T.R.
KAHRAMANMARAŞ SÜTÇÜ IMAM UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE

EFFECT OF OIL SUPPLEMENTATION ON GAS AND METHANE PRODUCTION OF SOME FEEDSTUFFS

DLSHAD NAMIQ KHURSHID

MASTER'S THESIS
DEPARTMENT OF BIOENGINEERING AND SCIENCE

KAHRAMANMARAS 2017

KAHRAMANMARAŞ SÜTÇÜ IMAM UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

EFFECT OF OIL SUPPLEMENTATION ON GAS AND METHANE PRODUCTION OF SOME FEEDSTUFFS

DLSHAD NAMIQ KHURSHID

Thesis submitted in candidature
for
The degree of Master in
Department of Bioengineering and Science

KAHRAMANMARAŞ 2017

M.Sc. thesis entitled “EFFECT OF OIL SUPPLEMENTATION ON GAS AND METHANE PRODUCTION OF SOME FEEDSTUFFS” and prepared by DLSHAD NAMIQ KHURSHID ZANGANA, who is a student at of Bioengineering and Science Department, Graduate School of Natural and Applied Sciences, Kahramanmaraş Sütçü İmam University, was certified by all the/majority jury members, whose signatures are given below at the date of 6/10/2017.

Prof. Dr. Adem KAMALAK (Supervisor) ……………………………
Department of Animal Science
Kahramanmaraş Sütçü İmam University

Prof. Dr. Emin ÖZKÖSE (Member) ………………………….
Department of Animal Science
Kahramanmaraş Sütçü İmam University

Assist. Prof. Dr. Ali İhsan ATALAY (Member) ……………………
Department of Animal Science
Iğdır University

I approve that the above signatures related to the members.

Assoc. Prof. Dr. Mustafa ŞEKKELİ ……………………………….
Director of Graduate School
Kahramanmaraş Sütçü İmam University

DECLARATION

I hereby declare that all information in the thesis has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

DLSHAD NAMIQ KHURSHID

Note: Uses of the reports in this thesis, from original and other sources, tables, figures and photographs without citation, subject to the provisions of Law No. 5846 of Intellectual and artistic works.

YAĞ İLAVESİNİN BAZI YEMLERİN GAZ VE METAN ÜRETİMİNE ETKİSİ
(YÜKSEK LİSANS TEZİ)

DLSHAD NAMIQ KHURSHID

ÖZET

Bu çalışmanın amacı, mısır yağının ruminant beslemede kullanılan bazı yemlerin gaz ve metan üretimine etkisini belirlemek için yapılmıştır. Bu çalışmada mısır yağının fermenmtasyonu üzerine etkisini belirlemek için Menke gaz üretim tekniği kullanılarak belirlenmiştir. Mısır danesi, arpa danesi, yonca otu ve buğday samanı ivesi koyunundan alınan rumen sıvısıyla üç tekerrürlü olarak 24 saatlik inkübasyona bırakılmıştır. İnkübasyon sonunda üretilen gazın metan içeriği metan analiz cihazıyla belirlenmiştir. Mısır yağının ilavesi gaz ve metan üretimini önemli derecede azaltmıştır. Yağ muamelesi yapılmayan yemlerin gaz üretimi 24.74 ile 79.74 ml, muamele edilen yemlerin gaz üretimi 21.33 ile 74.97 ml arasında değişmiştir. Yağ muamelesi yapılmayan yemlerin metan üretimi 4.60 ile 13.22 ml, muamele edilen yemlerin metan üretimi 3.75 ile 12.36 ml arasında değişmiştir. Sonuç olarak ruminant hayvan beslemede kullanılan bazı yemlerin mısır yağı ile muamele edilmesi gaz ve metan üretimini düşürmektedir. Bununla birlikte mısır yağının metan üretimi ve hayvan performansına etkisini belirlemek için daha fazla in vivo çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Gaz üretimi, metan üretimi, ruminant, yağ ilavesi.

Kahramanmaraş Sütçü İmam Üniversitesi
Fen Bilimleri Enstitüsü
Biyomühendislik ve Bilimleri Anabilim Dalı, Ekim/ 2017

Danışman: Prof. Dr. Adem KAMALAK
Sayfa sayısı: 63

EFFECT OF OIL SUPPLEMENTATION ON GAS AND METHANE PRODUCTION OF SOME FEEDSTUFFS

(M.Sc. THESIS)

DLSHAD NAMIQ KHURSHID

ABSTRACT

The aim of the current experiment was carried out to investigate the effect of supplementation of maize oil on the gas production and methane production of some feedstuffs used ruminant nutrition. In the current experiment the effect of maize oil supplementation on the fermentation of some feedstuffs used ruminant animals was determined using Menke gas production technique. The feedstuffs such as maize grain, barley grain, alfalfa hay and wheat straw were incubated in triplicate with rumen fluid from Awassi sheep for 24 h. At the end of incubation the methane contents of gas produced were determined with methane analyzer. The supplementation of maize oil has a significant effect on gas production and methane production. Gas productions for untreated feedstuffs ranged from 24.74 to 79.74 ml whereas gas production for oil treated feedstuffs ranged 21.33 to 74.97 ml. Methane productions for untreated feedstuffs ranged from 4.60 to 13.22 ml whereas gas production for oil treated feedstuffs ranged 3.75 to 12.36 ml. As a conclusion, supplementation of maize oil decreased the gas production and methane production of some feedstuffs used ruminant nutrition. However, further in vivo investigations are requested to determine the effect of oil supplementation on methane production and animal performance.

Key words: Gas production, methane production, ruminant, oil supplementation.

University of Kahramanmaraş Sütçü İmam
Graduate School of Natural and Applied Science
Department of Bioengineering and Science, October/ 2017

Supervisor: Prof. Dr. Adem KAMALAK
Page number: 63

ACKNOWLEDGMENT

In the name of God and before anything deepest thanks to Allah, the most gracious, beneficent, and merciful, for the help and guidance to achieve my goals by giving me this chance and allowing me to complete my study and reach this level.

Thanks to my supervisor Prof. Dr. Adem KAMALAK for his excellent supervision and supporting me until I finished my thesis.

I wish to express my gratitude to Emrah KAYA, Ali Ihsan ATALAY, Özer KURT and Yasemin AKAGÜNDÜZ for teaching me the laboratory analysis knowledge. My dear friend YADGAR GHALI SALIH for giving me useful information on thesis writing

For the financial support, I would like to thank my family for providing education and for their unconditional support and encouragement to pursue my interests, I am grateful to my sisters and brothers for their patience, psychological support and for reminding me that not everything in life is scientific.

Lastly, Thanks to everyone who learned me even one word in my life.

DLSHAD NAMIQ KHURSHID

TABLE OF CONTENTS

Page No

Page No

LIST OF FIGURES

Page No

LIST OF TABLES

Page No

LIST OF ABBREVIATIONS

AA : Acetic Acid
ADIN : Acid Detergent Insoluble Nitrogen
ADF : Acid Detergent Fiber
AOAC : Association of Official Analytical Chemists
BA : Butyric Acid
CO_2 : Carbon dioxide
CP : Crude Protein
CA : Crude Ash
DM : Dry Matter
FC : Fermentation Coefficient
FS : Fleig Score
ME : Metabolisable Energy
NDF : Neutral Detergent Fiber
NPN : Non-Protein Nitrogen
OM : Organic Matter
OMD : Organic Matter Digestibility
RDP : Rumen Degradable Protein
TMR : Total Mixed Ration
WSC : Water Soluble Carbohydrate
Mt : million tonnes
GP : Gas production
TDN : Total Digestible Nutrients
DE : Digestible Energy
GHG **:** Greenhouse Gas

1. INTRODUCTION

Nutrition is the foundation of animal production, in livestock system, the proper nutrition is essential in order to achieve high productivity (Cheeke and Dierenfeld, 2010; Fasae *et al*., 2010; Dryden, 2008). Indeed, the feed is the largest cost related to ruminant nutrition, usually represents 60% or more of all output costs (McDonald *et al*., 2010; Schroeder, 2009). However, for millennia, nutrition suffers from environmental factors such as climate change and habitat degradation or loss resulted from increased human population and human activity, Several studies indicated that the growth of the human population is a key risk factor in climate change, expected to be roughly 35% between 2006 and 2050 (Gerber *et al*., 2013; Goodland and Anhang, 2009; Koneswaran and Nierenberg, 2008). Blockhouse *et al* (2013) also have a similar opinion, by 2050, the number of animals will double from 60 billion to 120 billion and result in releasing more greenhouse gas (GHG) emissions, because the population of animals, in all around the world, is still increasing; whereas, in other industries, the greenhouse gas emissions will decrease.

On top of that, it is expected that the global use of meat will be increasing about 230 to 470 million tonnes (Mt) and milk from 585 to about 1050 Mt in 2050 (Knapp *et al*., 2014; Blokhuis *et al*., 2013; Morgavi *et al*., 2010) because the numbers of livestock that are reared for meat, egg, and dairy production will increase (Koneswaran and Nierenberg, 2008; Steeg *et al*., 2009). However, since 1990, the animal population in Iraq was decreased dramatically because of a bad health and husbandry of dairy cattle and equipment shortages, thus, the milk production and milk products have almost collapsed (Jaradat, 2003). Livestock contributes to climate change by emitting greenhouse gases or carbon footprints like CO_2, CH_4, and N_2O; either directly, for example from enteric fermentation, or indirectly, for example from feed-production activities, deforestation to create new pasture, etc. (Borhan *et al*. 2012).

In developing countries, the increase in CH_4 emissions from an animal is common; particularly where cattle numbers permanently increase and where there is a possibility for technological change (Herrero *et al*., 2008). Over 100 years the potential contribution of methane to global warming is estimated at about 21 times more than carbon dioxide (Dryden, 2008), this is because methane has a positive radiative force on the climate; even it is much shorter-lived in the atmosphere.

As well as it has a serious effect on the high atmosphere ozone formation (Koneswaran and Nierenberg, 2008; Steeg *et al*., 2009; Boadi *et al*., 2004; Pal *et al*., 2014). Hook *et al*. (2010) indicated that the contributions of CH_4 of the ruminant species to greenhouse gases are different. Methane is estimated from derived energy in the diet is different, ranging from 5.5 to 9.0 % of dairy cows, from 3.5 to 6.5 % for beef fattening, from 6.0 to 7.5 % for range-cattle, from 7.5 to 9.0 % for buffalo and from 7.0 to 9.0 % for camels. In addition, estimates of methane emissions also deepen on feed intake, forage quality, forage composition, the forage processing, and geographical location, the main source of enteric methane emissions to the atmosphere is the ruminant cattle (Morgavi *et al*., 2011), whereas manure has also the significant contribution of methane and nitrous oxide emissions to greenhouse gases. Generally, the contribution of N_2O and CH_4 to the total greenhouse gases is about 6 and 22% respectively. In the rumen fermentation process, enteric methane is produced as a waste product (Pal *et al*., 2014; Borhan *et al*., 2012). Ruminant animals exhale the 90 % of the methane produced in the rumen through the mouth and nose. No ruminant animals also produced CH_4 is in the lower gut (Hook *et al*., 2010). Herrero *et al*. (2008) also indicated that the ruminants, rice fields, burning of natural gas, biomass, wetlands and plants grow in aerobic environments are the most significant sources of methane in the worldwide.

Numerous articles indicated that the mitigation of methane emission is crucial, due to its contribution to global warming. Enteric methane emissions considered an economic loss to the farmer where forage is converted to methane instead of products (Koneswaran and Nierenberg 2008; Boadi *et al*., 2004). Therefore, there is a great need for more research to find out the factors affecting the enteric methane production and limit the uncertainty in GHG emission inventories, and to determine viable mitigation strategies of GHG. Makkar (2005) indicated that there are different methods available to measure the methane emissions from ruminants.

One of the problems our world faces today is global warming due to the augmentation of the greenhouse impact which arises from accumulating gases that trap heat in the atmosphere. Methane is considered the 2nd majorityproblem greenhouse gas emitted from anthropogenic sources (Wuebbles and Hayhoe, 2002) Animals, particularly ruminants, in their gastrointestinal tracts produce CH_4 from anaerobic fermentation as all pathways for the disposal of metabolic hydrogen produced during microbial metabolism.

Livestock ruminant is responsible for about 15-20% of the total anthropogenic emissions of CH_4 (Moss *et al*., 2000). The produced CH_4 from enteric fermentation of ruminant is associated with energy losses and related to the environmental problems, and hence reduction in their retention and use of energy, especially 6-8%, but up to 12% of the gross energy in feed is converted to CH_4 during microbial digestion in the rumen (Johnson and Johnson, 1995). Therefore, declining CH_4 production from ruminants is desirable for lessening greenhouse gas emissions and rising utilization of the digested energy (Jayanegara, *et al*, ,2009; Makkar, *et al.,* 2005). Methane is an effective greenhouse gas, of which about 11% - 17% of worldwide emissions consist of enteric methane produced by ruminants (Beauchemin *et al*., 2009).

Methane is one of the most important greenhouse gases and about 70% of methane *emission* is related to human activity, Animals are in charge of 25% of these productions, which are derived mainly from their gastrointestinal system but also from their manure, Reducing the emission of methane would be beneficial to animals since emission of methane is a process with which animal lost energy, It is well known that methane emission is a direct result of fermentation processes performed by arches, which scavenge hydrogen and use it to emissions methane, the production of methane is a straightforward energy loss to the ruminant as well as a source of contamination. Methane production from animals conduces to total global methane production, which is a major conduces to global warming (Lassey *et al*., 2001). Enteric methane conduces 30– 40% of Total Gas Production (TGP) from agricultural source (Moss *et al*., 2000). There are several ways of mitigating of methane emissions such as the use of prebiotics and proboscis (Mwenya *et al*., 2004), supplementation of fat (Van Nevel and Demeyer., 1996) and addition of plant extracts (Goel *et al*., 2008).

Methane is produced by archea in the rumen during anaerobic fermentation of soluble and structural carbohydrates contained in the feed (Kurihara *et al*., 1999). Indicated that CH_4 energy loss in cattle feed on tropical forage diets was greater than in those feed on clement forage diets, due to relatively high levels of fiber and lignin but a low level of non-fiber carbohydrate, however, ruminant in developing countries are preponderantly maintained on a high-roughage diet with little or no concentrate (Hariadi and Santoso, 2010).

Methanogenic archaea (known as methanogens) synthesize methane using carbon dioxide and hydrogen, which is produced mainly in the formation of acetate, and in some cases format (Wolin *et al*., 1997). The microbial population in both rumen and hindgut of ruminant animals produces methane, where methanogens bacteria reduce carbon dioxide to methane by using hydrogen (H_2) in the methanogenesis process (Mills *et al*., 2001).

The in vitro gas production technique is a useful method to determine the extent and rate of feed degradation (Blummel and Ørskov, 1999; Cone *et al*., 1997; Groot *et al*., 1996; Beuvink *et al*., 1992). Gas production (GP) provides more elaborate information on fermentation kinetics of ruminant feeds that can be achieved by estimating for the disappearing of feed from fermentation media and number of different *in vitro* techniques have been developed (France *et al*., 2001).

The aim of this experiment was to investigate the effect of supplementation of maize oil on the gas production and methane production of same feedstuffs such as barley grain, maize grain, alfalfa hay, wheat straw.

2. LITERATURE REVIEW

2.1. Potential Nutritive Value

Potential nutritive value is very popular in ruminant nutrition, associated forage with high quality considered highly significant for a proper diet of ruminant livestock due to their role in supplying minerals, protein, and energy, The feeding value of any forage is dependent on its content of nutrients critical to the body and its substance of energy-producing nutrients which can be; protein, minerals and vitamins (Oelberg, 1956).

