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KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE

ESTIMATION OF NUTRITIVE VALUES, METHANE EMISSION AND TANNINS OF SOME TREE LEAVES AROUND ERBIL CITY IN IRAQ

KAEFEE GIBBAR HASSAN

MASTER THESIS
DEPARTMENT OF ANIMAL SCIENCE

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A thesis submitted in partial fulfillment of the requirements for the degree of Master in Department of Animal Science

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M.Sc. thesis “ESTIMATION OF NUTRITIVE VALUES, METHANE EMISSION AND TANNINS OF SOME TREE LEAVES AROUND ERBIL CITY IN IRAQ” and prepared by KAEFEE GIBBAR HASSAN, who is a student at Department of Animal Science,,Graduate School of Natural and Applied Sciences, Kahramanmaraş Sütçü İmam University, 24.3.2015 was certified by all the majority jury members, whose signatures are given below.

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I declare and guarantee that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. Based on these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

KAEFEE GIBBAR HASSAN

IRAK ERBIL ŞEHRINDEN ELDE EDILEN BAZI AĞAÇ YAPRAKLARININ BESLEME DEĞERI, METAN EMISYONU VE TANIN IÇERIKLERININ BELIRLENMESI
(YÜKSEK LİSANS TEZİ)
KAEFEE GIBBAR HASSAN

ÖZET

Bu çalışmanın amacı, Irak'ın Erbil şehrinden toplanan bazı ağaç yapraklarının potansiyel besleme değerini, kimyasal kompozisyonunu, tanin içeriğini, in vitro gaz üretimini, metabolik enerji ve organik madde sindirim derecesini belirlemektir. Tür ağaç yapraklarının kompozisyonunu, in vitro gas üretimini, metan üretimini önemli derecede etkilemiştir. Ağaç yapraklarının ham protein içeriği %9.23 ile 16.23 arasında değişmiş olup en yüksek protein içeriği Robinia pseudoacacia 'dan elde edilmiştir. Ağaç yapraklarının NDF ve ADF içerikleri sırasıyla %17.93 ile 59.32 ve %10.43 ile 38.19 arasında değişmiş olup Punica granatum elde edilen yapraklar diğerlerinden farklı bulunmuştur. Kondense tanen içeriği %1.04 ile 14.45 arasında değişmiş olup en düşük tanen içeriği Sambucus nigra ağacından elde edilmiştir. Metabolik enerji ve organik madde sindirim derecesi sırasıyla 7.4 ile 10.76 MJ/kg ve %47.12 ile 71.14 arasında değişmiştir. Prunus armeniaca ağaç yaprakları diğerlerinden farklıdır. Metan gaz yüzdesi %17.54 ile 22.44 arasında değişmiş Prunus dulcis ağaç yaprakları en yüksek metan içeriğine sahip olmuştur. Toplam gaz ve metan üretimi 23 ile 44.5 ve 4.52 ve 8.7 ml arasında değişmiş olup en yüksek metan üretimi populous euphrtica ağaç yaprağından elde edilmiştir.

Bu çalışmaya konu olan ağaç yapraklarının çoğu ruminant hayvanların yaşama ve verim payını karşılayacak düzeyde olup protein içeriği, metabolik enerji içeriği ve organic madde sindirim derecesi yüksek bulunmuştur. Bununla birlikte *Juniperus oxycedrus* yapraklarının kondense tanen içeriği yüksek olmasından dolayı ruminant rasyonlarına katılırken kondense tanenin zararlı etkileri göz önüne alınmalıdır. Kondense tanen içerikleri %2'den düşük olan ağaç yapraklarını hayvanlarda gaz oluşumu önlemek ve metan emisyonunu azaltmak için kullanılabilir. Bu tezde ağaç yapraklarının kompozisyonu ve potensiyel besleme değeri ile ilgili elde edilen bilgiler Irak'ın Erbil şehrinde yaşayan çiftçilerin ruminant hayvan besleme de kullabileceği iyi bir bilgi sunacaktır.

Anahtar Kelimeler: Ağaç yaprakları, besin değeri, gaz üretimi, kimyasal kompozisyon,

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ESTIMATION OF NUTRITIVE VALUES, METHANE EMISSION AND TANNINS OF SOME TREE LEAVES AROUND ERBIL CITY IN IRAQ
(M.Sc. THESIS)
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ABSTRACT

The aim of this study was to determine the potential nutritive value of twelve tree leaves around Erbil province in northern Iraq using the chemical composition including condensed tannin, in vitro gas production, ME and OMD. Species had a significant effect on the chemical composition, *in vitro* gas production, metabolisable energy and organic matter digestibility of tree leaves from Erbil province in northern Iraq. The CP content ranged from 9.23 to16.23 %, with *Robinia pseudoacacia* having the highest content. The content of NDF and ADF ranged from 17.93 to 59.32 % and 10.43 to 38.19 % respectively. The content of *Punica granatum* was significantly lower than the others. The CT ranged from 1.04 to 14.45 % with *Sambucus nigra* having lowest tannin. The content ME and OMD ranged from 7.4 to 10.76 ml kg and 47.12 to 71.14 % respectively. The content of *Prunus armeniaca* was higher than others. The (CH_4%) content of tree leaves ranged from 17.54 to 22.44 % with *Prunus dulcis* having the higher methane. The content of TGP and CH_4 (ml) ranged from 23 to 44.5 ml and 4.52 to 8.7ml respectively. The content of *populous euphrtica* was lowest than the others.

The most of tree leaves studied in the current study have considerable amount of crude protein and metabılisable energy which are enough to meet the requirement of the ruminant animals for maintanence and production. However leaves from *Juniperus oxycedrus* have higher condensed tannin which would be detrimental to ruminant animals. Therefore condensed tannin content of leaves from *Juniperus oxycedrus* should be taken into consideration when leaves from *Juniperus oxycedrus* were included into ruminant diets. Condensed tannin contents of most of tree leaves studied in the current study was lower than 2%, which is suggesting that these tree leaves could be used as alternative forage to reduce the risk of bloating and methane emission in ruminants. These findings may offer good knowledge on the chemical composition and potential nutritive value of twelve plant species for farmers in Erbil province in northern Iraq to identify the best sources for their ruminant animals.

Key words: Tree leaves, Nutritive value, Gas production, Chemical, Tannin, Correlation

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADIN	Acid detergent insoluble nitrogen.
AOAC	Association of official analytical chemists.
CA	Crude ash
CF	Crude fat
CP	Crude protein
CT	Condensed tannins
DM	Dry matter
DMD	Dry matter digestibility
EE	Ether extract
HT	Hydrolysable tannins
ME	Metabolize energy
NDF	Neutral detergent fiber
OMD	Organic matter digestibility
PEG	Polyethylene glycol
S.E.M	Standard error mean
TGP	Total gas production
VFAs	Volatile fatty acids

1. INTRODUCTION

Fodders trees leave have always played a significant role in ruminant nutrition. Recently, these feed resources have been unnoticed by scientists because of insufficient knowledge of their possible use and the lack of initiatives to develop more inventive systems of feeding (Devendra, 1992). The conventional approach to trees is to study and utilize the "single" species. The reality is the case that, in many parts of the the world, animal eat or are fed with "mixtures" of tree fodder. Apart from showing the ruminants preference for actual fodder species, they also show that, had the opportunity, ruminants will feed on mixtures of forages (Paudel and Tiwari, 1992; Rangkuti et al., 1989).

Chemical analyses conducted in the laboratory contain basic nutritional analyses such as dry matter, crude protein, crude fiber, crude fat, crude ash, tannins and minerals (Monika et al., 2011).

Assessment of the nutritive value characteristics of tree leaves is very important because of the recent increase in the use of this material in ruminant nutrition (Reed, 1995). Tree leaves are known to include several kinds of polyphenols that affect the DM digestibility (Khazaal and Orskov, 1994).

The nutritive value of a ruminant fodder is determined by the compactness of its chemical composition, as well as their rate and extent of digestion. Determination of the digestibility of feeds *in vivo* is hard, expensive, requires a large quantity of feed, and is largely unsuitable for single tree leaves thereby making it unsuitable for routine feed assessment. The *in vivo* method is moreover subject to errors associated with the use of digest flow rate marker, microbial marker, and inherent ruminant variation. On the other hamd, the *in vitro* techmique is one of the new methods which provide more rapid and less expensive alternatives (Stern et al., 1997).

