelberg: Springer.

Vijverberg, J. and TH FRANK, H., 1976. The chemical composition and energy contents of copepods and cladocerans in relation to their size. *Freshwater Biology*, *6*(4), pp.333-345.

Kennedy, M., List, D., Lu, Y., Foo, L.Y., Newman, R.H., Sims, I.M., Bain, P.J.S., Hamilton, B. and Fenton, G., 1999. Apple pomace and products derived from apple pomace: uses, composition and analysis. In *Analysis of plant waste materials* (pp. 75-119). Springer, Berlin, Heidelberg.

Shang, H.M., Song, H., Xing, Y.L., Niu, S.L., Ding, G.D., Jiang, Y.Y. and Liang, F., 2016. Effects of dietary fermentation concentrate of Hericium caput-medusae (Bull.: Fr.) Pers. on growth performance, digestibility, and intestinal microbiology and morphology in broiler chickens. *Journal of the Science of Food and Agriculture*, *96*(1), pp.215-222.

Isah, O.A., Fayemi, P.O., Gazaly, M.B. and Aderinboye, R.Y., 2012. Nutritional characteristics of four browse plants consumed by free-ranging ruminants in Western part of Nigeria. *African Journal of Agricultural Research*, *7*(12), pp.1944-1949.

Adesogan, A.T., 2002. A critical evaluation of selected nutritive value methods. In *Proceeding of the 13th Annual Florida Ruminant Nutrition Symposium*.

Blu, M. and Ørskov, E.R., 1993. Comparison of in vitro gas production and nylon bag degradability of roughages in predicting feed intake in cattle. *Animal feed science and technology*, *40*(2-3), pp.109-119.

Boisen, S. and Moughan, P.J., 1996. Different expressions of dietary protein and amino acid digestibility in pig feeds and their application in protein evaluation: a theoretical approach. *Acta Agriculturae Scandinavica A-Animal Sciences*, *46*(3), pp.165-172.

Bruinenberg, M.H., Valk, H., Korevaar, H. and Struik, P.C., 2002. Factors affecting digestibility of temperate forages from seminatural grasslands: a review. *Grass and forage science*, *57*(3), pp.292-301.

Getachew, G., Blümmel, M., Makkar, H.P.S. and Becker, K., 1998. In vitro gas measuring techniques for assessment of nutritional quality of feeds: a review. *Animal Feed Science and Technology*, *72*(3-4), pp.261-281.

Dhliwayo, O., 2017. *The impact of rainfall extremes on smallholder farmers in rural communities: a case of Chitomborwizi, Makonde district* (Doctoral dissertation, BUSE).

Di Marco, O.N., Aello, M.S., Nomdedeu, M. and Van Houtte, S., 2002. Effect of maize crop maturity on silage chemical composition and digestibility (in vivo, in situ and in vitro). *Animal Feed Science and Technology*, *99*(1-4), pp.37-43.

Edson, C., Takarwirwa, N.N., Kuziwa, N.L., Stella, N. and Maasdorp, B., 2018. Effect of mixed maize-legume silages on milk quality and quantity from lactating smallholder dairy cows. *Tropical animal health and production*, *50*(6), pp.1255-1260.

Galyean ML. (2010). "Laboratory procedures in animal nutrition research". Department of Animal and Food Sciences Texas Tech University, Lubbock

Kamalak, A.D.E.M., Canbolat, O., Gurbuz, Y. and Ozay, O., 2005. Comparison of in vitro gas production technique with in situ nylon bag technique to estimate dry matter degradation. *Czech Journal of Animal Science*, *50*(2), pp.60-67.

Galyean, M.L., Cole, N.A., Tedeschi, L.O. and Branine, M.E., 2016. BOARD-INVITED REVIEW: Efficiency of converting digestible energy to metabolizable energy and reevaluation of the California Net Energy System maintenance requirements and equations for predicting dietary net energy values for beef cattle. *Journal of Animal Science*, *94*(4), pp.1329-1341.

Givens, D.I., Owen, E., Omed, H.M. and Axford, R.F.E., 2000. *Forage evaluation in ruminant nutrition*. CABI.

Kang, S., Wanapat, M., Phesatcha, K. and Norrapoke, T., 2015. Effect of protein level and urea in concentrate mixture on feed intake and rumen fermentation in swamp buffaloes fed rice straw-based diet. *Tropical animal health and production*, *47*(4), pp.671-679.