As the seasons change the ways and the types of the production also change, therefore the nutritive value of feeds is looked at the most appropriate measure which owns the ability to provide the nutritional requirements of the grazing animal (Ruyle, 1993). Nutrient value can be described as the animal production response to grazing forage in situations where forage availability does not draw a limitation for animal performance (Barry, 2013). It is pointed that the potential nutritive of feedstuffs is shown by the chemical composition and the digestibility (Dale, 2014; Saha *et al*., 2010; Kumara *et al*., 2009).

In fact, forage nutritive value is not a task that only consisted of chemical composition but really it also includes intake characteristics in addition to the effectiveness of extracted –nutrients from the fodder through digestion (Sanon *et al*., 2008; Barry *et al*., 1999). Furthermore, the nutritive value of diets is based on three general ingredients; digestibility, voluntary intake and the efficiency with which feed energy is utilized (Skenjana, 2011).

Crampton *et al*. (1960) showed that the digestion data which is one of the ways to assess diets taken into use largely for decades. In course of indicating the nutritive value of any forage, two measurements occupy our consideration, ruminal degradability and small intestinal digestibility (Woods *et al*., 2003a). In fact, the ruminant's productivity is tied to the capacity of forage to boost the effectiveness of rumen microbial fermentation and to supply relatively the necessary amounts and balances of nutrients desired by the animal tissues for different states of production reported by Norton *et al*. (1994).Scientifically and practically proven that some factors such as soil factors, climate plant species animal class, and the stage of maturity leave a big impact on the nutritive value of forage (Oelberg, 1956).

Dale, (2014) believes that nutrient concentration and digestibility are two major factors influencing the nutritive value. Sanon *et al*. (2008) according to his understanding the claims that the browse consists of lignin and anti-nutritional factors, and it could be toxic; that is why the consumption of browse has a limited rate. Two researchers Aganga and Tshwenyane (2003) after a precise observation came to the conclusion that the anti-nutritive factors could intersect with the description that believes they are materials growing in natural forages by the metabolism. It seems that the most suitable way to know and show the nutritive value of feeds is a wet chemistry, and it is very significant when it comes to quality assurance purposes and for development of new techniques (Dale, 2014). Evidence clarify that despite the importance of the chemical composition but recently it is only the biological measurement which is more dependable in terms of giving enough information about the nutritive value of fibrous feeds or waste Ørskov (1987). It has announced that the energy yield of a source of forage might be measured from DM weight per unit area (Ibrahim and Olaloku, 2000).

It is clear that the volatile fatty acid is the critical energy source for ruminants (VFA) (Getachew *et al*., 2004). Energy values of feed are normally marked as Total Digestible Nutrients (TDN) or Digestible Energy (DE). The sources of energy are mostly grasses and it is because they have high cellulose in their content. Eventually, when the reduction of intake and the reduction of total energy happen the digestibility of grasses goes so low (Ruyle, 1993). Metabolizable energy displays part of the forage energy which livestock might use it. Vivo is used for measuring metabolizable energy and it is estimated from 24 h *in vitro* gas production and chemical composition of diets because of the high correlation which exists among them, *In vitro* gas production technique is used to evaluate the energy value of numerous forage especially straws (Hamid *et al*., 2007).

Shrubs cannot be categorized as good sources of energy when fruit production state finishes; and, forbs are considered as an intermediate stage between grasses and shrubs. As a matter of fact, energy plays out constantly as are an astrictive factor of animal production on pasture that is crude protein (Ruyle, 1993; Fulkerson *et al*., 2007) think that the diets contribute in providing energy, frequently in form of carbohydrates carbohydrate forms 60-80% of dry matter.

The carbohydrates contain cellulose and bacteria conduct digestion process mostly in the rumen of ruminants, animal digests starch completely, and hemicellulose which occupies intermediate stage between starches and cellulose and crushed by weak acids and alkalis. Saha *et al*. (2010) looked at the neutral detergent fiber (NDF) and realized that it is the remains or an insoluble portion left after boiling a feed sample in neutral detergent solution. Neutral detergent fiber consisted of cellulose, hemicellulose, and lignin. On the other hand, acid detergent fiber (ADF) consists of cellulose and lignin and they are a less digestible portion of forage. Feeds which carry lower ADF can be marked with having higher digestibility values than that of feeds which carry higher ADF. This indicates remarkably whenever there is an increase of the level of ADF of diet, simultaneously a decrease in digestibility of diet occurs. Focus on both NDF and ADF increases when maturity increases (Kamalak *et al*., 2011).

Robinson and Getachew (2002) both conclude that the energy value and voluntary intake have a positive relationship, It is because the energy value which exists in most of the feeds critically affected by the level and fermentability of NDF. Normally when an increase occurs in the level of NDF in forage species, the decrease occurs in intake potential, and it is because; between NDF and digestible NDF there is a devastating negative correlation in most forage species, so within a species of forage, NDF level is a strong predictor of voluntary intake.

Crude protein amount is known when calculated by multiplying nitrogen content by factor 6.25 (Ruyle, 1993), on the other hand 25-50% of the total nitrogen could be non-protein, initially amides and amino acids. Protein value generally depends on the supply of certain amino acids that are necessary for the formation of protein in the body which cannot be produced by the body. The amount of digestible protein obtained from a plant depends on the species of plant as well as the class of livestock. In addition, the digestibility of protein varies potentially among domestic animal species and even between individuals of a given species (Oelberg, 1956).

Dale, (2014) pointed out that when the nitrogen balance comes into consideration all the sources that contribute to nitrogen loss are important. The urea amount in an animal’s blood, milk, and considered as a metabolic indicator of the higher concentration of nitrogen. Ruminants depend on microbial protein as their protein supplier and it undergoes a synthesis process in the rumen as a result of the decomposition of feed and bypass protein which is found in the small intestine (Fulkerson *et al*., 2007; Woods *et a*l., 2003b). Microbial protein and bypass protein are both called as a metabolizable protein which they help maintenance and production of ruminant animals.

Ibrahim and Olaloku (2000) argue that minerals also play a significant role in terms of tissue growth and regulation of ruminant's body functions, like calcium, phosphorous, magnesium, sodium, potassium, sulphur, chlorine, iron, copper, cobalt, iodine, manganese, selenium, zinc, chromium, fluoride, molybdenum, nickel, silicon and vanadium. When there is lack of any of these minerals in the diet symptoms of default definitely surfaces, Shrubs possess sufficient phosphorus which helps maintenance and gestation requirement, even when mature although the proportion of phosphorus in most forbs is less than shrubs (Ruyle, 1993). Vitamins are counted as having secondary importance for ruminants, and body needs a small quantity to regulate its function (Ibrahim and Olaloku, 2000).

Olteanu *et al*. (2005) proposes that the digestibility is a major factor in indicating the nutritive value of feeds, Nowadays, many methods and ways are available to evaluate the digestibility of ruminant forages. These ways shed light on the differences that exist between the stomach digestibility (actual, bypass, digestibility), the ruminal digestibility (degradability) and the apparent digestibility (overall digestive tract).

Kamalak *et al*. (2011) and Karabulut *et al*. (2007b) claim that chemical composition in combination with *in vitro* gas production technique is hugely used to mark the potential nutritive value of forages. Indeed chemical composition of rangeland plants is significant, specifically in combination with in vitro digestibility (Tufarelli *et al*., 2010; Kumara *et al*., 2009). The *in vitro* gas production technique displays the amount of gas produced over time that shows the end outcome of the fermentation of the feed substrate to unstable fatty acids, microbial biomass and the neutralization of the volatile fatty acids yielded (Osuga *et al*., 2006).

Getachew *et al*. (2004) argue that strong relationship exists between the extent of gas emission at 24 h and chemical composition, while at the same time a weak relationship is determined between the proportion of gas emission and chemical composition. Several studies confirm the correlation between CP and gas production. Crude protein and gas production has a negative correlation. Past studies found out that the browses' crude protein content properly correlates with potential gas production, for example (CP ranging from 32 to 487 g kg^{-1} DM) and with tropical browses (CP ranging from 70 to 300 g kg^{-1} DM) have demonstrated no effect of CP level on gas and VFA production in their study that has been implemented. On the other hand, Getachew *et al.* (2004) reviewed some studies and he came to a conclusion that there is a negative correlation between CP and gas production, they only aim to lower volatile fatty acids (VFA) production, Gas and VFA production hold a confirmative relationship between, and speculation of VFA production from gas production significantly improves if it combines with the CP content of the feeds me, On the other hand, there is irregular relationship between NDF level and gas production, however, several studies project the negative effect of NDF on digestibility.

2.1.1. Barley

Hordeum Vulgare L. (Poaceae), is classified as an annual monocotyledonous herb, It is one of the tribe Triticeae belongings, this herb is evolutionarily nearly tied to two other small-grain cereal species, wheat, and rye, although the genus Hordeum is famous for its development before 12 million years ago (von Bothmer and Komatsuda, 2011). It is said that the first time signs of the pre-agricultural gathering of wild barley were found in the land of Fertile Crescent in south-western Asia c. 22 000 years ago and domestication of barely started increasing independently also in Central Asia (Piperno *et al*. 2004). It was The early selection by environmental factors and man and it arrived into modern breeding which has resulted in hundreds of landraces and cultivars, they are grown from semi-arid subtropical to temperate climates, from equatorial to nearly circumpolar latitudes, and from sea-level to high altitudes, characteristically for a grain crop, barley grown today different from the barley cultivated ancient times nowadays the barley owns long heads and large grains which is odd to its wild ancestor, these features are helpful in producing high grain yield as well as quality. Today, barley is considered as one of the significant crop plants globally, and it can be used as feed or as a raw material for malt production, in Finland, feed and industrial uses of barley form 59 and 14% of total barley usage, respectively (Tike, 2013). Only a small amount of barley (0.7% in Finland) is consumed as food, while in northern Africa barley forms a significant part of food and it is a staple food and it has been discovered that in its content a sufficient amount of soluble dietary fiber is present and proven that health influences have boosted the status of barley as a food ingredient (Baik and Ullrich, 2008).

2.1.2. Maize

Is one of the most important food grain and it comes at third rank after wheat and rice, and there is a lot of demand for maize and it’s because its use in biofuel production has increased as well. Maize‘s main component is starch, and starch undergoes a wet milling process when produced, maize or corn is a cereal crop is grown widely around the world because of the specialty has in adjusting to different environments, maize is one of the grains produced annually than any other grain, nearly 50 species exist and have different colors, textures, and grain shapes and sizes. White, yellow and red are the most common types. People mostly prefer the white and yellow varieties and it depends on the region, maize was first brought into Africa in the 1500s and since that time became one of Africa's dominant food crops. Similarly, as many other places, it is used as a vegetable although it is a grain crop the grains have different vitamins A, C and E, carbohydrates, essential minerals, and contain 9% protein. They also have dietary fibers and calories which are considered a good source of energy (iita, 2016).

2.1.3. Alfalfa

Hay is a significant source for providing a protein and a fiber of high quality. Alfalfa is counted as legume hay while sometimes known as “Lucerne”. Their hays are rich in protein and minerals and tastier than grass hays. The energy exists in alfalfa is high and alfalfa is a good source of vitamins and minerals. If it’s treated well, it is the greatest of the legume hays from a nutrient standpoint. It owns the most feed value of all the perennial pasture forages. Alfalfa can be used as for horses, dairy cows, beef cattle, sheep, chickens, turkeys and other farm animals.

Because of its high nutritional quality, alfalfa hay is considered as one of the greatest and more popular horse feed varieties available. alfalfa is put in the rations for young growing animals, breeding animals, and adult working horses, having high protein, calcium and vitamin content makes it more useful in balancing rations for broodmares and young growing horses. Its tastiness makes it more popular especially for horses that are finicky about eating (Anderson, 2016).

2.1.4. Hay

it is classified as a grass, legumes, or other herbaceous plants are cut, dried, and stored for use as animal fodder, in particular given to grazing animals such as cattle, horses, goats, and sheep. Smaller animals such as rabbits and guinea pigs are fed hay, hay can also be given to pigs, but they have not ability to digest it as efficiently as more fully herbivorous animals hay can be consumed as animal fodder if enough pasture or rangeland is not available on which to graze an animal, when grazing is not available because of the weather (such as during the winter) or when lush pasture is too rich by itself and may leave impact on the health of the animal. It can be used as food in times when an animal is incapable of reaching pasture, especially in times when animals are kept in a stable or barn (Wikipedia, 2016).

2.2. Methane

Methane is a gas with no color, no taste, and it is also inflammable; basically, it is a natural gas. In weight perspective, it is pretty light and even its lighter than air and with a specific gravity of 0.554 (Hook *et al*., 2010).

Both the chemical formula CH_4 and the chemical compound of methane gas is one atom of carbon and four atoms of hydrogen. It has the simplest component of alkanet and it forms the composition of gases in nature. The good relation of methane makes methane tremendous and attractive fuel, therefore sometimes the way it is controlled and stored needs accuracy because of the gaseous state which finds more situations normally. Methane's location can be found under the sea floor or below ground, and from there it finds out its path to the surface earth and in the atmosphere of the earth (Staley, 2009).

This gas consists of a chemical compound with high combustion energy, 55.5 ML/kg (Crutzen *et al*., 1995). The ruminant animals play a major role in methane production and the process is considered very important to the global warming and the climate change (Mekuriaw, 2013). It is classified as the second major greenhouse gas and it is 25 times more effective than carbon dioxide as a greenhouse gas (Forster *et al*., 2007).

Enteric methane forms 30-40% of the methane that is produced from the agricultural sector, specifically from livestock production operations; the major source of methane is from ruminant animals (Moss *et al*., 2000). The microbial population in both rumen and hindgut of ruminant animals is responsible for producing methane, where methanogenesis bacteria transform carbon dioxide to methane by using hydrogen (H_2), in the methane gens process (Mills *et al*., 2001).

There is another process present in the hindgut called acetogenesis. In Both processes hydrogen is the main factor to produce methane or VFA. in some stoichiometric calculations which are based on VFA proportion, the value is predicted of CH_4 production in the hindgut is normally higher than that gained from CH_4 production in the hindgut, this difference likely occurs because of the role of acetogenesis and methanogenesis: in the rumen H_2 is widely used for methanogenesis, while in the hindgut more H_2 is used for acetogenesis,the amount of that methane which produced may vary with the farming system, the animal species, but the most significant factor is feed, specifically its nature and its digestibility (Fonty *et al*., 2007; Ramin and Huhtanen, 2013).

Furthermore, the production of methane is tied with considerable dietary energy losses from ruminant directing to decreasing energy gain and productivity. Control of methane production by ruminants would have important economic as well as environmental profits (Van-Nevel and Demeyer, 1996).

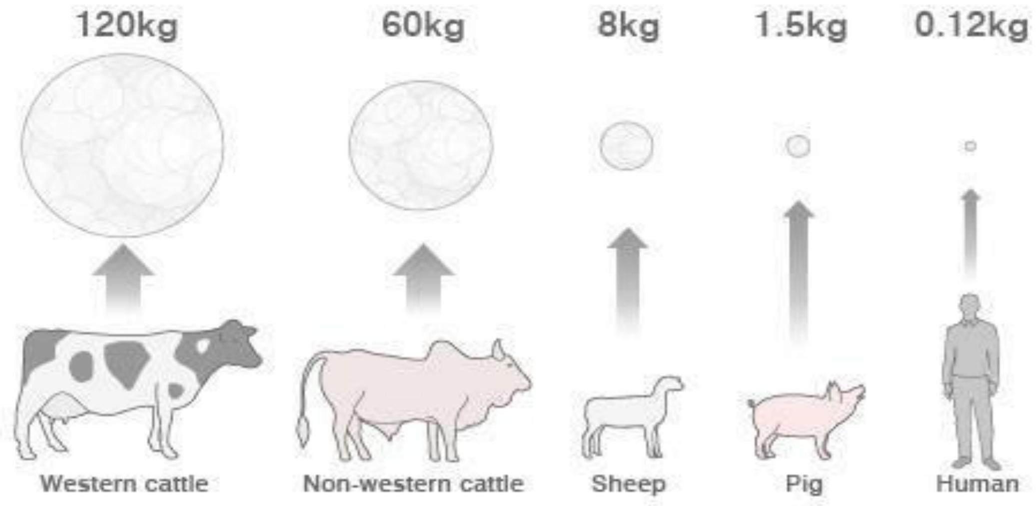


Figure 2.1. Methane emission per animal/human per year (Miller *et al*., 2006).