Methane is one of the most impotant greenhouse gasses 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 gas trointestinal system but also from their manure. Reducing the emission of methane would be benefit 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 archea, which scavenge hydrogen and use it to emissions methane. The production of methane is a straight for ward 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 (Hartung and Monteny, 2014; Lassey, 2007). Enteric methane conduces 30– 40% of total methane production (TGP) from agricultural source (Moss et al., 2000). There are several ways of mitigating of methane emission such as use of prebiotics and probiotics (Mwenya et al., 2004; Takahashi et al., 2005), supplementation of fat (Van Nevel and Demeyer, 1996; Ungerfeld et al., 2005) and addition of plant extcracts(Śliwiński et al., 2002; Patra et al., 2006; Goel et al., 2008).

The *in vitro* gas production technique is a useful method to determine the extent and rate of feed degradation (Blummel and Ørskov, 1999; Beuvink et al., 1992; Groot et al., 1996; Cone et al., 1997). Gas production (GP) provides more elaborate information on fermentation kinetics of ruminant feeds than can be achieved by estimating for disappearing of feed from fermentation media and number of different *in vitro* (GP) techniques have been developed (France et al., 1993).

Recently an effort of mitigating of enteric methane emission from ruminant animals has been a key priority, because this biological process accounts for 2–12% loss of dietary gross energy in ruminant (Johnson and Johnson, 1995). Moreover, methane is a potent greenhouse gas with a global warming potential 23 times higher than that of carbon dioxide (Boykoff et al., 2008).

Methane (CH_4) is produced by archea in the rumen during anaerobic fermentation of soluble and structural carbohydrates contained in feed (Kurihara et al., 1999; Hariadi and Santoso, 2010) 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).

Tannins are the plant secondary compounds that are present in any kind commonly consumed tree leaves by livestocks. Tannins are usually defined as water soluble polymeric that precipitately proteins (Haslam and Lilley, 1988). They are broadly classified into condensed tannins and hydrolyzable. Hydrolyzable tannins are allergic acid and gallic acid esters of a core molecule that comprises of polyols including phenolics and sugars (e.g. catechin), whereas condensed tannins (CT) consist of oligomers of flavan-3-ols and concerned flavourous residues, which produce anthocyanidins on acid degradation

(Reed,1995). Condensed tannins (CT) affect nutrient supply to the ruminant through complexion with digestive enzymes, dietary and endogenous proteins ((Barry and McNabb, 1999). On the other hand, the effect of hydrolyzable tannins may be less detrimental since it hydrolysed in the digestive tract (Getachew et al., 2008).

Tannin containing feed samples is incubated with polyethylene glycol (PEG) to determine the effect of tannin on the fermentation using the *in vitro* gas production technique developed by Makkar et al. (1979). As PEG is an inert molecule with a heights molecular weight and high affinity for consetant tannins (CT), it neutralizes some negative effects of tannins in rumen fermentation and increased GP (Makkar, 2003; Getachew et al., 2000). Effects of tannin from a different source in reducing ruminant gas production and ammonia levels have been reported. It has been suggested that tannins in the diet might help to decrease ruminant total methane production (Hess et al., 2006).

The aim of this thesis was to determine the effect of species on the chemical composition, potential nutritive value, gas production, methane production, interaction among the chemical composition and in vitro gas production parameters.

2. LITERATURE REVIEW

2.1 Nutritive Value and Feeding Value

The feeding value is the capacity of forage to promote ruminant production. This is a complex function of intake, food supply and animal metabolism. This is a highly interactive process. In ruminants, the utilization of nutrients derived from forage can be modified by the presence of other feeds in the rumen. Digestibility, in a broad sense, includes degradability and fermentation. These, in turn, are functions of the chemistry of the forages (Rosales, 1996).

2.2 Tree Leaves

Most tree species have been grown for fodder, and have always played an important role in ruminant nutrition in the most parts of world. Most of the multipurpose species have economic value other than as fodder but being grown for such purposes makes them readily available for ruminant feeding (Moog, 1991). Tree leaves are an integral part of the smallholder farming systems in the most parts of world (Devendra, 1992).

Tree leaves capture a higher of solar energy in high and low rainfall areas. They provide sustainable yields of biomass. They reduce erosion and improve soil structure and fertility. Some trees also provide green biomass of high protein content at a time when feed resources are scarce and mostly low in nitrogen. In marginal areas where dry seasons are prolonged, the deep roots of trees allow greater exploitation of water and mineral reserves in the soil profile (Preston and Leng, 1987).

Traditionally trees have been grown for specific purposes, among these are (Nitis, 1989).

- As live fences and windbreaks.
- Shade for crops and ruminant.
- Climbers for vine crops.
- Traditional medicine and religious ceremonies.
- Poles for livestock housing.
- To reduce wind and water erosion.
- To store crop residues and to dry grasses.
- As materials for farm implements, timber for housing and wood for handicrafts

- Fuel wood.
- Food production for human and livestock consumption.
- A source of pollen for bee keeping.

2.2.1 Diversity of tree and shrub

There is considerable diversity within genera and within species of tree. Lowry et al. (1994) reported 112 species of the genus *Erythrin.* Kass (1998) reported 100 species in the genus *Albizia* The diversity within species is exemplified by the 96 accessions of *Cajanus Cajan* and 26 accessions of *Aeschynomene Americana* (Larbi *et al*., 1996) listed in its forage germ plasma catalogue. There are a lot of reports about new fodder resources of trees from 45 species from Costa Rica, 16 species from the Philippines (Moog, 1991), 20 species from Colombia (Rosales et al., 1992) and 40 species from Guatemala, (Be-navides et al., 1994). A huge diversity of tree leaves and shrub leaves species has been recognized. Blair. (1989) presented a list (gathered from several sources) of trees leaves and shrubs leaves of nutritive value as ruminant fodders. The compendium included 270 different species from about 74 genera. Although there are several hundred trees and shrubs, the information about the contribution to animal nutrition is not well documented and limited (Rosales, 1996). The list of tree and shrubs used in livestocks are given in Table 2.1.

Table 2. 1. Some tree and shrubs for livestocks in the tropics (Devendra, 1992).

Common name	Scientific name
Acacia	*Acacia nilotica*
	Acacia catechu
	Acacia siberiana
Cassava	*Maniot esculents*
Calliandra	*Calliandra calothyrsus*
Erythrina	*Erythrina variegata*
Ficus	*Ficus bengalensis*
	Ficus exasperate
	Ficus religiosa
Gliricidia	*Gliricidia maculate*
	Gliricidia sepium
Jackfruit	*Artocarpus heterophyllus*
Leucaena	*Leucaena leucocephala*
Pigeon pea	*Cajanus cajan*
P rosopis	*Prosopis juli flora*
Sesbania	*Sesbania sesban*
	Sesbania grandiflora
Tamarind	*Tamarindus indica*

2.2.2 Foliage production

Regardless of the production systems of tree leaves , leafage yield is effected by more factor as plant density and type of harvesting, for example age at the tie first harvesting , tallness and frequency of cutting and the time season of harvesting (Ivory, 1990). Thus, only broad generalizations on the relative yields of different species can be made from values given in the literature. Trees may be grown in pure stands, for restricted browsing, or cut-and-carry systems. Yields of *Leucaena* foliage to 11.8 dry matter (DM)/ha have been reported for 3 different spacing's of K341 and K8 *Leucaena* grown in Hawaii (Pound and Martinez, 1983).

The amount of biomass will be available for use at a time when either ruminant demand is high or the availability of other feeds is low (Gill et al., 2007). Browse production is influenced by many ecological factors such as edaphic, climatic, and topographic conditions and management background affecting exploitation by animals, lopping and burning forested areas (Walker, 1980).

2.2.3 Nutritive value of fodder tree leaves

This success has also led some research worker from both developing countries and industrialized to the belief that the chemical compound of the feed is its nutritive value. It is not difficult to find articles in journals or seminar proceedings in which the nutritive value of trees is expressed as a simple chemical composition. Ration systems based on additive chemical compound may not be appropriate however with mixtures of tropical forage. The significance of conventional feeding standards to developing countries, in particular in the tropics, has been questioned from the economic (Jackson, 1981) and nutritional points of study (Preston and Leng, 1987).