Khan, M.A., Mahr-Un-Nisa, S.M. and Sarwar, M., 2003. Techniques measuring digestibility for the nutritional evaluation of feeds. *International Journal of Agriculture and Biology*, *5*(1), pp.91-94.

Kitessa, S., Irish, G.G. and Flinn, P.C., 1999. Comparison of methods used to predict the in vivo digestibility of feeds in ruminants. *Australian Journal of Agricultural Research*, *50*(5), pp.825-842.

López, S., 2005. In vitro and in situ techniques for estimating digestibility.

Mohamed, R. and Chaudhry, A.S., 2008. Methods to study degradation of ruminant feeds. *Nutrition Research Reviews*, *21*(1), pp.68-81.

Murillo, M., Herrera, E., Reyes, O., Gurrola, J.N. and Gutierrez, E., 2011. Use in vitro gas production technique for assessment of nutritional quality of diets by range steers. *African Journal of Agricultural Research*, *6*(11), pp.2522-2526.

Huhtanen, P., Kaustell, K. and Jaakkola, S., 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. *Animal Feed Science and Technology*, *48*(3-4), pp.211-227.

Mut, H., Gulumser, E., Dogrusoz, M.C. and Basaran, U., 2017. Forage yield and nutritive value of maize-legume mixtures. *Range Management and Agroforestry*, *38*(1), pp.76-81.

Ngongoni, N.T., Mapiye, C., Mwale, M. and Mupeta, B., 2006. Factors affecting milk production in the smallholder dairy sector of Zimbabwe. *Livestock research for rural development*, *18*(05), pp.1-21.

Moe, P.W., 1981. Energy metabolism of dairy cattle. *Journal of Dairy Science*, *64*(6), pp.1120-1139.

Ngongoni, N.T., Mapiye, C., Mwale, M. and Mupeta, B., 2007. Effect of supplementing a high-protein ram press sunflower cake concentrate on smallholder milk production in Zimbabwe. *Tropical animal health and production*, *39*(4), pp.297-307.

Riday, H. and Albrecht, K.A., 2008. Intercropping tropical vine legumes and maize for silage in temperate climates. *Journal of sustainable agriculture*, *32*(3), pp.425-438.

Saha, U. K., Sonon, L. S., Hancock, D. W., Hill, N. S., Stewart, L., Heusner, G. L., & Kissel, D. E., 2010. Common terms used in animal feeding and nutrition.

Terry, R.A. and Tilley, J.M.A., 1964. The digestibility of the leaves and stems of perennial ryegrass, cocksfoot, timothy, tall fescue, lucerne and sainfoin, as measured by an in vitro procedure. *Grass and Forage Science*, *19*(4), pp.363-372.

Tilley, J.M.A. and Terry, D.R., 1963. A two-stage technique for the in vitro digestion of forage crops. *Grass and forage science*, *18*(2), pp.104-111.

Uher, D., Horvatić, I., Jareš, D. and Maćešić, D., 2019. Influence of Intercropping Maize with Cowpea on Forage Yield and Quality.

Van Keulen, J.Y.B.A. and Young, B.A., 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *Journal of Animal Science*, *44*(2), pp.282-287.

Waller, J., Merchen, N., Hanson, T. and Klopfenstein, T., 1980. Effect of sampling intervals and digesta markers on abomasal flow determinations. *Journal of Animal Science*, *50*(6), pp.1122-1126.

Wei, M., Chen, Z., Wei, S., Geng, G. and Yan, P., 2018. Comparison among methods of effective energy evaluation of corn silage for beef cattle. *Asian-Australasian journal of animal sciences*, *31*(6), p.851.

Weiss, W.P. and Tebbe, A.W., 2019. Estimating digestible energy values of feeds and diets and integrating those values into net energy systems. *Translational Animal Science*, *3*(3), pp.953-961.

Zewdie, A.K., 2019. The different methods of measuring feed digestibility: A review. *EC Nutr*, *14*(1), pp.68-74.

Waite, W.C., 1943. Production and Processing of Dairy Products. *The ANNALS of the American Academy of Political and Social Science*, *225*(1), pp.206-209.

Asi.k-state.edu. 2021. [online] Available at: <https://www.asi.k-state.edu/research-and-extension/swine/swinenutritionguide/pdf/KSU%20Feed%20Sampling%20and%20Analysis%20fact%20sheet.pdf> [Accessed 23 December 2021].