2.2.1. Acetogenesis

Acetogenic bacteria are held accountable for the oxidation of the products which are created in the acidogenic phase into a substrate appropriate for the methanogenic microorganisms, In this way, acetogenic bacteria are the most significant part of an intermediate metabolic group that generates substrate for methanogenic microorganisms, those products produced by acetogenic bacteria are acetic acid, hydrogen and carbon dioxide, When the formation of acetic and propionic acids takes place, a sufficient amount of hydrogen is formed, making the pH in the aqueous medium downsize. Even we have two ways by which hydrogen is taken to use in the medium,, first, via the methanogenic microorganisms, which benefit from hydrogen and carbon dioxide to generate methane and the second one, via the formation of organic acids, such as propionic and butyric acids, their formation occurs through the reaction of hydrogen, carbon dioxide and acetic acid (De Limos Cherznicharo, 2007).

Among all the products undergo metabolization by the acidogenic bacteria, it is only hydrogen and acetate that are directly used by methanogenic microorganisms. Although at 50% of the biodegradable COD are changed into propionic and butyric acids, which later because of the decomposition process they change into acetic acid and hydrogen by the action of acetogenic bacteria (De Lemos and Chernicharo, 2007).

2.2.2. Methanogenesis

Methanogenic archaea in the last stage run anaerobic degradation process of organic compounds into methane and carbon dioxide. In this process, a small number of the substrate, comprising acetic acid, hydrogen/carbon dioxide, formic acid, methanol, methylamines and carbon monoxide is used. In light of their similarity for substrate and extent of methane production, methanogenic microorganisms can be displayed into two main groups, one which is responsible for forming methane acetic acid or methanol (acetate using microorganisms, aceticlastic methanogens) and the other which is responsible for making methane from hydrogen and carbon dioxide (hydrogen using microorganisms, hydrogenotrophic methanogens) (De LemosChernicharo, 2007).

2.2.2.1. Acetislastic methanogens

It is clear that few number of pathogenic species own that power to make methane from acetate. However a limited number of pathogenic species are able to make methane from acetate, it is simply the microorganisms which overcome in anaerobic digestion, they are held accountable for nearly 60 to 70 of all methane production and it starts with the methyl group of the acetic acid (Zinder, 1993).

2.2.2.2 Hydrogenotrophic methanogens

Different from acetoclastic organism, in fact methanogenic species which are well known have the capacity to make methane from hydrogen and carbon dioxide, the genre which undergoes isolation process in anaerobic reactors are methnanobacterium, and methanobrevibacter, both the aceticlastic and the hydrogenotrophic methanogenic microorganisms are very essential in preserving the course of anaerobic digestion, since they are in charge responsible for the essential function of consuming the hydrogen made in the previous phases (De LemosChernicharo, 2007).

2.3. Analysis of Methane

Methane is counted as one of the byproducts of ruminant chemical transformation of organic compounds through enzymes by methanogenic archaea from H_2 and CO_2 taken from fermenting sources of carbon, more precisely sugar (Demeyer and Fievez, 2000; Fonty *et al*., 1995; Jouany *et al*., 1995; Wolin and Miller, 1983). Belching is a great threat to methane and it causes its increase, representing a loss of between 5 and 8% energy in the gross contained in the feeds consumed by the ruminant (Blaxter,1962). Methane is categorized as a major factor in producing greenhouse gas production from agriculture (Moss *et al*., 2000; Johnson and Johnson, 1995).

2.4. Methanogenesis in Rumen and Ruminants Methane Formation

The process in which methane is produced in rumen called methanogenesis; there are some certain factors that play a role in the emission of methane in the rumen. The factors are energy content of feed, quantity, and quality of feed, type, and amount of the carbohydrates undergo fermentation in rumen, .the rate of propionic acid production to the rate of acetic acid, the live weight and age of the animal, the differences in animals (Nkrumah *et al*., 2006; Moss *et al*., 2000).

Methane's contribution to the greenhouse gases, global warming and its production from the ruminant animals all made methane be the most paid attention gas, obviously methane is produced from the feed fermentation and it's responsible for hydrogen disposal by archea in the rumen, any reduction of cofactors produced in different fermentation processes should be oxidised by the reaction of dehydrogenation that collected H_2 in the rumen (Martin *et al*., 2010).

The methane is produced by Methanogens (archaea) in the rumen by using carbon dioxide and hydrogen and it is mainly made in the formation of acetate, and some cases format. Methane cannot be produced with propionate, but with acetate and butyrate (Wolin *et al*., 1997). Disposal of hydrogen is one of the reasons that help the continuity of dehydrogenase engaged in the process of regeneration which ends up in low hydrogen partial pressure and the increase of the efficiency of the rumen (Martin *et al*., 2010). Methanogenesis can be reduced only partly till methane is the only source in hydrogen elimination. Maintaining that capacity to give a high-grade form of a diet based on roughages which are very significant for ruminant; thus, continuing their major role as a world supplier of nutrient (Chalupa, 1977).

The ruminants produce most of the CH_4 in the rumen; even it is true that a limited amount of fermentation happens after ruminal, the amount of CH_4 cattle produces is 7 and 9 times bigger than the amount produced by sheep and goats nearly. A rumen is a place where 87%-90% of methane emission happens and a smaller amount like 13%-10% of methane emission happen in the large intestine. (Dini *et al*., 2012; Murray, *et al*., 1999) A big amount around 95% of the CH_4 made in rumen sent to the lungs where animals breathe out, and it is a reason for the loss of 99% of the CH_4 emission through mouth and nostrils and the rest lost through rectum which is 1% (Murray *et al*., 1976).

2.5. Energy Losses and Methane Production in Digestive Tract of Ruminants

A small amount of the energy taken in feed gets lost in the process of digestion as chemical compounds in faces, urine, and fermentation gases (Crutzen, 1986). The synthesis of methane in ruminant shows energy lost and this goes back to the lack of the carbon dioxide (CO_2) by the methanogenic archea. After the digestion of feed in the rumen, the loss of energy happens in the form of heat or methane (McDonald *et al*., 2002). Currently, many types of research tried to discover this loss of energy as methane emission.

2.6. Factors Affect Methane Production:

2.6.1. Dietary fat supplements

Dietary fat seems to have a promising nutritional option for reducing ruminal methanogenesis without downsizing ruminal pH instead of concentrates (Sejian *et al*., 2011). Currently, CH_4 has been reduced by Fat. If the energy supplementation in a ruminant's diet experiences transformation from carbohydrate to fat, then less fermentation and CH_4 production will happen. Supplementation of fats to ruminant forages may result in a decrease of CH_4 production by up to 80% *in vitro* and around 25% *in vivo* (Singh., 2010). Even, Supplementation dietary lipid lessens CH_4 production by minimizing ruminal fermentation of organic matter fermentation, fiber digestibility, consequently the methanogenic pathway and by the direct prevention of methanogens in the rumen through the hydrogenation of unsaturated fatty acids (Johnson and Johnson, 1995). A meta-analysis of methane production with lipid supplementation in lactating dairy bovines found out a 2.2% decrease in methane emission (Eugene *et al*., 2008).

There are many reasons that may have roles on methane reduction, for example, the ruminant species, eating regimen and the kind of lipid utilized (Martin *et al*., 2010). In fact, methane reduction more likely can be between 10-25% of methane even the fact that lowers ≥ 40% are possible with increased amounts of lipid supplementation in practical. It is Common that total fat should not surpass 6-7% of the dietary dry matter; otherwise dry matter intake may lower (Beauchemin *et al*., 2008).

2.6.2. Diet composition

Diet composition is capable of influencing rumen fermentation and downsizing methane production as a result of more propionate present or less degradation of feed used expended in the rumen (Yates *et al*., 2001). Methane production can be lowered by giving high crude protein/low-cell wall proportions, more especially by giving more focus (Shibata *et al*., 2010). It is very important the diet components be considered, especially kind of carbohydrate because it is very important for methane production as they are also able to impact the microbial population and ruminal pH. Therefore, developing forage quality may result in better animal production and also lessening methane emission, and it is measured by a decline in methane emission per unit of ruminant animal product (Henry *et al*., 2009).

Any increase of starch amount concentration in the forage results in reduction of methane yield per kg of DML, the nature of the relationship which exists between amount of concentrate in the forage and methane emission is curvilinear with a labeled reduction in methane emission when dietary starch ranges higher than 40% (Sauvant and Giger-Reverdin, 2007). The attempted that aimed at Supplementing plant fiber in the forage with starch ended up in a change of VFA production from acetate towards propionate, which caused to less hydrogen production (Singh, 2010). The decrease of methane emission experienced when the amount of that grain put in the diet increased (McAllister and Newbold, 2008).

Because that roughage has more cell wall and concentrate has more fermentable nutrients, for this reason, the replacement of roughage by concentrate in animal diets ended up in low acetate production but the high amount of propionate production (Kreuzer *et al*., 2008).

When advance happens in forage, an increase of methane emission happens too, despite the fact in a few trials no change in methane production has been reported (Pinares-Patiño *et al*., 2003). CH_4 production can be affected by one of the important factors which are Forage quality. There are differences among forages in terms of digestibility and time of harvest (Hristov *et al*., 2013). Forage is responsible for processing such as grinding, pelleting and chopping which may further lower methane production. Lifting passage rate of the processed forage probably lowered methane production (Jhonson and Ward., 1996).

Mix up of high sugar grasses into diets influenced CH_4 production (Kim *et al*., 2011). Mix up of high sugar grasses backs microbial growth in the rumen, by directing food N into microbial protein and redirecting H_2 to microbial cells instead of CH_4 production (Hristov *et al*., 2013).

2.6.3. Defaunation

Protozoa is listed one of the microorganisms in the rumen which has responsibility for 50% of fibrinolytic activity within the rumen (Coleman, 1986). The removal of protozoa from the rumen results in a decrease of CH_4 emission by various ways which meet in the lowered fiber digestion, the lowered methanogen populations that are symbiotically associated with protozoa and the reduced hydrogen production (Hegarty, 1999).

Defaunation is a disruptive process that dislocates the cross-feeding between ruminal protozoa and Archaea in the rumen (Eugene *et al*., 2004). Defaunation by dietary or chemical agents lowers enteric CH_4 production by about 20 to 50% despite the fact that it depends on the diet composition (Van-Nevel and Demeyer, 1996). The decline in CH_4 production by 26% per kg of dry matter intake (DMI) in protozoa-free lambs is linked to a slump in a number of methanogens in the total bacterial population of the whole ruminal content (McAllister and Newbold, 2008). It is well known that the secondary components named as saponins carry antiprotozoal activity which could change with the sort of saponin. Saponins are glycosides that have interaction with the cholesterol which already exists in the membrane of protozoa and causes cell lysis (Hess *et al*., 2003). Supplementation of spooning indirectly prevents methane production with no reverse effect on rumen function (Goel *et al*., 2008).

2.6.4. Ionophores

Ionophores are listed as lipid-soluble molecules. Some ionophores work as antibiotics or as growth-enhancing feed additives to help grow cattle (Moss *et al*., 2000). It is known that Supplementation of ionophores to ruminants diets influence CH_4 emission in two ways; Firstly supplementation of ionophoresusized feed conversion efficiency and this lowers CH_4 output per unit of product and, secondly Supplementation of ionophores reduced CH_4 emission per unit of dry matter used due to their impact on rumen fermentation (Lascan and Cárdenas, 2010).

Van-Nevel and Deymeyer, (1992) explain that ionophoric antibiotics, for instance, monensin decrease methane emission by mixed rumen microbes *in vitro*. This stumble in methanogenesis is not because of the direct effect of the ionophores on methanogenic bacteria but rather comes out from a change in bacterial population from gram-positive to gram-negative organisms with concurrent shift in the fermentation from acetate to propionate and limits the development of bacteria and protozoa (Moss *et al*., 2000; Newbold, *et al*., 1988).

Supplementations of monensin into diets at a dose of < 20 mg kg-1 do not have any effect on the methane emission (Beauchemin *et al*., 2009). In upper doses of monensin such as 24-35 mg kg^{-1} diet, resulted in methane production by 4-10 % (Odongo *et al*., 2007). These studies show that ionophores antibiotics, especially Monessen, can function as a reducer of methane production for the short-term decrease and also can develop the feed consumption (Giuburunca *et al*., 2014). Even though, with the increase of public concerns and health authorities concerns about the use of chemical or antibiotic feed additives in animal production, the consumption of ionospheres to lessen CH_4 production may not be the best believable option (Boadi *et al*., 2004).

2.7. Methods of Determination Methane Production in Ruminants

There are many ways applied in determination of CH_4 from individual ruminants, there are two ways to measure methane emissions which is *in vitro* and *in vivo* methods, *In vitro* technique usually methane inhibitors are tested and it's because of the uniform exploratory conditions which can be kept up in a lower demanding path than *in vivo* trials and they are not too expensive and not taking too much time (Bhatta *et al*., 2006). *In vivo* ways are regularly selected, as they will mark what actually occurs in the animal. It is clear that each technique owns own qualities and shortcomings and the methods need to be chosen depending on the inquiry (Johnson and Johnson, 1995).

2.7.1. Respiration chamber

A respiration chamber is the best way among the rest to measure CH_4 emission (Beauchemin and McGinn, 2005). The respiration chamber is a system which is more accurate for determining CH_4 production by ruminants (Johnson and Johnson, 1995). These chambers can be in closed or open circuit, for open circuit chambers the shift in a CH_4 gas of passway and way out air is resolved. In closed chambers, the CH_4 development is measured

(Beauchemin and McGinn, 2005). It is a rule when outflows are quantified from enteric and respiratory methodologies, animals are forced out from the feedlot (or eating browsing system) and held in digestion system stalls, and encased in calorimeters chambers (Summer *et al*., 2004).

The principle of the respiration chamber is to gather all blown breath from the animal and to assess gas, e.g. CH_4 concentration. The animal is put in a chamber for about 2-4 days with ventilation for intake and exhaust air, to maintain the air moving within the chamber, fresh air flow is also used to the recycling fan (McGinn *et al*., 2006). When the measurement takes a place in Methane accumulates in the chambers, and the CH_4 production is measured from the difference in the concentration of CH_4 in the samples taken before and after the measurement period and the volume of the chamber minus the estimated volume of the animal (Blaxter, 1965).

2.7.2. The sulfur hexafluoride (SF_6) tracer technique

The sulfur hexafluoride (SF_6) method is another method it works as an alternative method for measuring CH_4 emission *in vivo* (Johnson *et al*., 1994). According to the principle exists in the method when the emission rate of SF_6 from the rumen is known then methane emission can be measured (Hegarty, 2013). The SF_6 can be taken into many comparisons, because of an extremely low detection limit (Muñoz *et al*., 2012). It is essential for the SF_6 to mix with rumen air in the same way as methane. The SF_6 path includes the consumption of a SF_6permeation tube dosed into the reticulorumen (Lassey *et al*., 2001).

The CH_4/SF_6 ratio fitted for background concentrations is taken into use for daily CH_4 emission calculation (Vlaming *et al*., 2005). The SF_6 is sent into small permeation tubes. First of all the measurement of the diffusion rate of SF_6 from the permeation tubes takes a place through sinking them in a bath water at 39 °C and the daily weight loss is measured until it is stable. After diffusion rate of SF_6 is determined, the permeation tube including SF_6 is then moved into the rumen of an experimental animal and air sample collection may start (Martin *et al*., 2008).

The sampling apparatus contains a collection canister, a halter, and capillary tubing.

A representative of breath gas sample, which consisting of respired and eructated gas is gathered through a capillary tube situated at the nose of the animal, fitted to a halter, or at the back of the head and tied with the evacuated canister (approximately 2.5 L); the tubing carries out regulation of the sampling rate for 24 hours (Lassey *et al.*, 2001).