2.2.4 Methods of analysis of the nutritive value of feedstuffs

The anticipation of the quality of the tree fodder, expressed as the nutritive value, is obviously a key factor in the development of feeding systems and many methods of qualitative analysis have been proposed.

However the since middle of 19th century, scientific method in moderate development countries has been assayed to develop systems to analytical forage to qualify the value of feed to ruminant. They contain measures of the chemical compound of the tree leaves and its digestibleness (Apper et al., 2006).

The system which is most frequently cited in the literature is that of proximate analysis. As can be seen from Table 2.2. The type of analysis system divides the forage into six fractions (McDonald et al., 1981).

Table 2. 2. The components in the proximate analysis of forages.

Fraction	Component
Moisture	Water (H_2O) , volatile acids and bases if present
Ash	Essential elements major: Ca, K, Mg, Na, S, P, Cl. Trace: Cu, Cr, F , Fe, Mn, Zn, Si, Co, Se, V, Ni, Sn , As, I, Mo.
Crude protein	Nitrates, nitrogenous glycosides, proteins, amines, glycolipids, B-vitamins, nucleic acids, urea, ammonia, amino acids.
Ether extract	Ether extract, fats organic acids, oils, pigments, , waxes sterols, vitamins E,D,A,K
Crude fiber	Hemicellulose, fiber cellulose ,lignin
Nitrogen	Sugars, free extractives fructans, pectins, organic acids resins, starch, pigments, water soluble proteins.

The dry matter (DM) content is determined as the loss in weight which results from drying a known weight of food at 100 °C to constant weight. This method is acceptable for most feedstuffs.

However the ash content is determined by ignition of a known weight of the feed at 500 °C until all carbon has been removed. This residue is the ash including the inorganic constituent of the food. However, some loss of volatile material in the form of chloride, sodium, phosphorus potassium, and sulphur will take place during ignition. The ash content is thus not truly illustration of the inorganic material in the forage either quantitatively or qualitatively.

The crude protein (CP) content is calculated from the nitrogen content of the food, determined by a modification of the Kjeldahl sulphuric acid digestion technique. In this method, all nitrogen present in the forage, except that in the form of nitrite and nitrate, is conveted to ammonia. This ammonia is liberated by addition sodium hydroxide to the digest, distilling off and collection in standard acid solution. The nitrogen content is multiplied by 6.25 to obtain the (CP) value, based on the assumption that plant proteins contain about 16 percent nitrogen. This is not "true protein" since the method includes nitrogen from sources other than protein, such as nucleic acids, alkaloids, etc.

The ether extract (EE) fraction is determined by subjecting the feed to a continuous extraction with petroleum ether for a defined period. The residue is obtained by evaporation of the solvent. As well as true fat, it contains organic acids, waxes, organic alcohols, acids, pigments; designation of the fraction as "oil" or "fat" is therefore wrong. The ether extract determination is generally omitted in forage analysis as the amount of lipid present in feeds is very low.

The original crude fiber (CF) method has been replaced by NDF and ADF analysis Van Soest (1994). NDF consists essentially of, cellulose, lignin and hemicelluloses and was regarded as a measure of the cells of plant and material. Also, most of the silica is removed during extraction. ADF stand for essentially the cellulose and crudee lignin fractions of plant material but also include silica. ADF determination is in particular useful for feeds since ADF content of feedstuffs is negatively correleted with digestibility (McDonald et al., 1981).

Energy is required for maintanence and production of livestocks. Energy is released when nutrients are oxidized. The amount of total energy in forage is termed as the total energy. In practice, not all of this gross energy is available to the ruminant animals due to a result of losses by feces or urine and methane emission. When these losses are deducted from the GE, the amount of total energy that may potentially be absorbed by the ruminant is obtained. This is termed as metabolize energy (ME). The efficiency of energy utilization is different for maintenance and production (Makkar, 2005).

2.3 Forage as Feed

Ruminants have evolved a capacious set of stomachs, a fermentation tub, which harbors microorganisms capable of digesting fibrous materials like cellulose. The microbial fermentation in the rumen precedes host enzyme digestion in the abomasums and small intestine and thus allows the ruminants to utilize plants which have a more fiber content and a few nutritional values for simple-stomached animals (Forbes and France, 1993).

Although ruminant animal itself has no ability to digest or degrade the cell wall contents of forage such as celluloses and hemicelluloses, rumen microorganisms are able to break down the cell wall contents of forage such as celluloses and hemicelluloses. However, bacteria in the intestine of monogastric animals are able to digest limited amounts of cellulose and hemicelluloses (Van Soest, 1994). Animal microorganisms, protozoa, bacteria, and fungi, ferment feed constituents (sugars, polysaccharides, proteins, etc.) producing the adenosine triphosphate (ATP) they need for growth and maintenance and the latter involving the use of energy for the synthesize of monomers and their polymerization (e.g., for synthesis of amino acids from carbon compounds and ammonia, and for the extension of polypeptide chains) in addition to the end products of the fermentation (Nolan, 1993).

2.4 *In Vitro* and *In Vivo* Digestibility Methods

The analytical methods can give an indication of the potential nutritive value for supplying a particular nutrient, but the real value of the feed to the ruminant can be obtained only after making allowances for availability of these feed during digestion.

Recently the *in vitro* and *in vivo* digestibility techniques with combination of chemical analysis have been used to determine the nutritive value of feedstuffs. In *in vitro* dry matter digestibility technique, feefstuffs were incubated with buffered rıumen fluid for 24 or 48 hours. The composition of micro-organisms in inoculants taken from different animals affects the digestibility of feedstuffs. The estimated digestibility values are a nice indication of nutritive value (McDonald et al., 1981). In *in vivo* digestibility technique, the known amount of feedstuffs are given to animals, the digestibility of feedstuffs were calculated using the dry matter loss of feces and dry matter intake. These *in vivo* digestibility values are actual nutritive value of feedstuffs.

It has ben reported that *in vitro* and *in vivo* digestibilities of some tree leaves ranged from 17 to 67% and 44 to 60% respectively. (McKay and Frandsen, 1969; Walker, 1980, Vercoe, 1987). *In vitro* and *in vivo* digestibility values of some tree leaves has been also given in Table 2.3.

Table 2. 3. Digestibility of some tree leaves compared to *Panicum maximum* (Smith, 1992).

Fodder Species	*In vivo* Organic Matter Digestibility (%)	*In vitro* Organic Matter Digestibility (%)
Gliricidia sepium	53.9	-----
Sesbania grandiflora	31.8	66.7
Panicum maximum	53.2	37.0
Leucaena	64.9	57.5
Albizia lebbeck	53.2	59.5

2.5 Mixtures of Fodder Tree Leaves

Farmers in many parts of the world feed mixtures of fodder tree leaves to their ruminant as a supplement or as the whole ration (Paudel and Tiwari, 1992; Rangkuti et al., 1990; Devendra and Pun, 1993; Gill and Powell, 1993). It was suggested that use of mixtures ensures a more diverse supply of tree leaves and therefore decrease the risk of dependence on a single tree species.

Fodder tree leaves are chemically completed feedstuffs which give them the potential for all type of interactive processes in the ruminant. The interaction, at the digestive steps, of nutrients and ant-nutritional factors can have an important role play on animal productivity. They are proposed that the harmful effect of secondary compounds can be getting over by the simple approach of reducing toxicity by feeding some toxic plant in a mixture with other plants, thus adulterating effective stage of each compotation. The effect of condensed tannins (CT) can be overcome by complexion them with polyethylene glycol (PEG). Lowry (1990) reported that natural analogues"soluble, non-degradable polyhydroxy compounds occurs in plants, and there is the possibility of a positive mutual action between tannin and PEG analogue when the two plants are fed together. Information on the nutritive value of trees leaves and shrubs is limited, there is even less information on the nutritive and feeding value of mixtures of leaves. Most of the information available involving use of two or more fodder tree leaves kinds relate to their replacing value (Van Eys et al., 1986; Richards et al., 1994; Adejumo, 1995).

2.6 The Rumen

It is necessary to understand the certain concepts about the rumen anatomy and physiology as well as the interaction with the rumen micro-organisms (Lingnau, 2011).