The concentration of SF_6 and CH_4 in the canister is explained by using gas chromatography, the estimation of methane emission is fulfilled from the release rate of SF_6 and concentration of SF_6 and CH_4 in the containers in excess of background level (Storm *et al.*, 2012). The SF_6 technique is used in daily CH_4 emissions estimation for individual animals, and in general measurements are conducted for 2-4 days, It is believed that the longer measurement period takes it provides a more accurate estimate of emissions than short periods, this method can be used for all ruminant animals such as sheep, deer, and cattle (Swainson *et al*., 2008).

The values taken into estimation for CH_4 emissions are lesser than that attained in respiration chamber since CH_4 emissions from the hindgut are not accounted for by the SF_6 technique. CH_4 emissions are measured similarly by taking into use the SF_6 technique which is 93 to 95 of those measured with respiration chambers (McGinn *et al*., 2006). It is discussed that the difference felt between SF_6 and respiration chamber results linked to high CH_4 production and release from the hindgut (Grainger *et al*., 2007).

Feeding a diet that doubles hindgut fermentation could build high uncertainty with SF_6 measurements (McGinn *et al*., 2004). When The SF_6 technique is used it is necessary that animals wear equipment to estimate CH_4 production, from a practical view it has two negative sides, firstly, this needs animals to be trained on wearing the equipment, and this requires labor and time. Secondly, while animals wearing the equipment it prevents animals from eating and drinking regularly (Pinares and Patino, 2008).

2.7.3. *In vitro* gas production technique

This technique is one of the common techniques being used to simulate ruminal fermentation of feed and feedstuffs for years (Rymer *et al*., 2005). The traditional IVGPTs have met modifications just to include measurement of methane production therefore in current days great interests find in greenhouse gas (GHG) emissions from agriculture (Navarro-Villa *et al*., 2011). Lately, the high interest in the professional utilization of roughage diets has ended up in a boost in the use of this technique due to the benefits of studying fermentation kinetics. Gas measurement is a good source which provides a good data relating to digestion kinetics of both soluble and insoluble fractions of feedstuffs (France *et al*., 2000).

The measuring process for the total amount of the gas produced during incubation takes a place and the methane content of the produced gas is figured out by using GC and other equipment's, this technique needs fresh rumen fluid, and it is widely obtained from fistulae ruminants animals such as cow and sheep etc. In addition, rumen fluid also exists in the rumen of intact animals or slaughtered animals and it can be obtained from. There is also Focus on the use of feces or cultures as substitute inoculants and it is being used and compared to fresh rumen fluid but only for total gas production and feed degradation (Mould *et al*., 2005). If the system and other laboratory constraints allow, so it will be possible to conduct up simultaneously to several hundred parallel incubations as well, this paves way for the occurrence of enough amount of repetition in experiments to back statistically important differences between treatments.

Outstanding variation among repeated measurements of methane production carried out with IVGPT is not well mentioned in the literature. A recent ring-test comparing *in vitro* gas production kinetics between laboratories reports a repeatability (variation between duplicates within a series expressed as coefficient of variation (CV) for asymptotic gas production ranging from 1.8% to 7.6% (Cornou *et al*., 2013).

The *in vitro* gas production technique is significantly used to demonstrate total gas production, as well as organic matter fermentation and methane emission. It is possible for quick checking of feedstuffs and additives for methane synthesis and total gas production. Reports show that good agreements exist between the SF_6 and *in vitro* gas production technique in terms of methane emission (Bhatta *et al*., 2008; Bhatta *et al*., 2006). There is also present a good agreement between respiration chamber and *in vitro* gas production technique (Patra and Saxena, 2010).

Despite the fact that *in vitro* gas production technique owns some disadvantages because it only replicates the ruminal fermentation of feed, not emissions and digestibility by the entire animal. In addition, under normal conditions, it does not embrace long-term adaptation of the ruminal microorganisms to the tested feedstuffs. Rumen fluid use from animals on a standard feed ration is common. During live animal experiments, it is well-known practice to have adaptation times to new feeds of at least 14 days. The animals' output is not called stable before that time. For the methane-producing population of ruminal microbes, there are signs that the adaptation period after shifting to a new feed is more than 30 days (Williams *et al*., 2009).

2.7.4. Rumen fermentation

In fact, ruminants take the responsibility of producing CH_4 from the agricultural sector and it is because of the biochemistry of plant substances by the rumen microbial community (Wright and Klieve, 2011). In general, because the rumen is an anaerobic and methanogenic ecology where the production of fermentation of forges CO_2 and H_2 take a place which is the wide electron acceptor and donor, straight, in the system (Morgavi *et al*., 2010).

Wright and Klieve (2011), mark that the rumen is a unique environment that inserts a bunch of species of bacteria, fungi, protozoa, methanogenic archaea, and viruses. In addition according to the estimations using molecular biology methods which only the bacteria have at least between 300 and 400 phenotypes (Morgavi *et al*.,2010). Similarly a complex culture of a microorganism that cooperates to have a significant role in the digestion of forage and gives energy and protein to the host in the form of volatile fatty acid and microbial protein (Wright and Klieve, 2011).

In relation to the rumen bacteria nature for H_2 Production, it can be put into three categories: bacteria which produce butyrate, ethanol, lactate without H_2, and propionate; bacteria that make acetate as well as H_2, and microorganisms for methanogenic. Bacteria which are under the first category forming those bacteria that have capacity theoretically to experience total fermentation with no need to get rid of H_2 and count on CH_4 formation; while in contrast, the second category seems more reliant on the third category for the fermentation efficiency thereby CH_4 formation (Bodas *et al*., 2012).

Borhan *et al*. (2012) argue that the organic remains via the fermentation of anaerobic are deformed biologically in the absence of O_2 to methane, carbon dioxide, nitrogen, and hydrogen sulfide. Methanogenic fermentation of organic substances occurs under precisely anaerobic and low redox potential situations where nitrate and nitrate concentrations are not high. Complex and simple carbohydrates go under the process of hydrolyzing in the reticulorumen and hindgut to 5- and 6-carbon sugars by microbial enzyme activity. Sugars which are under fermentation process to VFA through multiple-step pathways which produce limiting equivalents (i.e., metabolic hydrogen) (Knapp *et al*., 2014; Bodas *et al*., 2012; Boadi *et al*., 2004), which may be summarized in the following equations described by (Knapp *et al*., 2014; Yunta, 2010; Boadi *et al*., 2004):

Glucose → 2 pyruvates + 4H

(Carbohydrate metabolism) [1]

Pyruvate + H_2O → acetate + CO_2 + 2H [2]

Pyruvate + 4H → propionate + H_2O [3]

2 acetate + 4H → butyrate + $2H_2O$ [4]

In addition, they also pointed out that the process of conversion of 'the metabolic hydrogen occurs and it changes to H_2 by hydrogenate-expressing bacterial species, and the H_2 also changes to CH_4 by archaea in the combined reaction':

CO_2 + 8H → CH_4 + $2H_2O$ (methanogenesis) [5] Consequently, Boadi *et al*., (2004) argues that H_2 is not stored in the rumen, but instead it is used by using other bacteria, remarkably the methanogens which are present in the environment of the mixed microbial, despite that it is classified as one of the main final products of the protozoa fermentation, bacteria, and fungi. (Knapp *et al*.,2014) claims that accumulated H_2 terminates ruminal fermentation pathways through undesirable feedback mechanisms, and if not taken away by the methanogens, it will lower down the complete extent of carbohydrate degradation, the synthesis of microbial protein and the rate of microbial growth.

Dietary protein degradation and assimilation of microbial protein could direct to either a net production of hydrogen or net consumption, those Methanogens that use methyl groups of H_2 have been lately found in the rumen. This set and other unidentified organism have the capacity to illuminate the volatility in the effects of different rumen manipulations that have been noticed in the past, a great number of studies show that gastrointestinal tract methanogens normally vary according to feed and the geographical area of the host, methanobrevibacterphylo types are the main methanogens in ruminants. Almost 89% of enteric CH_4 made by ruminants belch, energy losses in ruminants according to the estimations is around 2 and 14 % of the total energy used by ruminants and this forms an economic loss to the farmers because feed energy is spent by CH_4 emission instead of milk, and meat production (Wright and Klieve, 2011).

3. MATERIAL AND METHODS

Feedstuffs used in the current experiment

In the current experiment maize grain, barley grain, alfalfa hay, and wheat straw were used. The feedstuffs were milled to pass through 1 mm screen size. The effect of oil on the gas and methane production was tested using oil from maize grain. Maize oil was dissolved in the same amount of ether extract for even distribution of oil on the surface of feedstuffs. The maize oil in the level of 6 % was added to maize grain, barley grain, alfalfa hay and wheat straw.

3.1. Dry Matter Analysis (AOAC, 1990)

Green forages consist of dry matter (DM) and moisture, after removing of water, the remaining part of forages is called dry matter, it includes essential nutrients required by the animal for maintenance, growth, pregnancy and lactation. Determination of feed dry matter provides a measure of the nutritive value of the feed. The productivity of the animal depends on the nutritive value of the feed that the animal utilized it. Two temperature are used for drying feed samples, for forages contain a high amount of volatile acids. 105 ^{0}C for 16-24 hours is used, and for forages contain a low amount of volatile acids 135 ^{0}C for 2 hours is used.

Figure 3.1. Desiccators

Equipment and tools:

1. Sensitive scale for measurement.
2. Aluminum foil container.
3. Drying Oven.
4. Desiccators

Procedure:

Empty containers were weighed and recorded (W1). Approximately 20 g of each sample was placed in the containers and weighed (W). The samples contained containers were put in the drying oven at 105^0C for 24 hours. After drying the containers were removed from the oven and put in the desiccators for cooling and reweighed (W2). Then the dry matter content of the samples was calculated as follows:

DM (%) = (W2-W1) / W * 100

W1 = Weight of containers.

W2 = Weight of containers with samples after 24 drying.

W = Weight of samples

3.2. Crude Ash Analysis (AOAC, 1990)

Ash includes inorganic part of the dry matter, which carbon does not donate in its composition, involving minerals and inorganic salts present in dry matter and remaining part of feed materials after burning at 550 °C for 5-6 h in the burning oven.

Organic matter includes a non-metallic portion of the dry matter which carbon donates in its chemical structure. However, it converts to water vapor and carbon oxides by burning at 550 ^{0}C and entirely lost, it involves carbohydrates, proteins, and fats.

Equipment and tools

1. Sensitive scale for measurement.

2. Crucible: A cup-shaped container made from any materials with high-temperature resistance such as (Porcelain).

3. Burning Oven: Usually electrically heated and modified automatically to the required level of temperature, works between 0-1200 ^{0}C.

4. Desiccators.

Procedure

Clean crucible containers were burnt at 550 ^{0}C in the burning oven then removed and cooled in desiccators and weighted (W1), around1-2 g from dried ground samples were put in the crucibles and weighted (W) then the sample contained crucibles were placed in the burning oven and burnt at 550^{0}C for 5-6hours, then the containers were removed and placed in the desiccators and cooled to the room temperature. After that, the containers with samples were weighted (W2). The ash content of the samples was determined as follows:

Ash% = (W2-W1/W) *100

W1= Weight of containers

W2= Weight of sample contained containers after 5-6 hours of burning

W= Weight of samples

3.3. NDF Analysis (Van Soest and Wine, 1967)

In order to make a solution for NDF analysis, 18.61 g of ethylenedinitrilotetraaceticacid disodium salt dehydrates (EDTA) and 6.81 g of Sodium tetraborate decahydrate were weighted and put in a big beaker. Then 300 ml of distilled (pure) water were added and the chemicals were dissolved after that 30 g of Sodium dodecyl sulfate and 10 ml of Triethyleneglycol were dissolved in another beaker. Then the two solutions were mixed together. 4.56 g of Di-Sodium phosphate were dissolved in a small beaker with distilled (pure) water by heating. After all the solutions were combined in one beaker, distilled water was added to the solution to become 1 lit. The pH of the prepared solution was set to be 6.9-7.1

The crucibles were dried in the drying oven at 105 ^{0}C for 2 hours and weighed (W1). Approximately 0.5-1 g (W) of dried ground samples were weighed into the crucibles then 100 ml of neutral detergent solution were added. After that, the crucibles with samples were boiled for 1 hour in NDF-boiling apparatus after heating. When boiling is finished the content was filtered. After filtering, the content was washed with distilled water and acetone twice. Then crucibles were removed and dried at 80^{0}C for 10-12 hours and cooled in the desiccators and reweighed (W2) then NDF content of samples was determined as follows:

NDF (g/kg DM) = (W2-W1) / W *100

W1= Weight of crucibles

W2= weight after drying crucibles with samples

W=Samples weight

3.4. ADF Analysis (Van Soest, 1963)

For ADF analysis 20 g of Cetyl-trimethylammonium bromide were dissolved in 1 lit 1N (Normality) of sulfuric acid. Crucibles were dried at 105^{0}C for 2 hours and weighed (W1). Then approximately 0.5-1gfrom the ground samples for each replication were put in the crucibles and weighted (W). 100 ml of ADF solution were added to each sample, and then the sample contained crucibles were boiled for 1 hour in ADF-boiling apparatus. After boiling was finished, the crucibles were filtered then the samples were washed with distilled water and acetone twice. After that, crucibles were removed and dried at 80^{0}C for 10-12 hours and after drying the crucibles with samples were cooled in the desiccators and reweighed by the sensitive scale and recorded (W2). Then ADF content of the samples was determined as follows:

ADF (g/kg DM) = (W2-W1) / W *100

W1=weight of crucibles

W2= weight after drying crucibles with samples

W= Samples weight.

Figure 3.2. NDF-Boiling Apparatus

3.5. Crude Protein Analysis (AOAC, 1990)

For the analysis of crude protein in feeds, the Kjeldahl method was used. The method involves three steps: First, samples were digested in sulfuric acid in the presence of a catalyst to convert nitrogen to ammonia, second, ammonia was distilled, and in the third step, ammonia was determined by titration with a standard solution (HCl).

Equipment and tools

1. Sensitive scale for measurement.
2. Kjeldahl flasks, 800 ml.
3. Kjeldahl digestion unit and distillation device.
4. Kjeldahl rack.
5. Conical flask 250-500 ml.
6. Digital Burette.

Chemicals

1. Concentrate sulfuric acid H_2SO_4 (%98and D=1.84g/cm^3).
2. Sodium hydroxide (titrant), concentration (C=0.1mol/l).

3. H_3BO_3 (Boric acid) solution (w/w); 40 g pure boric acid weighed in1000 ml beaker and add 800 ml of distilled water then slightly mixing then add 10 ml from metal red (0.1 g from metal red powder in 100 ml Ethanol) and 7 ml from Bromisel Green (0.1g from Bromisel Green into100 ml Ethanol)
4. Kjeldahl tablets composed of 10 g anhydrous potassium sulfate (K_2SO_4), 0.3 g copper sulfate ($CuSO_4$) anhydrous, 0.1 g pumice.
5. Hydrochloric acid (HCl). The Kjeldahl method was used

Working technique

Digestion:

For digestion step, approximately 0.5-1 g of sample was weighted on filter paper and recorded then the sample was put in Kjeldahl flasks. After that, 2 Kjeldahl tablets and 20 ml sulfuric acid were added to each Kjeldahl flask. Then the mixture was heated with burner device at about 370°C-400°C for 2-3 hours, then the burner was turned off and let flasks sit for 5-10 minutes. The flasks were removed from the burner and placed on the Kjeldahl rack. The water aspirator was turned off and the flasks were cooled for 30 minutes. Digestion step is to break down the bonds between the polypeptides together and convert them to simple chemicals such as water, carbon dioxide, and ammonia.

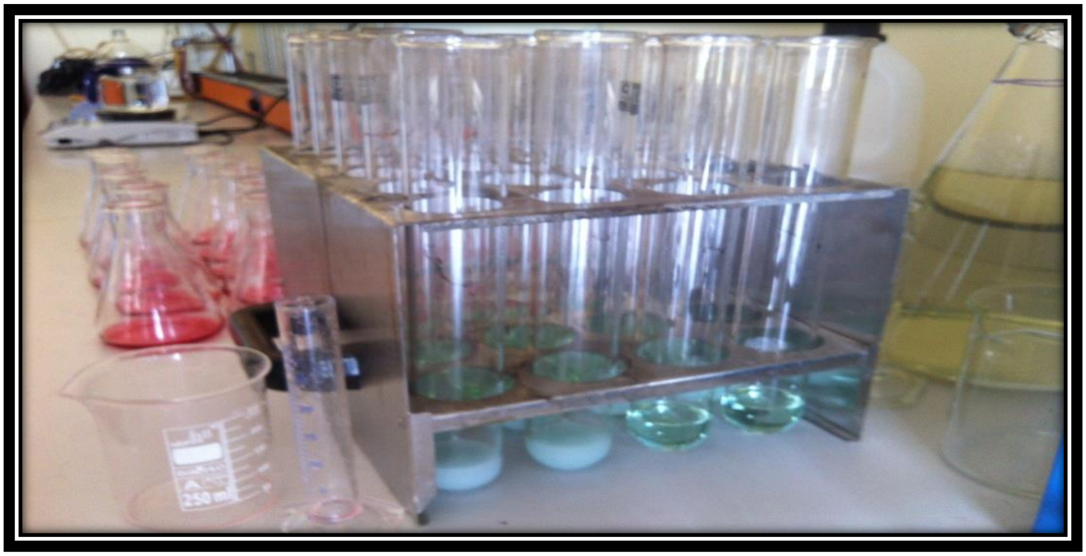


Figure 3.3. Kjeldahl Rack

$$\text{Organic-N}+H_2SO_4 \longrightarrow CO_2+H_2O+NH_4^{+}+SO_4$$
$$2NH_4^{+}+SO_4^{-2} \longrightarrow (NH_4)_2SO_4 \quad \text{(Digestion)}$$

Distillation:

For distillation step, 30 ml of boric acid were added to 500 ml Erlenmeyer flask for each sample after digestion. The flasks were put on the Kjeldahl unit for distillation; sodium hydroxide and pure water were connected with distillation unit and turned on the water for condensing. The samples were distilled for 3minutes; the color of boric acid was converted from red to blue color. After distillation was completed, the Erlenmeyer flasks were removed and prepared to be used for Titration. The aim of the titration was to separate ammonia from the digested mixture by raising the pH with sodium hydroxide, which changes ammonium ions into ammonia gas. Ammonia is collected through boiling and distillation of the gas.

$$(NH_4)_2SO_4 + 2NAOH \longrightarrow Na_2SO_4 + 2NH_4OH$$
$$NH_4OH \longleftrightarrow NH_3 + H_2O$$

$$NH_3 + H_3BO_3 \longrightarrow NH_4H_2BO_3 \quad \text{(Distillation)}$$

Figure 3.4. Kjeldahl Distillation Unit for Protein Determination

Titration

The liquid in the Erlenmeyer flask was titrated with 0.1 N HCl until the blue color of the liquid returned to a purplish pink color. The amount of HCl used on digital burette was recorded.

$$2NH_4H_2BO_3 + H_2SO_4 \longrightarrow 2H_3BO_3 + (NH_4)_2SO_4 \text{ (Titration)}$$

$$CP\ \% = \frac{N * me * 6.25 * (\text{Acid ml used in titration} - \text{Acid ml used in Blank})}{W} * 100$$

W = Weight of sample.

Me= Milligram equivalent weight of nitrogen which is 0.014.

N= Normality of the acid used in the titration of distillate (use 0.1N).

Acid= Usually HCl or H_2SO_4 are used for titration

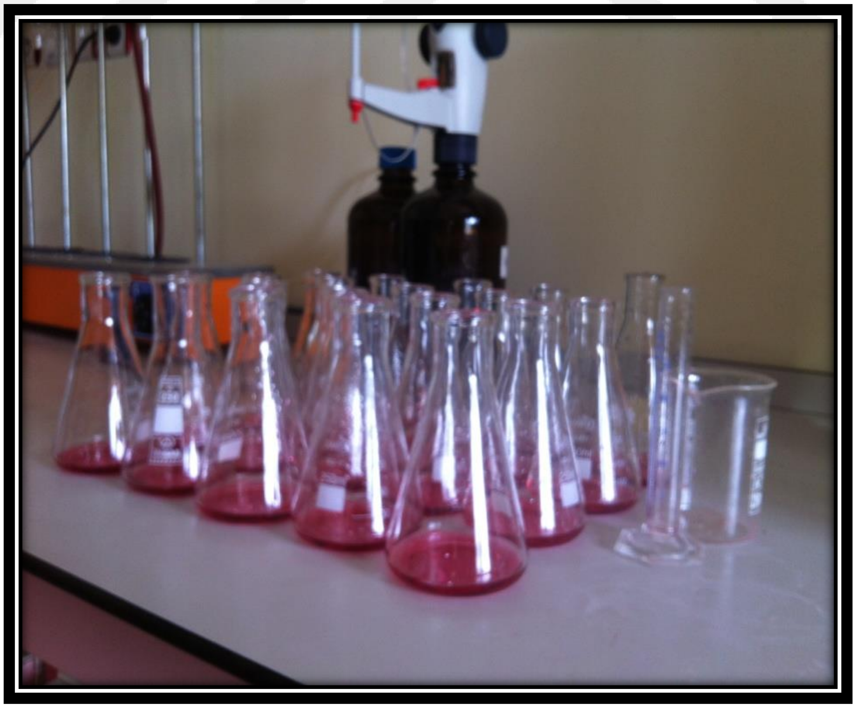


Figure 3.5. Soveril Beaker

3.6. Determination of Ether Extract (AOAC, 1995)

Equipment

1- Sensitive scale for measurement.
2- Porous cellulose thimbles.
3- Beakers (100 ml).
4- flasks
5- Soxhlet apparatus.
6- Drying Oven.
7- Desiccator.

Chemicals

Anhydrous ethyl ether.

Procedure: (AOAC, 2002)

Empty flasks were weighted and recorded (W1), two grams from dry silage sample were put into a separate extraction thimble and recorded (W).Then thimbles were placed on Soxhlet apparatus for extracting with ethyl ether for about 4 hours. After that, the flasks were put in drying oven at 65^0C for about 4 hours. Then the flasks were removed from the oven and placed in desiccators to be cooled. After cooling the flasks were reweighted and recorded (W2). Ether extract in flasks was calculated as follows:

Ether extract % = (W1-W2) / W * 100

W1= Weight of flask before extraction (gram)

W2= Weight of flask after fat extraction (gram)

W= Weight of silage sample (gram)

In this method, the dried, ground samples are placed in porous cellulose thimbles. The thimbles are placed in an extraction chamber, which is suspended in a flask containing the solvent and below a condenser. The flasks are heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. At the end of the extraction process, which lasts nearly four hours, the flasks containing the solvent and lipid are removed and placed in drying oven for few hours. After drying the mass of the remaining lipid can be measured.

Figure 3.6. Complete the Soxhlet Apparatus

3.7. *In Vitro* Gas Production Technique

3.7.1. Equipment and tools required

1. Elastic tube (about 5 cm in length per glass syringe; pushed onto the inlet) fixed

With a plastic clamp

2. Semi-automatic pipette or a dispenser (30 ml capacity)

3. Incubation (Water bath maintained at 39 °C)

4. Thermostat with circulation pump

5. Conical flask (800-1000 ml)

6. Beaker, 500-600 ml

7. Glass syringe

8. Plastic clips

9. Hotplate

10. Balance

Chemical

1. Sodium hydrogen phosphate (Na_2HO_4)
2. Potassium dihydrogen phosphate (KH_2PO_4)
3. Magnesium sulfate ($MgSO_4.7H_2O$)
4. Sodium hydrogen carbonate ($NaHCO_3$)
5. Ammonium hydrogen carbonate (NH_4HC_3)
6. Calcium chloride ($CaCl_2.2H_2O$)
7. Manganese chloride ($MnCl_2.4H_2O$)
8. Cobalt chloride ($CoCl_2.6H_2O$)
9. Iron chloride ($FeCl_3.6H_2O$)
10. Resazurin (0.1%)
11. Sodium hydroxide (1n NaOH)
12. Sodium sulfide ($Na_2S.H_2O$)

Procedure

Approximately 0.200 gram of samples was weighed carefully in 100 ml calibrated glass syringe with pistons oiled with Vaseline. All incubation was carried out in quadruplicate. Four syringes without samples were also incubated as blank. 30 ml of buffered rumen fluid was transferred into syringes containing samples.

Macromineral solution: 5.7 g Na_2HPO_4 + 6.2 g KH_2PO_4 + 0.6 g $MgSO_4.7H_2O$ were dissolved in 1 liter distilled water.

Micro mineral solution: 13.2 g $CaCl_2.2H_2O$ + 10.0 g $MnCl_2.4H_2O$ + 1.0 g

$CoCl_2.6H_2O$ + 0.8g $FeCl_2.6H_2O$ were dissolved in 100 ml distilled water.

Bicarbonate buffer: 35 g $NaHCO_3$ + 4 g (NH_4) HCO_3 were dissolved in 1 liter

Distilled water

Resazurin: 100 ml resazurin were dissolved in 100 ml distilled water.

Reducing solution: 2 ml 1.0 N (Normal) NaOH + 285 mg $Na_2S.7H_2O$ + 47.5 ml were dissolved in 100 ml distilled water.

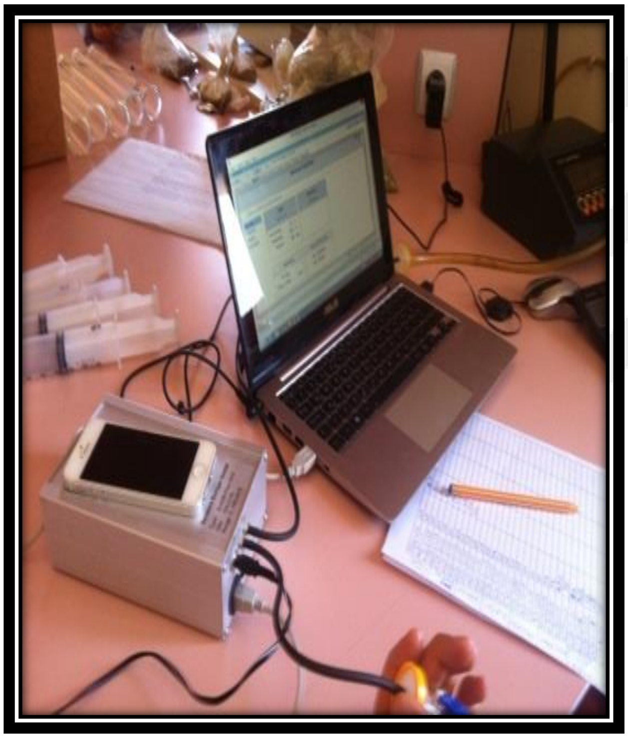
Figure 3.7. Water bath with holder for syringes

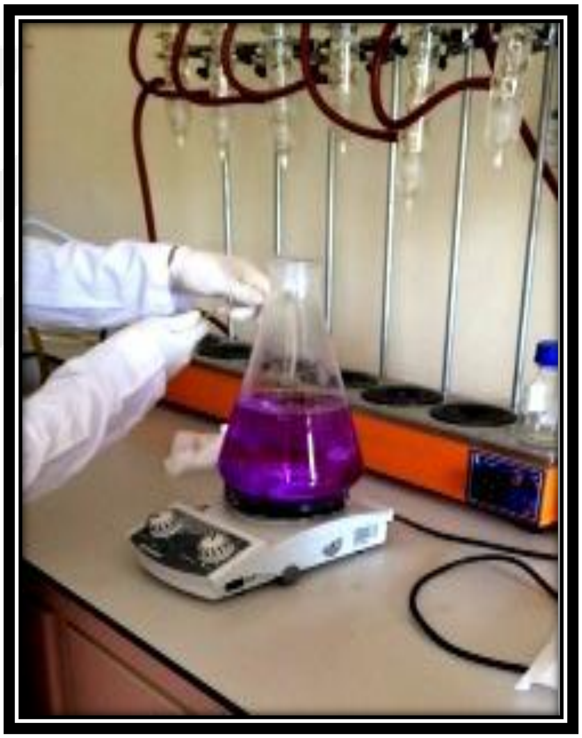
3.7.2. Rumen fluid

Rumen fluid was obtained from three fistulated Awassi sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%) with a free access to water and mineral block. Rumen fluid was collected before morning feeding and filtered through four layers of cheesecloth under flushing with CO_2. Rumen fluid was combined with the buffered mineral solution at 1:2 respectively. After that closing the clips on the silicon tube at the syringe tip, syringes were mildly shaken and tubes opened to remove gas by pushing the piston to a higher level to achieve completely all removal gas emission. The clip was closed carefully. The initial volume was recorded. Then syringes were placed in a water bath set at 39^0C. The gas measurement was carried out for 24 h incubation. Methane gas content of total gas produced at 24 h fermentation was measured using an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goet *et al*., 2008).

After measuring gas produced at 24 h incubation, gas samples were transferred into the inlet of the infrared methane analyzer using the plastic syringe. The infrared methane analyzer displays methane as a percent of total gas. Methane production (ml) was calculated as follows.

Methane production (ml) = Total gas production (ml) X Percentage of Methane (%)