2.6.1 Anatomy and physiologyof rumen

A ruminant animal has four stomach compartments, the reticulum, the rumen, the omasum, and the abomasums (Figure 2. 1). Of these, the rumen is the largest and can contain up to 150 to 230 litres of material (Ishler et al., 2001). The reticulo-rumen (reticulum and rumen are often considered together) constitutes up to 85 % of the total capacity of the stomach (Mcdonald et al., 2002). The rumen acts as a fermentation vat and is the site of microbial activity. There are an estimated 150 billion microorganisms present in every teaspoon of rumen fluids (Ishler et al., 2001).

In ruminant animals microbial digestion takes place prior to enzymatic digestion and it is this characteristic of the ruminant's digestive physiology which enables them to utilize forages and fibrous roughages as a food source which monogastric animals are not able to do (Van Soest, 1994).

Another characteristic of ruminant animals is that of rumination. Rumination is the ability of an animal to complete eating at a rapid rate and finish chewing at a later stage. It involves the regurgitation of feed in the form of a bolus or cud, the re-mastication thereof, re-salivation and finally the re-swallowing of the rumen digesta. A major secretion into the digestive tract is that of saliva. The volume of saliva produced is directly related to the time spent eating and ruminating by dairy cows (Ishler et al., 2001).

Balch (1958) found that for every 5kg of hay fed to dairy cows they produced only 21-28 kg of saliva while for concentrate this was only 6-8 kg of saliva. Beauchemin et al. (2008) indicated that forage source did not affect the rate of salivation (213 g/min). The eating rate did however differ between forage sources (g of DM/min). In an earlier study done by Beauchemin and Buchanan (1989) who indicated that increasing NDF content of the feed quadratically increased rumination and chewing time. Increases in chewing time in turn lead to differences in ensalivation of forages, in other words gram of saliva produced per gram of DM and gram of saliva produced per gram of NDF (Beauchemin et al., 2008). Saliva produced was greatest for straw (7.23g saliva/g DM) and was similar for barley silage, alfalfa silage, and alfalfa hay at 4.15, 3.40, and 4.34 g saliva/g of DM, respectively.

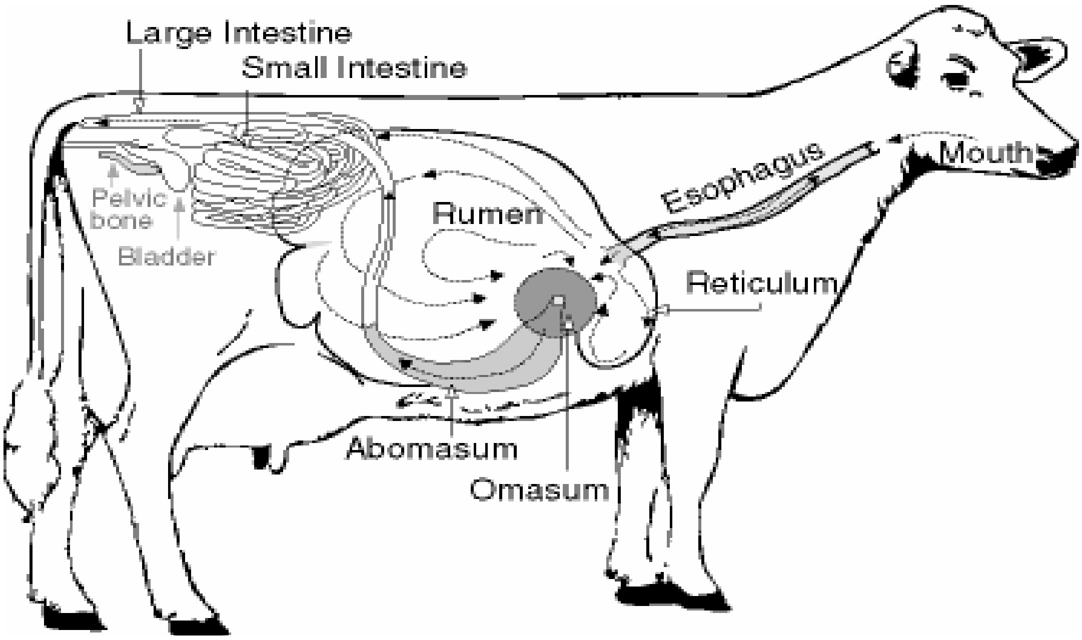


Figure 2. 1. Rumen digestive system (Weimer et al., 2009).

2.6.2 Rumen microbiology

To obtain a rumen environment that maximizes microbial growth and production, nutritionally balanced diets are needed and both the needs of the rumen microbes as well as that of the cow need to be considered.

The rumen microbial population consists of bacteria, protozoa and fungi of which the bacteria are the majority with numbers ranging between 10^{10} to 10^{11}cells/gram rumen contents. Bacteria can be categorized into distinct groups according to the type of substrate they utilize. These include cellulose, hemicellulose, starch, sugars, intermediate acids, protein, and lipids. However, most species are capable of utilizing more than one type of substrate (Ishler et al., 2001).

Most of the bacteria groups are specialized in polysaccharide hydrolysis and the fermentation of sugars resulting from the hydrolysis, and for this reason animals fed the same diets would have microbe populations that are very similar (Firkens and Yu, 2006).

However, as shown by Weimer et al. (1999) microbial populations can differ between animals fed the same diet making the integration between rumen microbiology and dairy cow nutrition a very difficult matter (Weimer et al., 2009).

2.6.3 Rumen pH

One of the most variable factors that can influence rumen microbial populations and volatile fatty acid production is rumen pH (Ishler et al., 2001).The most severe changes in rumen microbe population occur with dietary changes, such as a ratio between roughage and a high grain concentrate, or pasture to concentrate feeding. The change to higher amounts of highly fermentable carbohydrates is done to improve production. This may however result in acidosis, with the pH decreasing in the rumen as a result of large increases in lactic acid production (Tajima et al., 2000).

There are two groups of bacteria which function at different pH levels in the rumen. The starch digesters are better suited to more acidic environments at a pH of 5.2 to 6 (Ishler et al., 2001). The major cause of lactic acidosis is *Streptococcus bovis*, which easily ferments starch and produces lactate (Tajima et al., 2000).

Fiber digesters in contrast thrive at a pH of 6.2 to 6.8, and cellulolytic and methanogenic bacteria decrease as the pH decreases below 6.0 (Ishler et al., 2001).Only three commonly found bacterial species in the rumen are considered cellulolytic although many others have been reported. These are *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *Ruminococcus albus* and strains of these bacteria cause the solubilisation of cellulose and hemicellulose (Flint, 1994).

2.6.4 Fermentation of protein in rumen

A function of rumen microbes is to synthesize microbial protein. Rumen ammonia nitrogen is utilised for protein production. Ammonia nitrogen is derived from three sources, namely dietary protein and non protein nitrogen, hydrolysis of recycled urea and degradation of microbial protein. Rumen ammonia is removed by way of protein synthesis by microbes, absorption through the rumen wall and flushing to the omasum. Figure 2.2 is a schematic summary of nitrogen utilization by ruminants. Microbial protein is of better quality than that of plant protein and often rivals that of animal protein. Microbes can however not produce certain essential amino acids and therefore supplementation is needed to achieve high levels of milk production (Ishler et al., 2001).

Rumen ammonia utilization is affected by two factors. One is the amount of bacteria in the rumen, and the second is how rapidly the microbial population grows. Microbial mass and growth and therefore utilisation, will depend on the amount of energy available to bacteria. Therefore feeds high in total digestible nutrients are more fermentable and thus provide more energy to bacteria. Rumen degradable protein is not utilized when ruminal ammonia nitrogen is in excess of 5mg/dL. Therefore, when rumen ammonia exceeds 5mg/dL nothing more is gained from further supplementation of degradable protein (Satter and Slyter, 1974).

Under typical ryegrass and tropical high protein pastures, concentrates containing more than 12-13% CP is not efficiently utilized, however lactating dairy cows in the first third of lactation can benefit from dietary protein levels as high as 16 -17 % under nitrogen limiting conditions microbial (Satter & Roffler, 1974).