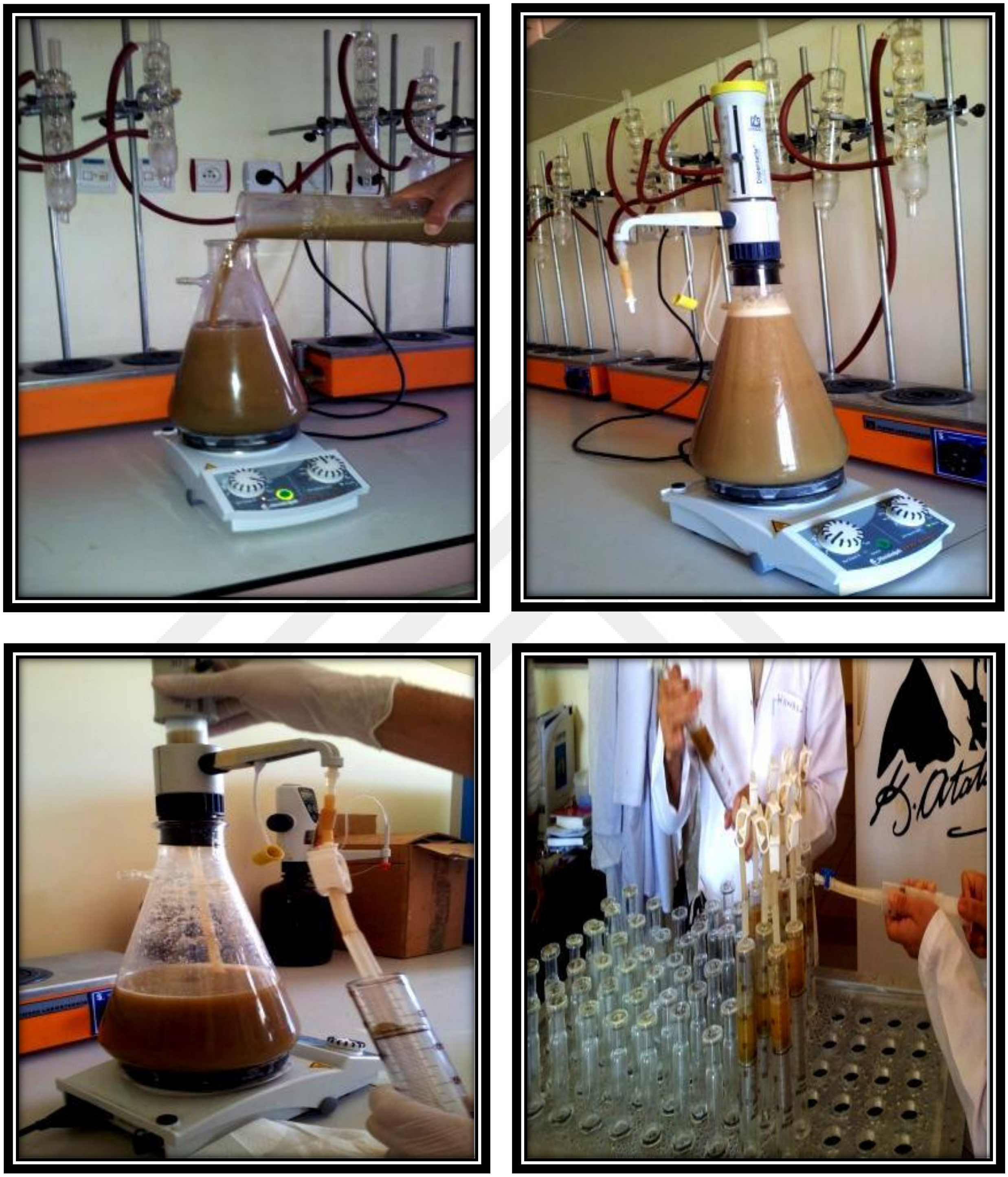


Figure 3.8. Process to Preparation the Rumen Fluid and Methane Measurement

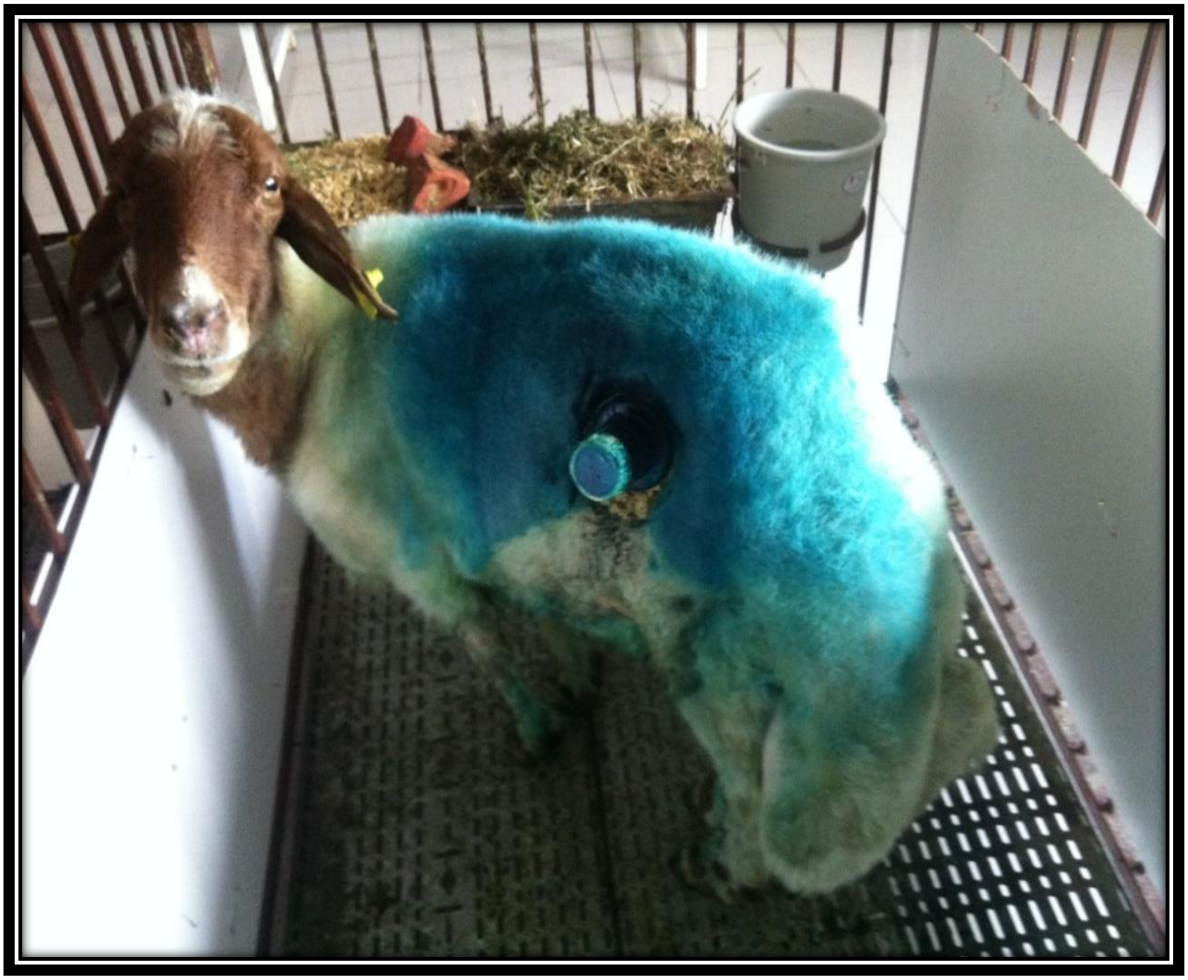


Figure 3.9. Fistula Awassi Sheep

3.8. Statistical Analysis

The effect of maize oil supplementation on gas production and methane production of barley, hay, alfalfa, and maize were tested using T-test. Standard errors (SE) of means were calculated from the residual mean square in the analysis of variance.

4. RESULTS AND DISCUSSION

4.1. Results

4.1.1. The chemical composition of some feedstuffs

The chemical composition of some feedstuffs is given in Table 1. According to the results of this study, there were significant differences ($P<0.001$) among feedstuffs used in the current study in terms of chemical composition. Dry matter (DM) ranged between 91.32% and 95.63%. Wheat straw had a dry matter content (95.63%) whereas maize grain had lowest dry matter content (91.32%).

There are also significant differences among feedstuffs As presented in Table 1, the highest percentage of CA was recorded again for wheat straw with about (9.83%) followed by alfalfa hay with (7.46%) then maize grain (6.53%) and finally the lowest percentage was recorded with barley grain with nearly (3.88%).

The acid detergent fiber (ADF) and NDF ranged from 5.17% to 55.39%, 17.77 to 88.03 respectively. The highest value of ADF was recorded for wheat straw and the lowest value was recorded from maize grain.

Crude Protein ranged from 3.22% to 16.52%. The wheat straw (WS) had the lowest (3.22%) while the alfalfa hay (AH) had the highest value (16.52%) then followed by barley grain and maize grain (10.85% & 8.59% respectively).

Ether Extract contents of feedstuffs ranged from 2.98% to 5.60%. The highest value of EE was recorded with maize Grain and the lowest value was recorded with wheat straw.

Table 4.1. The chemical composition of some feedstuffs used in ruminant nutrition

	FEEDSTUFFS					
Parameters	**MG**	**BG**	**AH**	**WS**	**SEM**	**Sig**
DM	91.32^d	92.15c	94.39^b	95.63^a	0.256	***
CA	6.53^b	3.88^c	7.46^b	9.83^a	0.531	***
ADF	5.17^d	7.12^c	40.83^b	55.39^a	0.586	***
NDF	17.77^d	27.50^c	53.10^b	88.03^a	0.971	***
CP	8.59^c	10.85^b	16.52^a	3.22^d	0.044	***
EE	5.60^a	3.92^b	3.11^b	2.98^b	0.564	***

$^{a\ bc}$ Column means with common superscripts do not differ (P>0.05); S.E.M: standard error means; DM: Dry matter(%), CA: Crude ash, CP: Crude protein (%), NDF: Neutral detergent fiber(%), ADF : Acid detergent fiber(%), EE: Ether extract(%), P<0.001

4.1.2. The effect of oil supplementation on gas production of some feedstuffs

As can be seen from Table 2, feedstuffs in terms of maize oil supplementation in order to reduce gas production showed significant differences (P<0.001). The differences between controls and all components were found to be significant especially from maize grain (MG) which was treated by 6% maize oil. This content was able to decrease gas production from 79.74% in control to 74.79% in treated oil contents.

It can be easily observed from Table 2 that there was a significant difference between control and barley grain (BG) treated by 6% maize oil which was able to reduce gas production from 75.24% in control to71.94% in oil-treated or after maize oil supplementation. Alfalfa hay or (AH) also showed significant differences between control and contents that was with 6% oil in terms of decreasing rate of gas production from 47.43% to 43.40% in oil treated.

Finally, there was a significant difference between control and wheat straw (WS) treated by 6% maize oil which decreased gas production from 24.74% in control to 21.33% in oil treated.

Table 4.2. The effect of oil supplementation on gas production of some feedstuffs used in ruminant nutrition

Feedstuffs	**Control**	**Oil treated**	**SEM**	**Sig**
MG	79.74^{a}	74.97^{b}	0.999	*
BG	75.24^{a}	71.94^{b}	0.2889	***
AH	47.43^{a}	43.40^{b}	0.865	*
WS	24.74^{a}	21.33^{b}	0.272	***

$^{a\ bc}$ Row means with common superscripts do not differ (P>0.05); S.E.M: standard error mean, MG: Maize Grain, BG: Barley grain, AH: Alfalfa hay, WS: Wheat straw

4.1.3. The effect of oil supplementation on methane production (ml) of the feedstuffs used in ruminant nutrition

In terms of the effect of adding oil to feedstuffs on the methane production measured by (milliliters ML), the feedstuffs divided between significant and non-significant differences compared to controls. While both maize grain (MG) and barely grain (BG) recorded no significant differences in methane production by (ml) when treated with maize oil 6%, the rest contents (AH & WS) had significant difference rates when treated with 6% oil in comparison to controls. The oil treated alfalfa hay (AH) was able to reduce methane production from (8.50) in controls to about (7.67) by (ml) and the wheat straw (WS) also was able to reduce it from (4.60) to (3.75) after (6%) oil supplementation (Table 3).

Table 4.3. The effect of oil supplementation on methane production (ml) of some feedstuffs used in ruminant nutrition

Feedstuffs	**Control**	**Oil treated**	**SEM**	**Sig.**
MG	13.22	12.36	0.350	**NS**
BG	12.08	11.09	0.275	**NS**
AH	8.50^{a}	7.67^{b}	0.112	***
WS	4.60^{a}	3.75^{b}	0.104	***

*$^{a\ bc}$ Row means with common superscripts do not differ (P>0.05); **S.E.M**: standard error mean, MG: Maize grain, BG: Barley grain, AH: Alfalfa hay, WS: Wheat straw*

4.1.4. The effect of oil supplementation on methane production (%) of the feedstuffs used in ruminant nutrition

Apart from the wheat straw (WS), the effect of oil supplementation on methane production by percentage (%) had no significant differences between controls and other feedstuffs when treated by maize oil 6%. However, the three feedstuffs including maize grain (MG), barely grain (BG) and alfalfa hay (AH) showed some reduction in the percentage of methane production but there were no significant differences with controls (Table 4).

As it clearly appears on Table 4, the only feedstuff that had a significant difference in terms of methane gas reduction after supplementing (6%) oil was the wheat straw (WS) which was able to decrease it from (18.61%) with control to about (17.60%).

Table 4.4. The effect of oil supplementation on methane production (%) of the feedstuffs used in Ruminant nutrition

Feedstuffs	Control	Oil treated	SEM	Sig
MG	16.58	16.47	0.273	**NS**
BG	16.05	15.42	0.397	**NS**
AH	17.94	17.68	0.141	**NS**
WS	18.61^{a}	17.60^{b}	0.256	*****

*$^{a\,bc}$ Row means with common superscripts do not differ (P>0.05); **S.E.M**: standard error mean, MG: Maize grain, BG: Barley grain, AH: Alfalfa hay, WS: Wheat straw*

4.2. Discussion

The chemical composition of feedstuffs differs from one component to another according to their original materials. This specificity of feedstuffs gives different degrees of variations in the effect of this material. This study conducted in order to study and measure the effect of adding maize oil on different foodstuffs like corn, barley, wheat straw and alfalfa hay in order to evaluate the ability of each component to reduce methane production. The effect of the chemical composition of feeds is known to cause variation in nutritive values as it represents the most important indices of these values of feed (Aghajanzadeh-Golshani *et al*., 2008; Maheri-Sis *et al*., 2008; Mupangwa *et al*., 1997).

Methane production is contributing to climate change as there is a great interest in reducing its emission from livestock production (Euge`ne *et al*., 2008). The amount of energy that has been lost through methane ($CH_4$) estimated to vary between 2-12% of total energy intake (Euge`ne *et al.*, 2008; Benchaar, 2008; Machmüller and Kreuzer, 2003).

Reducing methane production from rumen is the main issue that most of the scientists looking for and one of the reasons that reduce this gas production is the use of fat-enriched diets (Jalč *et al*. 2006).

Adding lipids to dairy cow diets has been used widely in order to raise the energy content of the diet and also to increase energy utilization for milk production (Euge`ne *et al.,* 2008; Chiquette *et al.,* 2004; Benchaar *et al.,* 2004). Lipids are known to reduce and inhibit methane production but the effects are variable according to adding oils, the chain length of fatty acids and the between lipids and the basal diet composition (Euge`ne *et al.,*2008). It was found that a significant reduction occurred in methane production when 10% coconut oil was added into a diet (Machmüller *et al.,* 2004; Kreuzer and Soliva, 2004).

However, some scientists showed that unsaturated fatty acids that originate from oil may disturb the rumen ecosystem which should be taken into consideration (Jalč *et al*., 2006).

As it can be seen in Table 1, there are significant differences (P<0.001) among the chemical composition of the feedstuffs that used in the study. Variations in the chemical composition of these materials might be due to several climatic and growing conditions like seasonality, soil characteristics, growing issues and stages, processing and measuring methods. The study revealed that the chemical composition of dry matter (DM) was ranged from 91.32% to 95.63%. The wheat straw (WS) had highest DM content in comparison to others, these values are considered high as dry matter represents most of the material base components in feed and is the indicator of the nutritional amounts that are giving usually to animals. Dry mater also has most of the nutrients parts in the feed which are required by animals for growing, lactating, pregnancy and general maintaining and body health (Holden, *et al*., 1999).

The results for crude ash (CA) from the study were significantly different (p<0.001) between multiple feedstuffs used. Wheat straw (WS) recorded the highest (9.83%) followed by (AH) (7.46%) and maize grain (MG) with (6.53%) and finally, the lowest rate was from bare grain with about (3.88%). Crude ash is known to be difficult or not digested by animals in spite of the fact that it is the inorganic (mineral matter) content of feed which remains essential to the animal health and production.

There were significant differences in the acid detergent fiber (ADF) content in the study and were ranged from 5.17% to 55.39%. From which the highest value was for wheat straw (WS) with about 56% while the maize grain (MZ) was the lowest with only 5.17% ADF range, however, the other grain compound (barely grain BG) also had a lower rate with about 7.12%. On the other hand, the nutrient detergent fiber (NDF) content also reported significant differences (P<0.001). The NDF from wheat straw (WS) recorded 88.03% which was by far the most (NDF) rate compared to the three other components. And like what was shown for (ADF), the maize grain (MG) also recorded the lowest with about (17.77%).

The crude protein (CP) content of feedstuffs was significantly different (p<0.001) and the recorded the highest value of CP with Alfalfa (16.52%) and the lowest value was recorded by using wheat straw (3.22%). The variety of CP levels and values from each feedstuff used in the study might be due to the diversity in some aspects that have a great effect during growth process (Rubenza, *et al.*, 2003).

The crude protein levels of feedstuffs were above the 7% CP requirement for ruminants, except wheat straw WS with about 3.22, % that should provide ammonia required by rumen microorganism to maintenance the ideal microbial growth. Additionally, these results for CP seem to meet the CP requirements for some animals especially in ewes for growth and lactation aspects which are ranging from 7-9% CP for maintains and 10-12% for lactation reported by (WL-Shatnawi and Mohawesh, 2000). A crude protein presents all the nitrogenous compounds that present in animal feed, and all contents are related directly to the ability to digest food, vitamins, and minerals (Granskopp and Bohner, 2001).