Protein is also degraded by microbes in the rumen. The amino acids and peptides produced are used for microbial protein growth. The feed and originating from within protein is subjected to changing degrees of proteolysis and demonization in the reticulo or olivary tract in ruminant producing ammonia (NH_3) Ammonia is the most important source of nitrogen for protein synthetic thinking in the rumen (Lingnau , 2011).

2.6.5 Fermentation of carbohydrates

Dietary structural and non structural carbohydrates are the main fermentation substrates in the rumen. They are degraded to their constituent such as pentoses and hexoses and before being fermented to volatile fatty acids (VFAs). The VFAs are principally acetic, butyric and propionic. The other main end products of the fermentation are methane and carbon dioxide (Czerkawski, 2013), (Figure 2.2). To the microbes, the VFAs are waste products but to the host ruminant they represent the major source of energy and with most diets account for near 80% of the energy disappearance in the rumen (The remainder being lost as heat and methane). The VFAs are absorbed across the epithelium the reticulo-rumen CH_4 and CO_2 and are lost by eructation (Forbes and France, 1993).

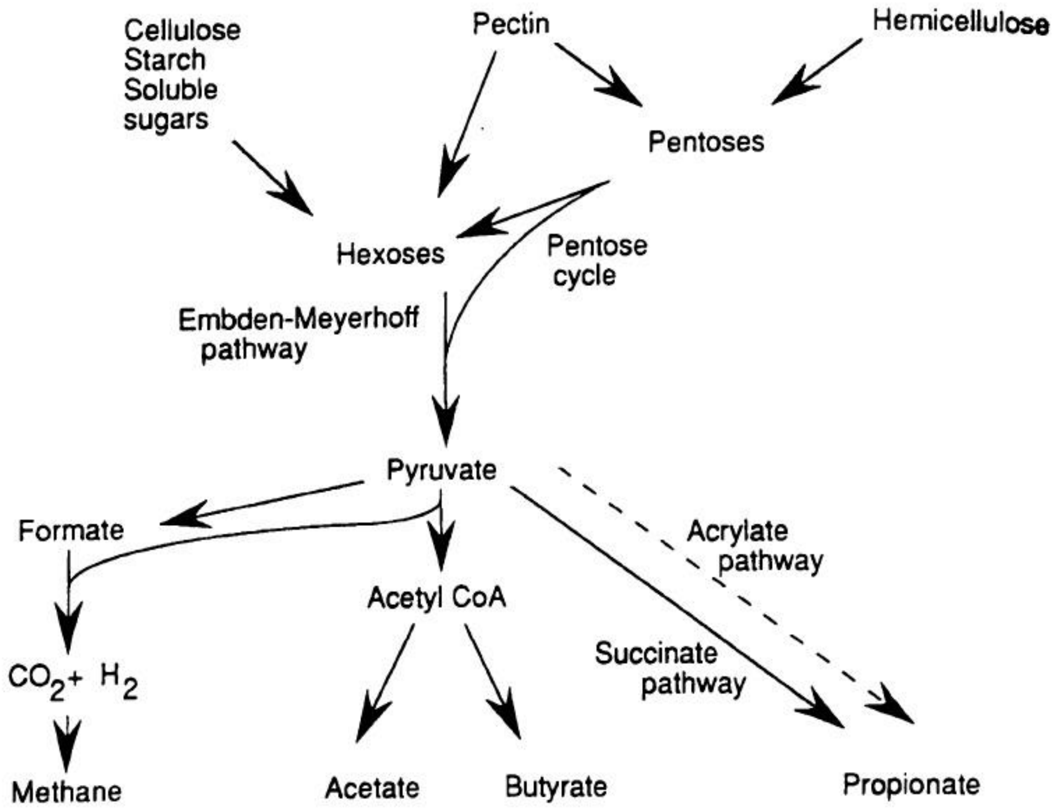


Figure 2. 2. A schematic representation of the major pathways of carbohydrate metabolism in the rumen (Forbes and James, 1993).

2.6.6 Rumen ammonia-nitrogen

Three of the studies mentioned above included rumen studies and recorded rumen ammonia levels (Van Vuuren et al., 1986; Khalili and Sairanen, 2000; Sayers et al., 2003). These are summarized in Table 2.4. There were differences for rumen ammonia nitrogen, with the high starch concentrate resulting in significantly higher ruminal ammonia nitrogen (NH_3-N) levels than the low starch concentrate (Khalili and Sairanen, 2000).

Van Vuuren et al. (1986) found that ruminal NH_3-N content did not differ significantly between supplementation types when cows were given 7kg (5.2-5.4kg actual intake) supplement, however the 1kg supplementation level had a significantly higher NH_3-N value. Van Vuuren et al. (1986) indicated the fact that more pasture was taken in at a lower level of supplementation than at the higher levels of supplementation, and that the pasture had a higher rumen degradable protein content than the supplement had and therefore increased the rumen ammonia concentration (Lingnau , 2011).

2.7 Methane

The chemical compound of methane gas with the chemical formula CH_4 is one atom of carbon and four atoms of hydrogen. It is the component simplest alkane and the main component of gases in natural. The relation copiousness of methane make the grade an attractiveness fuel, though gaining control and store it poses challenge due to state of gaseous found more circumstances normally . Generally in the nature, methane is under the sea floor founding or below ground, where it often find its way to the surface Earth and in the atmosphere of Earth is given in Figure 2.3 (Staley, 2009).

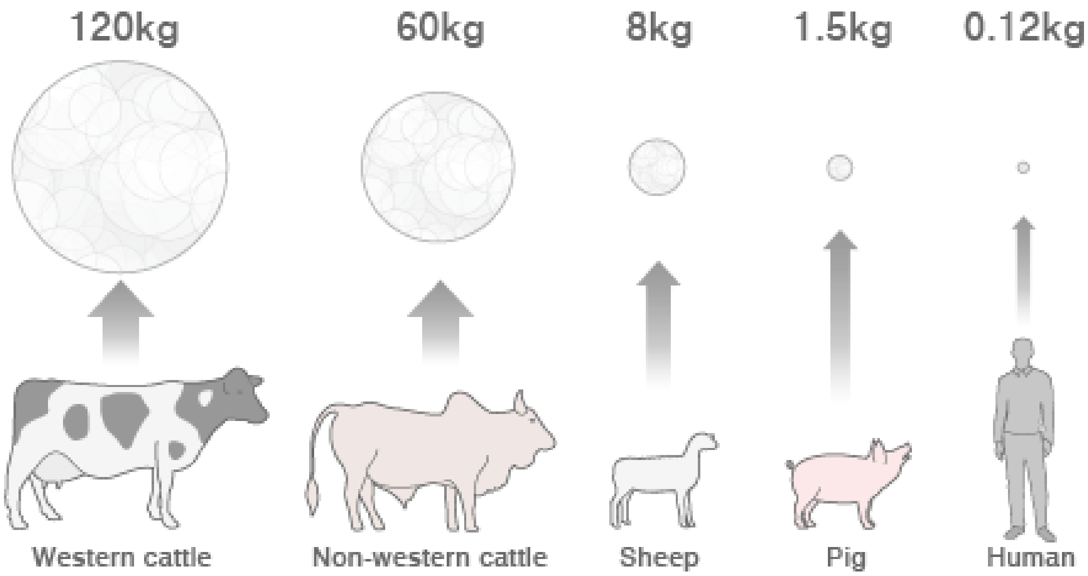


Figure 2. 3. Methane emission per animal / human per year (Miller et al., 2006).

2.7.1 Analysis of methane

Methane is one of byproducts of ruminant chemical conversion of organic compounds by means of enzymes by methanogenic archea from H_2 and CO_2 derived from the fermenting sources of carbon, particularly sugars (Demeyer and Fievez, 2000; Fonty et al., 1995; Jouany et al., 1995; Wolin and Miller, 1983). The Methane gas is lastly eliminated by belching, representing a loss of between 5 and 8% energy in the gross contained in the feeds consumed by the ruminant (Blaxter,1962). Also, methane is considered a major source of greenhouse gas production from agriculture (Johnson and Johnson, 1995; Moss et al., 2000).