In some researchers, it was found that there was a marked increase the amount of microbial protein synthesis (MPS) after treating diets of sheep with lensed oil, while in earlier studies no changes were found when treated with cod liver oil (Ikwuegbu and Sutton, 1982). Mentioned reduction in the digestion of organic matter (OM) in the rumen which can be due to decreases in fiber digestion .This reduction in fiber digestion in the rumen seems to be the main factor that affects the amount of oil supplementation to ruminant diets.

Unlike other components, the ether extract (EE) content from wheat straw (WS) was significantly lower than those of other species. In the current study (WS) recorded only (2.98%) while the result from maize grain (MG) was the highest with about (8.59%). Feeds need a suitable amount of energy for body maintains and production which mainly comes from using fat derivatives. The high-fat rate in the feed the more energy is produced when added to the animal diet in order to increase calories and produce fatty acids.

The study measured the rate of and the effect of oil supplementation (maize oil) on several feedstuffs. The results showed significant differences between feedstuffs for both control and tested contents. Maize grain (MG) that treated with 6% oil decreased the rate of gas production with about 5% comparing to control, while barely grain (BG) which was treated with the same amount of oil decreased the rate of gas production with about 4% from 75.24% for control to about 71.94% alfalfa hay (AH) also recorded significant differences with nearly the same results for BG while wheat straw (WS) that treated with 6% maize reduced the rate of gas production significantly.

In terms of evaluating the effect of oil supplementation on gas production by milliliters for all four feedstuffs used in the study, there were no significant differences between controls and two of the contents. Both maize grain (MG) and barely grain (BG) showed no significant differences despite the fact that they were able to reduce gas production measuring by milliliters. Noticeably, the other two contents (AH & WS) showed significant differences in terms of reducing the production of gas after oil supplementation Alfalfa hay (AH) was able to decrease methane production (ml) from 8.5% to about 7.67% whereas wheat straw(WS) effect on gas production differed significantly from 4.6% to 3.75% when treated with 6% maize oil.

Same feedstuffs tested in the study for evaluating the effect of adding maize oil on the percentage (%) of methane production in ruminant nutrition. The percentage of methane production for three of the feedstuffs maize grain (MG), barely grain (BG) and alfalfa hay (AH) was not significant for all contents comparing to controls after treating with 6% of maize oil. The only content that its percentage of gas reduction was significantly different ($p<0.001$) with controls was the wheat straw (WS) decreasing it from 18.61% to 17.60%. In a study conducted by (Jalč *et al*., 2006), it was found that in a diet that supplements with oils there was a significant reduction in the rate of methane production with about 23-24%.

In the same study, there was no significant effect on methane production after treating mixed concentrate-forage diet with oil. Additionally, methane release was not significantly decreased by linseed oil (12%) and was raised by repressed and fish oils (26% & 0.5%) respectively (Jalč *et al*., 2006).

Many factors determine the rate and amount of gas production during the fermentation process, which mainly affected by the nature of fiber and the existence of secondary metabolites (Babayemi *et al*., 2004). It was found that *in vitro* gas production is related to the presence and the production of volatile fatty acids (VFA) after the process of substrate fermentation. Thus the rate of gas production is associated with the rate of substrate fermentation (Blummel and Ørskov 1993).

5. CONCLUSION

The current study will provide the valuable information about the effect of oil supplementation on the *in vitro* gas and methane production. The supplementation of oil has a significant effect on the gas and methane production. However, further, *in vivo* investigations are requested to determine the effect of the oil supplementation on animal performance.

REFERENCES

Aerts, R.J., Barry, T.N., McNabb, W.C., 1999. Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. Journal of Agriculture, Ecosystems, and Environment, 75 (1), 1-12.

AOAC. 1990. Official Methods of Analysis, Association of Official Analytical Chemists. 15th ed. Washington, USA.

AOAC. 1995. Official Methods of Analysis, Association of Official Analytical Chemists. Animal feed sample preparation (950.02), 16thed. Washington, USA.

AOAC. 2002. Official Methods of Analysis, Association of Official Analytical Chemists.17th ed. Arlington, Virginia, USA.

Babayemi, O.J., Demeyer, D., Fievez, V., 2004a. *In vitro* rumen fermentation of tropical browse seeds in relation to their content of secondary metabolites. Journal of Animal and Feed Sciences, 13 (1), 31-34.

Barry, T.N., 2013. The feeding value of forage brassica plants for grazing ruminant livestock. Animal Feed Science and Technology, 181 (1), 15-25.

Beauchemin, K.A., Eriksen, L., Nørgaard, P., Rode, L.M., 2008. Salivary secretion during meals in the lactating dairy cattle Journal of Dairy Science, 91, 2077-2081.

Beauchemin, K.A., McAllister, T.A., McGinn, SM., 2009. Dietary mitigation of enteric methane from cattle. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 4, 1-18.

Beauchemin, K.A., McGinn, S.M., 2005. Methane emissions from feedlot cattle fed barley or corn diets. Journal of Animal Science, 83 (3), 653-661.

Bhatta, R., Enishi, O., Takusari, N., Higuchi, K., Nonaka, I., Kurihara, M., 2008. Diet effects on methane production by goats and a comparison between measurement methodologies. Journal of Agricultural Science, 146 (6), 705-715.

Bhatta, R., Tajima, K., Takusari, N., Higuchi, K., Enishi, O., Kurihara, M., 2006. Comparison of sulfur hexafluoride tracer technique, rumen simulation technique and *in vitro* gas production techniques for methane production from ruminant feeds. In International Congress Series, 1293, 58-61

Blaxter, K.L., 1962. The energy metabolism of ruminants.

Blaxter, K.L., Clapperton, J.L., 1965. Prediction of the amount of methane produced by ruminants. British Journal of Nutrition, 19 (1), 511-522.

Blokhuis, H., Miele, M., Veissier, I., Jones, B. (Eds.), 2013. Improving farm animal welfare: science and society working together: the welfare quality approach. Wageningen Academic Pub.

Blu, M., 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.

Boadi, D., Benchaar, C., Chiquette, J., Massé, D., 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: an updated review. Canadian Journal of Animal Science, 84 (3), 319-335.

Bodas, R., Prieto, N., García-González, R., Andrés, S., Giráldez, F.J., López, S., 2012. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Animal Feed Science and Technology, 176 (1), 78-93.

Borhan, M.S., Mukhtar, S., Capareda, S., Rahman, S., 2012. Greenhouse gas emissions from housing and manure management systems at confined livestock operations. In: Waste Management – An Integrated Vision, Chapter: 12. Intech Open Access Publisher, 259-296p

Carulla, J.E., Kreuzer, M., Machmüller, A., Hess, H.D., 2005. Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Crop and Pasture Science, 56 (9), 961-970.

Chalupa, W., 1977. Manipulating rumen fermentation. Journal of Animal Science, 45 (3), 585-599.

Cheeke, P.R., Dierenfeld, E.S., 2010. Comparative animal nutrition and metabolism Centre for Agriculture and Biosciences International CABI

Coleman, G.S., 1986.The distribution of carboxymethylcellulose between fractions taken from the rumens of sheep containing no protozoa or one of five different protozoal populations. Journal of Agricultural Science, 106 (1), 121-127.

Cornou, C., Storm, I.M.D., Hindrichsen, I.K., Worgan, H., Bakewell, E., Ruiz, D.R.Y., Hansen, H.H., 2013.A ring test of a wireless in a *vitro gas* production system. Journal of Animal Production Science, 53 (6), 585-592.

Crampton, E.W., Donefer, E., Lloyd, L.E., 1960. A nutritive value index for forages. Journal of Animal Science, 19 (2), 538-544.

Crutzen, P.J., 1995. The role of methane in atmospheric chemistry and climate. In ruminant physiology: Digestion, Metabolism, Growth, and Reproduction (Editors: Von-Engelhardt, W., Leonhard-Marek, S., Breves, G., Giesecke, D.). 18th International Conference on Ruminant Physiology, Ferdinand Enke Verlag, Stuttgart, pp. 291-316.

Dale L.M., 2014. Using NIR spectrometry and NIR-HSI to study forage chemical and botanical composition (Ph.D. Dissertation in English). University of Liège, Gembloux Agro-Bio Tech, Gembloux, Belgium,

Demeyer, D., Fievez, V., 2000. March. Ruminants et environnement: la methanogenese. In annales de zootechnie (Vol. 49, No. 2, pp. 95-112).

Dini, Y., Gere, J., Briano, C., Manetti, M., Juliarena, P., Picasso, V., Astigarraga, L., 2012. Methane emission and milk production of dairy cows grazing pastures rich in legumes or rich in grasses in Uruguay. Journal of Animals, 2 (2), 288-300.

Dryden, G. M., 2008. Animal nutrition science. CABI, 203p

Eugène, M., Archimedes, H., Sauvant, D., 2004. Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake, and digestion in ruminants. Livestock Production Science, 85 (1), 81-97.

Eugène, M., Massé, D., Chiquette, J., Benchaar, C., 2008. Meta-analysis on the effects of lipid supplementation on methane production in lactating dairy cows. Canadian Journal of Animal Science, 88 (2), 331-337.

Fasae, O.A., Sowande, O.S., Popoola, A.A., 2010. Evaluation of selected leaves of trees and foliage of shrubs as fodder in ruminant production. Journal of Agricultural Science and Environment, 10 (2), 36-44.

Fonty, G., Joblin, K., Chavarot, M., Roux, R., Naylor, G., Michallon, F., 2007. Establishment and development of ruminal hydrogenotrophs in methanogen-free lambs. Applied and Environmental Microbiology, 73 (20), 6391-6403.

Fonty, G., Jouany, J.P., Forano, E., Gouet, P., 1995. L'écosystème microbien du réticulo-rumen. Nutrition des ruminant's domestiques. INRA, Paris, France, 299-347.

Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D.W., Van Dor-land, R., 2007. Changes in atmospheric constituents and in radiative forcing. Chapter 2. In climate change 2007. The Physical Science Basis.

France, J., Dijkstra, J., Dhanoa, M.S., Lopez, S., Bannink, A., 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: derivation of models and other mathematical considerations. British Journal of Nutrition, 83 (2), 143-150.

Fulkerson, W.J., Neal, J.S., Clark, C.F., Horadagoda, A., Nandra, K.S., Barchia, I., 2007. Nutritive value of forage species grown in the warm temperate climate of Australia for dairy cows: grasses and legumes. Livestock Science, 107 (2), 253-264.

Ganskopp, D., Bohnert, D., 2001. Nutritional dynamics of 7 northern great basin kinds of grass. Journal of Range Management, 640-647.

Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Tempio, G., 2013. Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO).

Getachew, G., Robinson, P.H., DePeters, E.J., Taylor, S.J., 2004. Relationships between chemical composition, dry matter degradation and in vitro gas production of several ruminant feeds. Animal Feed Science and Technology, 111 (1), 57-71.

Giuburunca, M., Criste, A., Cocan, D., Constantinescu, R., Răducu, C., & Mireşan, V., 2014. Methane Production in the Rumen and its Influence on Global Warming. ProEnvironment/ProMediu, 7 (18), 64-70

Goel, G., Makkar, H.P.S., Becker, K. 2008. Effect of Sesbania sesban and Carduus pycno-cephalus leaves Fenugreek (Trigonella foenum-graecum L) seeds and their extract on the partitioning of nutrients from roughage and concentrate-based feeds to methane. Animal Feed Science Technology, 147 (1-3): 72-89, 2008

Goodland, R., Anhang, J., 2009. Livestock and climate change: what if the key actors in climate change are... cows, pigs, and chickens? World Watch, 10-19.

Grainger, C., Clarke, T., McGinn, S. M., Auldist, M.J., Beauchemin, K. A., Hannah, M.C., Eckard, R. J., 2007. Methane emissions from dairy cows measured using the sulfur hexafluoride (SF6) tracer and chamber techniques. Journal of Dairy Science, 90 (6), 2755-2766.

Hegarty, R.S., 1999. Reducing rumen methane emissions through the elimination of rumen protozoa. Crop and Pasture Science, 50 (8), 1321-1328.

Hegarty, R.S., 2013. Applicability of short-term emission measurements for on-farm quantification of enteric methane. Animal, 7 (s2), 401-408.

Henry, B., Eckard, R., 2009. Greenhouse gas emissions in livestock production systems. Tropical Grasslands, 43 (4), 232.

Herrero, M., Thornton, P. K., Kruska, R., Reid, R. S., 2008. Systems dynamics and the spatial distribution of methane emissions from African domestic ruminants to 2030. Agriculture, Ecosystems Environment, 126 (1), 122-137

Hess, H.D., Kreuzer, M., Dıaz, T. E., Lascano, C.E., Carulla, J.E., Soliva, C.R., Machmüller, A., 2003. Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated rumen fluid. Animal Feed Science and Technology, 109 (1), 79-94.

Hook, S.E., Wright, A.D.G., McBride, B.W., 2010. Methanogens: methane producers of the rumen and mitigation strategies. Archaea, (2010), 11.

Hristov, A.N., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., Oosting, S.J., 2013. Mitigation of greenhouse gas emissions in livestock production a review of technical options for non-CO_2 emissions. Animal Production and Health paper, 177 (177), 231.

Ibrahim, H., Olaloku, E., 2000. Improving cattle for milk, meat, and traction (vol. 4). ILRI (aka ILCA and ILRAD). Institute of Biology and Animal Nutrition, vol. 8.

Ikwuegbu, O.A., Sutton, J.D., 1982. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. British Journal of Nutrition, 48(2), pp.365-375.

In Arizona Ranchers' Management Guide, Arizona Cooperative Extensio0n, 84p.

Jalč, D., Potkański, A., Szumacher-Strabel, M., Kowalczyk, J., Cieślak, A., 2006. The effect of a forage diet and different fat sources on rumen fermentation *in vitro*. Journal Animal Feed Science, 15, pp.129-134.

Jaradat, A.A., 2003. Agriculture in Iraq: Resources, potentials, constraints, research needs, and priorities. Food, Agriculture, and Environment, 1 (2), 160-166

Jayanegara, A., Sofyan, A., H.P.S. Makkar., K. Becker., 2009. Gas production kinetics, organic matter digestibility and methane production *in vitro* in hay and straw diets supplemented by tannin-containing forages, 32:120-129.

Johnson, K., Huyler, M., Westberg, H., Lamb, B., Zimmerman, P., 1994. Measurement of methane emissions from ruminant livestock using a sulfur hexafluoride tracer technique. Journal of Environmental Science and Technology, 28 (2), 359-362.

Johnson, K.A., Johnson, D.E. 1995. Methane emissions from cattle. Journal of Animal Science 73, 2483-2492.

Joseph, S.D., Camps-Arbestain, M., Lin, Y., Munroe, P., Chia, C.H., Hook, J., Van Zwieten, L., Kimber, S., Cowie, A., Singh, B.P. and Lehmann, J., 2010. An investigation into the reactions of biochar in soil. Soil Research, 48(7), pp.501-515.

Karabulut, A., Canbolat, O., Ozkan, C.O., Kamalak, A., 2007b. Determination of nutritive value of citrus tree leaves for sheep using in vitro gas production technique. Asian Australasian Journal of Animal ScienceS, 20 (4), 529.

Kim, E.J., Newbold, C.J. Scollan, N.D., 2011. Effect of water-soluble carbohydrate in fresh forage on growth and methane production by growing lambs. Advances in Animal Biosciences, 2, 270.

Knapp, J.R., Laur, G.L., Vadas, P.A., Weiss, W.P., Tricarico, J.M., 2014. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. Journal of Dairy Science, 97 (6), 3231-3261

Koneswaran, G., Nierenberg, D., 2008. Global farm animal production and global warming: impacting and mitigating climate change. Environmental Health Perspectives, 116 (5), 578

Kreuzer, M., Soliva, C.R., 2008. Nutrition: the key to methane mitigation in ruminants. In Proceedings of the Society of Nutrition Physiology, (17), pp. 168-171.