2.7.2 Measurement of methane production

2.7.2.1 Measurement of total gas production

There are several *in vitro* methods employed for estimation of the gas produced from feedstuffs incubated with buffered ruminal fluids using gas-tight culture bottles or syringes. In these methods buffer, macro and mineral solutions are used to maintane the growth of ruminal micro-organisms (Goering and Van Soest, 1970). These two *in vitro* methods allow determination the other end products such as volatile fatty acids and ammonia (Menke et al., 1979; Theodorou et al., 1994).

2.7.2.2 Sampling of gas

Some of produced gas needs to be sampled for further examination. This can be done by a specisific gas-tight syringe with disposable needles. Such syringes allow the collected samples to be stored and locked.

For batch cultures, sampling could be done with a specific glass syringe (gas-tight) and its disposable needles. This type of syringes is particularly useful because its push button has a valve can safely store and lock the sample. Samples can be collected from the headspace throught few steps briegly the needle is inserted during the septum of the stiper, sample is taken into the syringe, and thevalave is lockedand finalythe total producted gas is measured (Makkar and Vercoe, 2007).

2.7.2.3 Calculations

Prior sample analysis suitable measurements and linearity tests are carried out. For this purpose, standard gas mixture is used to identify linearity and confirm it using few sample concetrations (Makkar and Vercoe, 2007).

2.7.3 Methods of measuring methane production

They are sevaral methods used for measuring methane emissions from ruminant. The advantages and disadvantages are discussed by Storm et al. (2012).

2.7.3.1 Measuring methane by means of chambers

Respiration chambers or chamber systems are used for the energy metabolism of ruminant since 1900 (Johnson et al., 2003; McLean and Tobin, 2007). Diagram of open circuit respiration chamber is given in Figure 2.4.

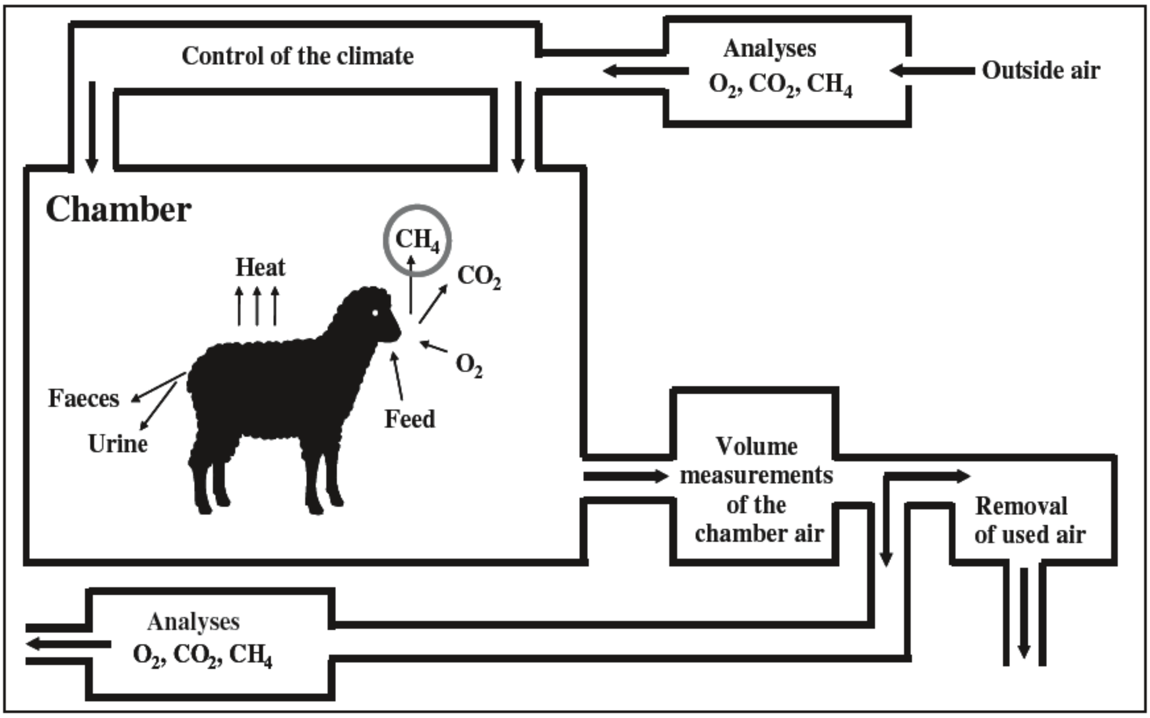


Figure 2. 4. Diagram of open circuit respiration chamber (Makkar and Vercoe, 2007).

2.7.3.2 Measuring methane with the SF6 tracer technique

SF6 tracer technique is described in detail by (Johnson et al., 2003; Zimmerman, 1993). Illustration of the SF6 tracer technique reprinted with permission is given in Figure 2.5. The main objective of the method was to investigate energy effectiveness in free range ruminant animals since respiration chambers could not be applied to free ranging ruminant (Johnson et al., 2003; O'Kelly, 1992).

The SF6 method is used widely in Canada (Lassey et al., 1997), Australia (Grainger et al., 2010) New Zealand (Lassey et al., 2011) and the US (Johnson et al., 2003) and also north European countries e.g., Norway (Hindrichsen et al ., 2005) and Sweden (Patel et al ., 2011).

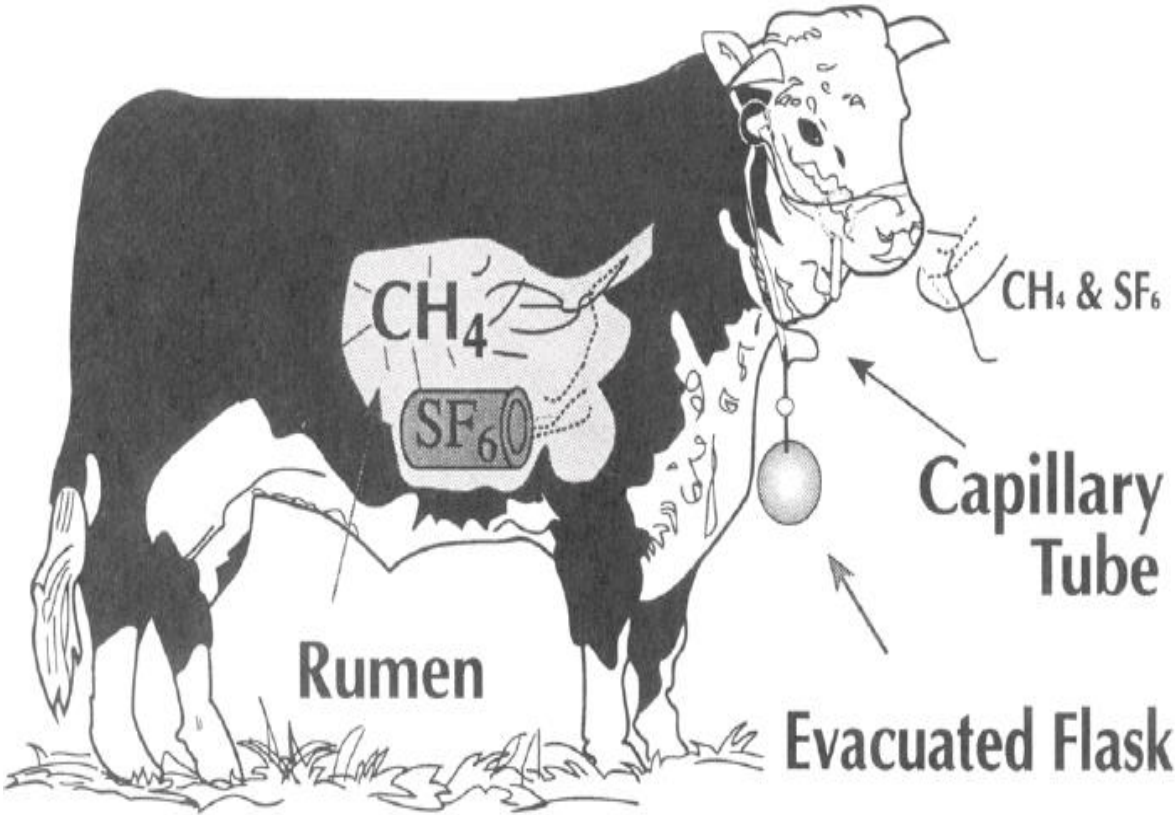


Figure 2. 5. Illustration of the SF6 tracer technique (Johnson et al., 2003), copyright (1994) American Chemical Society.

2.8 Methanogenesis in the Ruminants

The methane emission from animal contributes to global wormig because of its contribution to methane product of fermentation and represents of forage to get rid of hydrogen emesioned by rumen microbes in the rumen (Figure 2. 6). Decreased all factors produced in the time of different fermentation processes are re-oxidised of dehydrogenation reactions that accumulate hydrogen in the rumen (Martin et al., 2010).

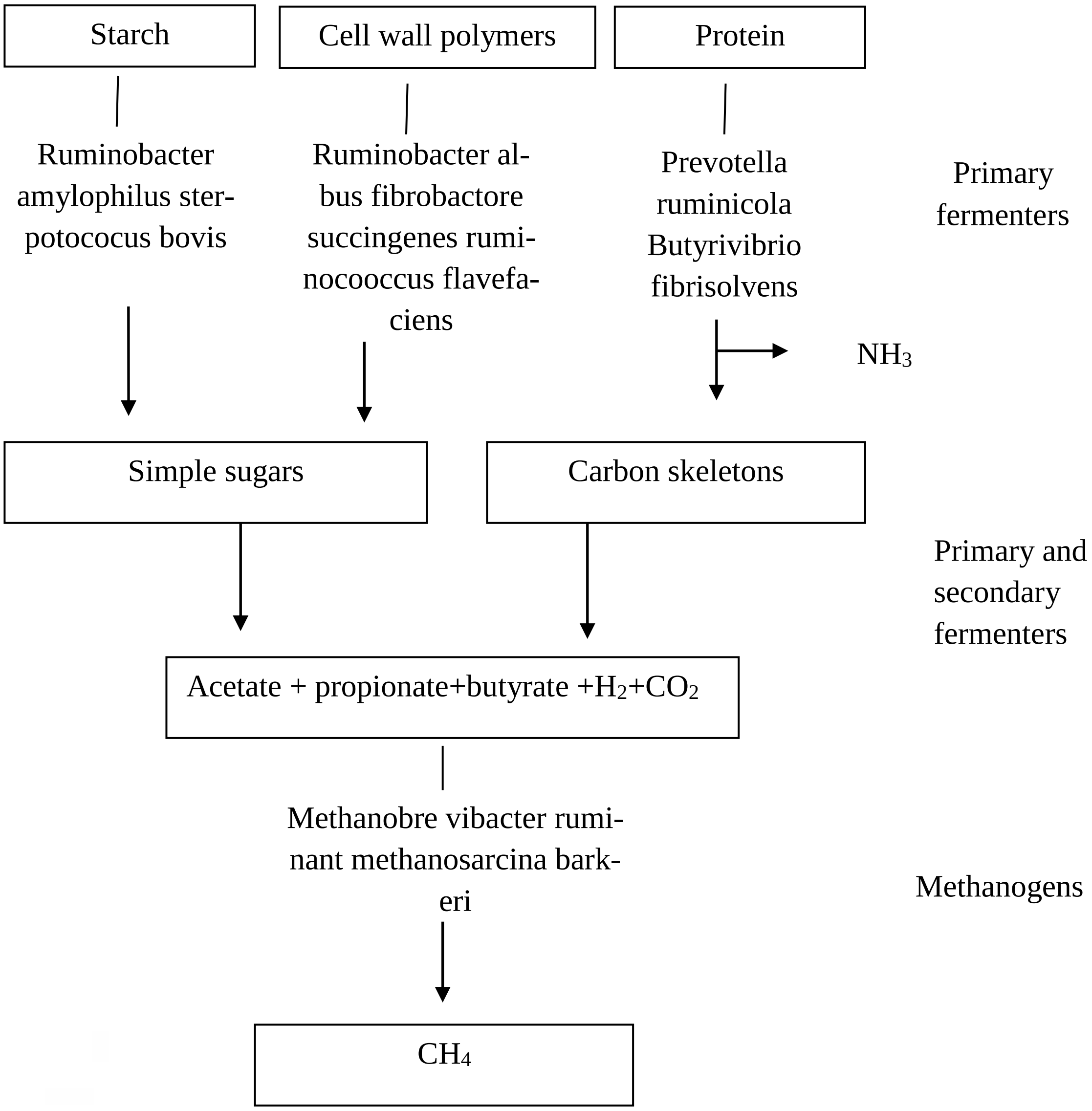


Figure 2. 6. Microbial fermentation in the ruminant (McAllister et al., 1993).

At the past decades concern has to begin in over the gathering of gases in the atmosphere that are capable of trapping heat (Table 2.4), leading to enhanced average global temperatures. It is very high probable that these so-called greenhouse gases have increased in concentration in the atmosphere due to the increased size of the human population and its co-occurrence activities (Forster et al., 2007).

Table 2. 4. Annual enteric methane emission from the main domesticated livestock species (Sauvant, 1992).

	Methane emission (kg) CH_4 animal-1 year-^{1})	Assumed average Body weight (kg)	Methane emission (g kg BW-1 year-1)
Ruminants			
Dairy cows	90	600	150
Beef cattle	65	400	163
Sheep	8	50	160
Goats 8 50 160	8	50	160
Non-ruminants			
Swine	1	80	13
Poultry	< 0.1	2	-
Horses	18	600	30

2.9 Strategies to Reduce Methane Emission of Ruminants

There are several strategies to reduce enteric methane production. These stratigies have been reviewed in the literature (Cottle et al., 2011; Eckard et al., 2010; Martin et al., 2010). These stratigies are illustrated in Figure 2.7.

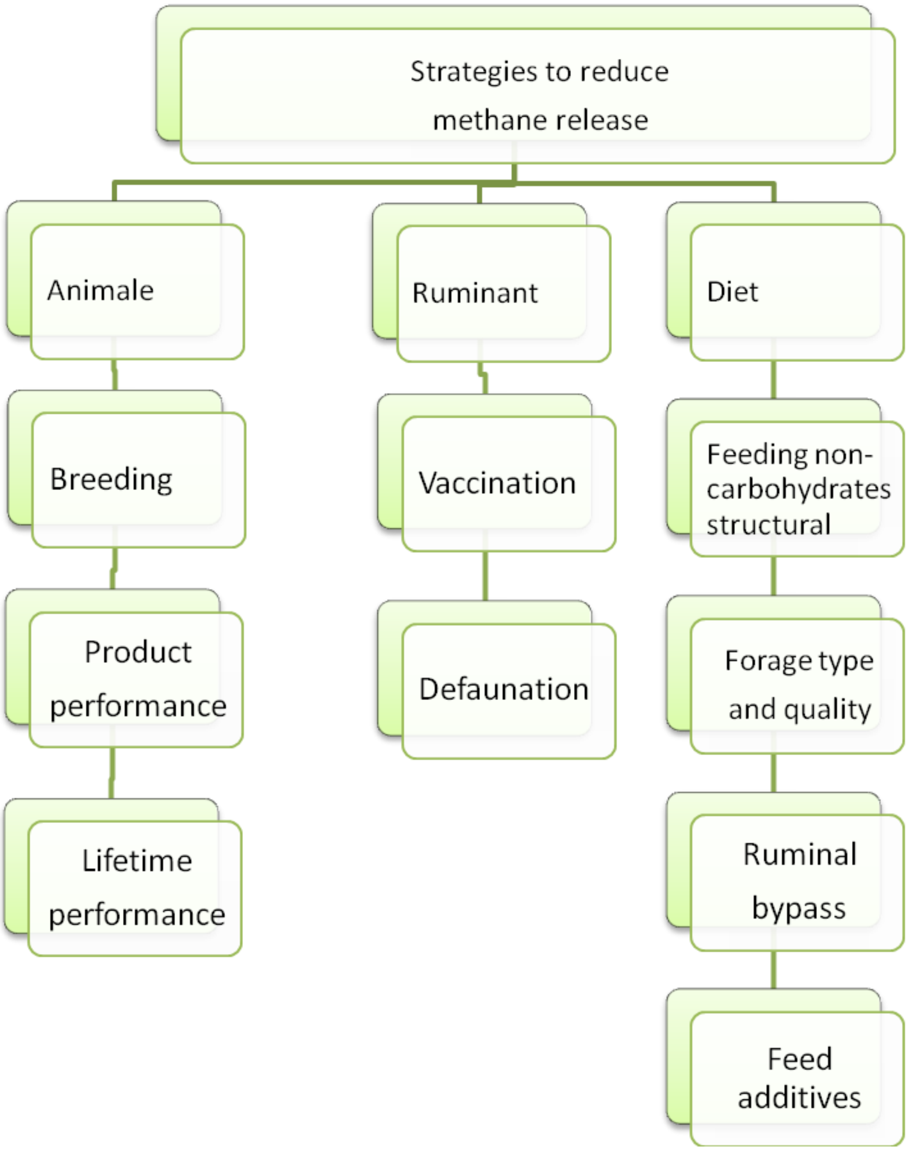


Figure 2. 7. Strategies to reduce enteric methane emission of ruminants (Eckard et al., 2010).