Kumara Mahipala, M.B.P., Krebs, G.L., McCafferty, P., Gunaratne, L.H.P., 2009. Chemical composition, biological effects of tannin and *in vitro* nutritive value of selected browse species grown in the West Australian Mediterranean environment. Animal feed science and technology, 153 (3), 2

Kurihara, M., Magner, T., Hunter, R.A., McCrabb, G.J., 1999. Methane production and energy partition of cattle in the tropics. British Journal of nutrition, 81(03), 227-234.

Lascano, C.E., Cárdenas, E., 2010. Alternatives for methane emission mitigation in livestock systems. Revista Brasileira de Zootecnia, 39, 175-182.

Lastly, K.R., Walker, C.F., McMillan, A.M.S., Ulyatt, M.J., 2001. On the performance of SF6 permeation tubes used in determining methane emissions from grazing livestock. Chemosphere-Global Change Science, 3 (4), 367-376.

Maheri-Sis, N., Chamani, M., Ali-Asghar, S., Mirza-Aghazadeh, A., Aghajanzadeh-Golshani, A., 2008. Nutritional evaluation of kabuli and desi type chickpeas (Cicer arietinum L.) for ruminants using *in vitro* gas production technique. African Journal of Biotechnology, 7 (16), 2946-2951.

Makkar, H.P., 2005. *In vitro* gas methods for evaluation of feeds containing phytochemicals. Animal Feed Science and Technology, 123, 291-302.

Martin, C., Rouel, J., Jouany, J.P., Doreau, M., Chilliard, Y., 2008. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. Journal of Animal Science, 86 (10), 2642-2650.

McAllister, T.A., Newbold, C.J., 2008. Redirecting rumen fermentation to reduce methanogenesis. Australian Journal of Experimental Agriculture, 48 (2), 7-13.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., Morgan, C. A., Sinclair, L. A., Wilkinson, R. G., 2010. Animal nutrition. 7th edn. San Francisco, Calif.; London: Benjamin Microbial Ecosystem 467-491.

McDonald, P., Edwards, R.A., Greenhalgh, J.F. D., Morgan, C.A., 2002. Animal nutrition. Four edition. Pearson education limited Harlow Essex, UK, pp. 515-531.

McGinn, S. M., Beauchemin, K.A., Iwaasa, A.D., McAllister, T.A., 2006. Assessment of the sulfur hexafluoride (SF6) tracer technique for measuring enteric methane emissions from cattle. Journal of Environmental Quality, 35 (5), 1686-1691.

McGinn, S.M., Beauchemin, K.A., Coates, T., Colombatto, D., 2004. Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. Journal of Animal Science, 82 (11), 3346-3356.

Mekuriaw, Y., 2013. Reducing livestock-related methane emissions using rumen biotechnology: Overview of possibilities for Ethiopia. Journal of Agriculture Science Research, 1 (1), 1-8.

Menke, K.H., Raab, L., Zalewski, A., Steingass, H., Fritz, D., Schneider, W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. The Journal of Agricultural Science, 93(01), 217-222.

Miller, R. L., Cakmur, R. V., Perlwitz, J., Geogdzhayev, I. V., Ginoux, P., Koch, D., Tegen, I., 2006. Mineral dust aerosols in the NASA Goddard Institute for Space Sciences Modele atmospheric general circulation model. Journal of Geophysical Research: Atmospheres (1984–2012), 111(D6).

Mills, J.A.N., Dijkstra, J., Bannink, A., Cammell, S.B., Kebreab, E., France, J., 2001. A mechanistic model of whole-tract digestion and methanogenesis in the lactating 61

Morgavi, D.P., Forano, E., Martin, C., Newbold, C.J., 2010. Microbial ecosystem and methanogenesis in ruminants. Animal, 4 (07), 1024-1036.

Moss, A.R., Jouany, J.P., Newbold, J., 2000. Methane production by ruminants: its contribution to global warming. In Annales de Zootechnie, 49 (3), 231-253.

Mould, F.L., Kliem, K.E., Morgan, R., Mauricio, R.M., 2005. In vitro microbial inoculum: A review of its function and properties. Journal of Animal Feed Science and Technology, 123, 31-50.

Muñoz, C., Yan, T., Wills, D. A., Murray, S., Gordon, A. W., 2012. Comparison of the sulfur hexafluoride tracer and respiration chamber techniques for estimating methane emissions and correction for rectum methane output from dairy cows. Journal of Dairy Science, 95 (6), 3139-3148.

Mupangwa, J.F., Ngongoni, N.T., Topps, J.H., Ndlovu, P., 1997. Chemical composition and dry matter degradability profiles of forage legumes Cassia rotundifolia cv. Wynn, Lablab purpureus cv. Highworth and Macroptilium atropurpureum cv. Siratro at 8 weeks of growth (pre-anthesis). Journal of Animal Feed Science and Technology, 69 (1), 167-178.

Murray, P.J., Moss, A., Lockyer, D.R., Jarvis, S.C., 1999. A comparison of systems for measuring methane emissions from sheep. The Journal of Agricultural Science, 133 (4), 439-444.

Murray, R.M., Bryant, A.M., Leng, R.A., 1976. Rates of production of methane in the rumen and large intestine of sheep. British Journal of Nutrition, 36 (1), 1-14.

Mwenya, B., Santoso, B., Sar, C., Gamo, Y., Kobayashi, T., Arai, I., Takahashi, J., 2004. Effects of including β1–4 galacto-oligosaccharides, lactic acid bacteria or yeast culture on methanogenesis as well as energy and nitrogen metabolism in sheep. Animal Feed Science and Technology, 115(3), pp.313-326.

Myers, R.H., Montgomery, D.C Anderson-Cook, C.M., 2016. Response surface methodology: process and product optimization using designed experiments. John Wiley & Sons.

Navarro-Villa, A., O'Brien, M., López, S., Boland, T. M., O'Reilly, P., 2011. Modifications of a gas production technique for assessing *in vitro* rumen methane production from feedstuffs. Journal of Animal Feed Science and Technology, 166, 163-174.

Newbold, C.J., Wallace, R.J., Watt, N.D., Richardson, A.J., 1988. Effect of the novel ionophore tetronasin on ruminal microorganisms. Journal of Applied and Environmental Microbiology, 54 (2), 544-547.

Nkrumah, J.D., Okine, E. K., Mathison, G.W., Schmid, K.Li, C., Basarab, J.A., Moore, S.S., 2006. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. Journal of Animal Science, 84 (1), 145-153.

Norton, B.W., Gutteridge, R.C., Shelton, H.M., 1994. The nutritive value of tree legumes. Forage tree legumes in tropical agriculture., 177-191.

Odongo, N. E., Bagg, R., Vessie, G., Dick, P., Or-Rashid, M. M., Hook, S. E., McBride, B. W., 2007. Long-term effects of feeding monensin on methane production in lactating dairy cows. Journal of Dairy Science, 90 (4), 1781-1788.

Oelberg, K., 1956. Factors affecting the nutritive value of range forage. Journal of Range Management, 220-225.

Olteanu M., Dihoru A., Dragomir C., Pop S., Voicu I., 2005. Comparative studies on the *in vivo* and *in vitro* assessment of bulk forage digestibility. Archiva Zootechnica vol. 868

Osuga, I.M., Abdulrazak, S.A., Nishino, N., Ichinohe, T., Fujihara, T., 2006. Potential nutritive value of selected browse species from Kenya using *in vitro* gas production technique and polyethylene glycol. Livestock Research for Rural Development, 18, 12.

Pal, K., Patra, A.K., Sahoo, A., Mandal, G.P., 2014. Effect of nitrate and fumarate in *Prosopis cineraria* and *Ailanthus excels* leaves-based diets on methane production and rumen fermentation. Small Ruminant Research, 121 (2), 168-174.

Patra, A.K., Saxena, J., 2010. A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. Phytochemistry, 71(11), 1198-1222.

Pendry, J.B., Holden, A.J., Robbins, D.J., Stewart, W.J., 1999. Magnetism from conductors and enhanced nonlinear phenomena. IEEE transactions on microwave theory and techniques, 47(11), pp.2075-2084.

Pinares-Patiño, C S., Clark, H., 2008. Reliability of the sulfur hexafluoride tracer technique for methane emission measurement from individual animals: an overview. Journal of Animal Production Science, 48 (2), 223-229.

Pinares-Patiño, C.S., Baumont, R., Martin, C., 2003. Methane emissions by Charolais cows grazing a monospecific pasture of Timothy at four stages of maturity. Canadian Journal of Animal Science, 83 (4), 769-777.

Ramin, M., Huhtanen, P., 2013. Development of equations for predicting methane emissions from ruminants. Journal of Dairy Science, 96 (4), 2476-2493.

Ruyle, G. (1993). Nutritional value of range forage for livestock. Range Cattle Nutrition.

Rymer, C., Huntington, J.A., Williams, B.A., Givens, D.I., 2005. *In vitro* cumulative gas production techniques: History, methodological considerations, and challenges. Journal of Animal Feed Science and Technology, 123, 9-30.

Saha, U.K., Simon, 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. University of Georgia Cooperative Extension Bulletin, 1367, 1-19.

Şahin, M., Üçkardeş, F., Canbolat, O., Kamalak, A., Atalay, A., 2011. Estimation of partial gas production times of some feedstuffs used in ruminant nutrition. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 17 (5), 731-734.

Sanon, H.O., Kabore-Zoungrana, C., Ledin, I., 2008. Nutritive value and voluntary feed intake by goats of three browse fodder species in the Sahelian zone of West Africa. Animal Feed Science and Technology, 144 (1), 97-110.

Sauvant, D., Giger-Reverdin, S., 2007. Empirical modeling by meta-analysis of digestive interactions and CH_4 production in ruminants. Publication-European Association for Animal Production, 124, 561.

Schroeder, J.W., 2009. Forage nutrition for ruminants. Fargo, ND: NDSU Extension Service.

Sejian, V., Lakritz, J., Ezeji, T., Lal, R., 2011. Forage and flax seed impact on enteric methane emissions in dairy cows. Research Journal of Veterinary Sciences, 4 (1), 1-8

Shibata, M., Terada, F., 2010. Factors affecting methane production and mitigation in ruminants. Animal Science Journal, 81 (1), 2-10.

Singh, B., 2010. Some nutritional strategies for mitigation of methane emissions. In: International Conference on ―Physiological capacity building in livestock under changing climate scenario. Physiology and Climatology Division, Indian Veterinary Research Institute, Izatnagar, 243122, Uttar Pradesh, India, pp 142-158.

Skenjana, A., 2011. The potential nutritive value of waste products from the sub-tropical fruit processing industry as livestock feed (Doctoral dissertation, University of Pretoria).

Sommer, S.G., McGinn, S.M., Hao, X., Larney, F.J., 2004. Techniques for measuring gas emissions from a composting stockpile of cattle manure. Atmospheric Environment, 38 (28), 4643-4652.

Staley, B.F., 2009. Environmental spatial factors affecting microbial ecology and metabolic activity during the initiation of methanogenesis in solid waste.

Steeg, J., Notenbaert, A., Herrero, M., Thornton, P., 2009. Livestock and climate change. Rural 21, 43 (6), 19-21

Storm, I.M., Hellwing, A.L.F., Nielsen, N.I., Madsen, J., 2012. Methods for measuring and estimating methane emissions from ruminants. Animals, 2(2), 160-183.

Swainson, N.M., Hoskin, S.O. Clark, H. Pinares-Patino, C.S., Brookes, I.M., 2008. Comparative methane emissions from cattle, red deer, and sheep. Proceedings of the New Zealand Society of Animal Production, 68, 59-62

Tufarelli, V., Cazzato, E., Ficco, A., Laudadio, V., 2010. Evaluation of Chemical Composition and in vitro digestibility of appennine pasture plants using yak (*Bos grunniens*) rumen fluid or fecal extract as an inoculum source. Asian-Australian Journal of Animal Science, 23, 1587-1593.

Van Nevel, C.J., Demeyer, D.I., 1992. Influence of antibiotics and a deaminase inhibitor on volatile fatty acids and methane production from detergent washed hay and soluble starch by rumen microbes in vitro. Journal of Animal Feed Science and Technology, 37 (1), 21-31.

Van Nevel, C.J., Demeyer, D.I., 1996. Control of rumen methanogenesis. Environmental Monitoring and Assessment, 42 (1-2), 73-97.

Van Soest, P.J. 1963. Use of detergents in the analysis of fibrous feeds. ii. a rapid method for determination of fiber and lignin. Journal of Association of Official Analytical chemists and Agricultural Chemicals.,46: 829-835.

Van Soest, P.J., Wine, R.H. 1967. Use of detergents in the analysis of fiber feeds.IV. Determination of Plant Cell-Wall Constituents. Association of Official Analytical Chemists. J.50, 50–55.

Vlaming, J.B., Clark, H. Villalobos, N.L., 2005. The effect of SF6 release rate, animal species, and feeding conditions on estimates of methane emissions from ruminants. New Zealand Society of Animal Production, 65, 4-8.

Williams, Y.J., Popovski, S., Rea, S.M., Skillman, L.C., Toovey, A.F., Northwood, K.S., Wright, A.D.G., 2009. A vaccine against rumen methanogens can alter the composition of archaeal populations. Applied and Environmental Microbiology, 75 (7), 1860-1866.

Wolin, M.J., Miller, T.L., Stewart, C.S., 1997. Microbe-Microbe interactions. In the rumen microbial ecosystem, Springer Netherlands, pp. 467-491.

Woods, V.B., Moloney, A.P., O’Mara, F.P., 2003b. The nutritive value of concentrate feedstuffs for ruminant animals: Part II: *In situ* ruminal degradability of crude protein. Animal Feed Science and Technology, 110 (1), 131-143.

Woods, V.B., O’Mara, F.P., Moloney, A.P., 2003a. The nutritive value of concentrate feedstuffs for ruminant animals: Part I: *In situ* ruminal degradability of dry matter and organic matter. Animal Feed Science and Technology, 110 (1), 111-130.

Wright, A.D.G., Klieve, A.V., 2011. Does the complexity of the rumen microbial ecology preclude methane mitigation? Animal Feed Science and Technology, 166, 248-253.

Wuebbles, D.J., and K. Hayhoe. 2002. Atmospheric methane and global change. Earth-Science. Review. 117-210.

Yates, C.M., Mills, J.A.N., Kebreab, E., Crompton, L.A., France, J., 2001. An integrated modeling approach to providing cost-effective means of reducing methane emissions from dairy cows. Journal of Agriculture Sciences, 137, 120-121.

Zinder, S.H. 1993. Physiological ecology of methanogens. In Methanogenesis (pp. 128-206). Springer US.

CURRICULUM VITAE

Personal Identity

Name, Surname: Dlshad Namiq Khurshid ZANGANA

Nationality: Iraqi

Date of birth: July 13, 1983

Place of birth: Sulaymaniyah, Iraq

Marital Status: Single

Telephone: (+90) 5367811880 - (+964) 7701234582

E-mail : dlshadnamiq2 @gmail.com

Education

Date	Department	University	Degree
2017	Animal Science.	KSU	M. Sc.
2010	Animal Production	University of Slemani	B.Sc.
2006	----------------------	Shahid Aram High School	High School Diploma

Work Experience Year	**Place**	**Position**
2011-2017	University of Garmian - Faculty of Agriculture	Asst. Teacher

Language Skills

Kurdish, English, Turkish, Arabic

Published Article

Kaya, E., Atalay, A.I., Kurt, O., **Zangana, D.N.**, Salih, M.J.M., Kamalak, A., 2016. Determination of effects of microwave irradiation on fermentation of oak nut (*Quercus coccifera*) using Hohenheim gas production technique. KSU Journal of Natural Sciences, 19(3), 268-271.