2.10 Tannins

The effect of tannin on the protein metabolism is illustrated in Figure 2.8. Tannins may combine with dietary protein and structural carbohydrates. This affects the digestibility in forages used for ruminants (Mueller-Harvey and McAllan, 1992). The effect of tannin depens on the amount of tannin in forages. Tannin will be toxic when the amount of tannin exceeds more than 50–100 g /kg DM of forages. On the other hand low level of tannin may have beneficial effect when the amount of tannin is lower than 2 % of diet since low level of tannin increases the bypass protein (Albrecht and Muck, 1991, Reed, 1995).

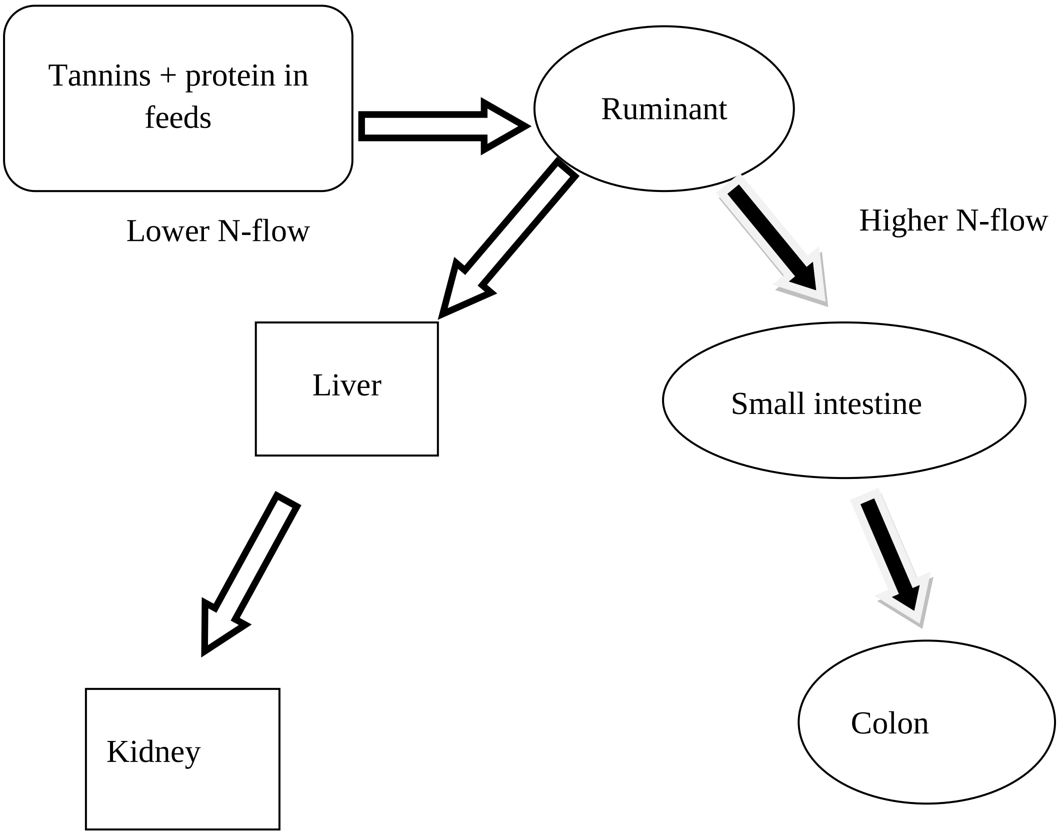


Figure 2. 8. The effect of tannin on the protein metabolism (Mueller-Harvey, 2006).

2.10.1 Chemical composition of tannin

Tannins are contain water soluble polyphenolic polymers of proportionately more molecular weight and have a big capacity to form complexes mainly with proteins due to the presence of a high number of phenolic hydroxyl groups. They take place in many nutritionally significant forage shrubs, trees, and legumes, fruits, cereals and grains. Tannins are usually classified into two groups (Fig. 2.9) condensed tannins (CT) and hydrolysable tannins (HT), (Patra and Saxena, 2010).

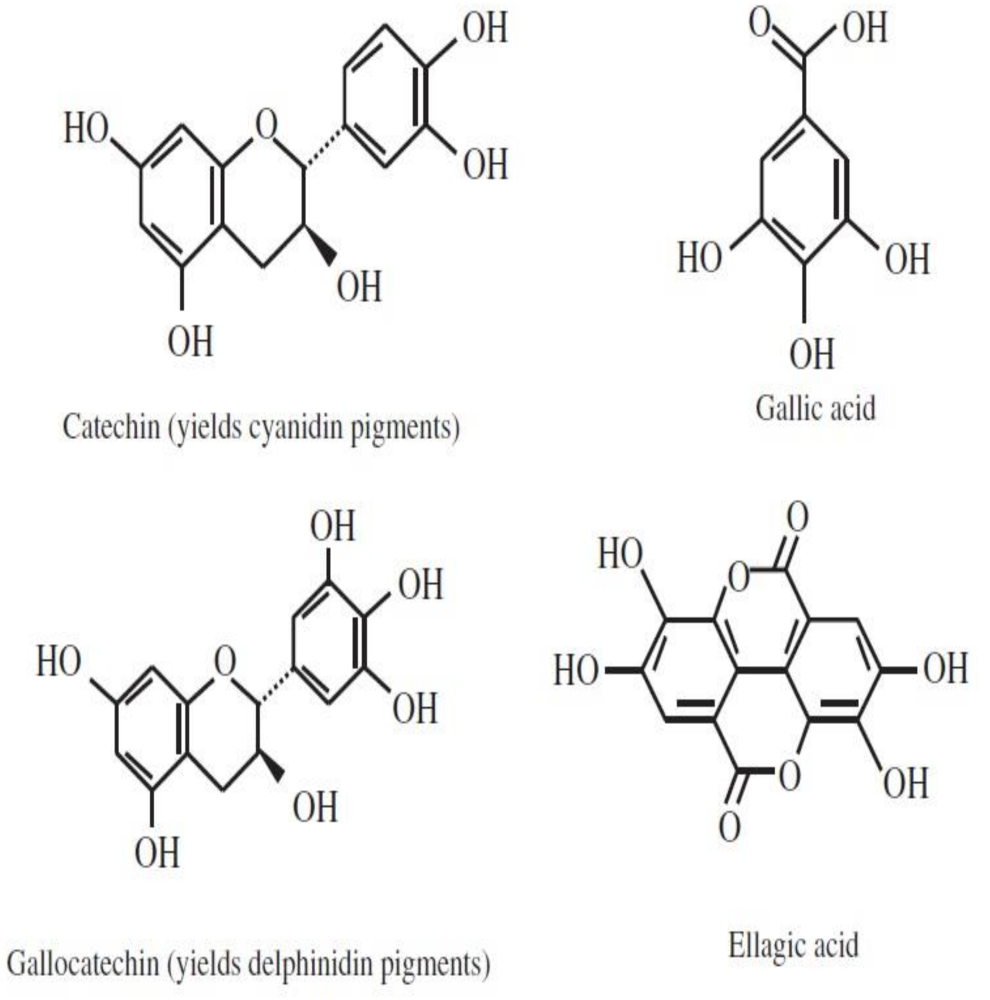


Figure 2. 9. Monomeric units of condensed (Catechin and gallocatechin) and hydrolysable tannins (Gallic and ellagic acid) (Patra and Saxena, 2010).

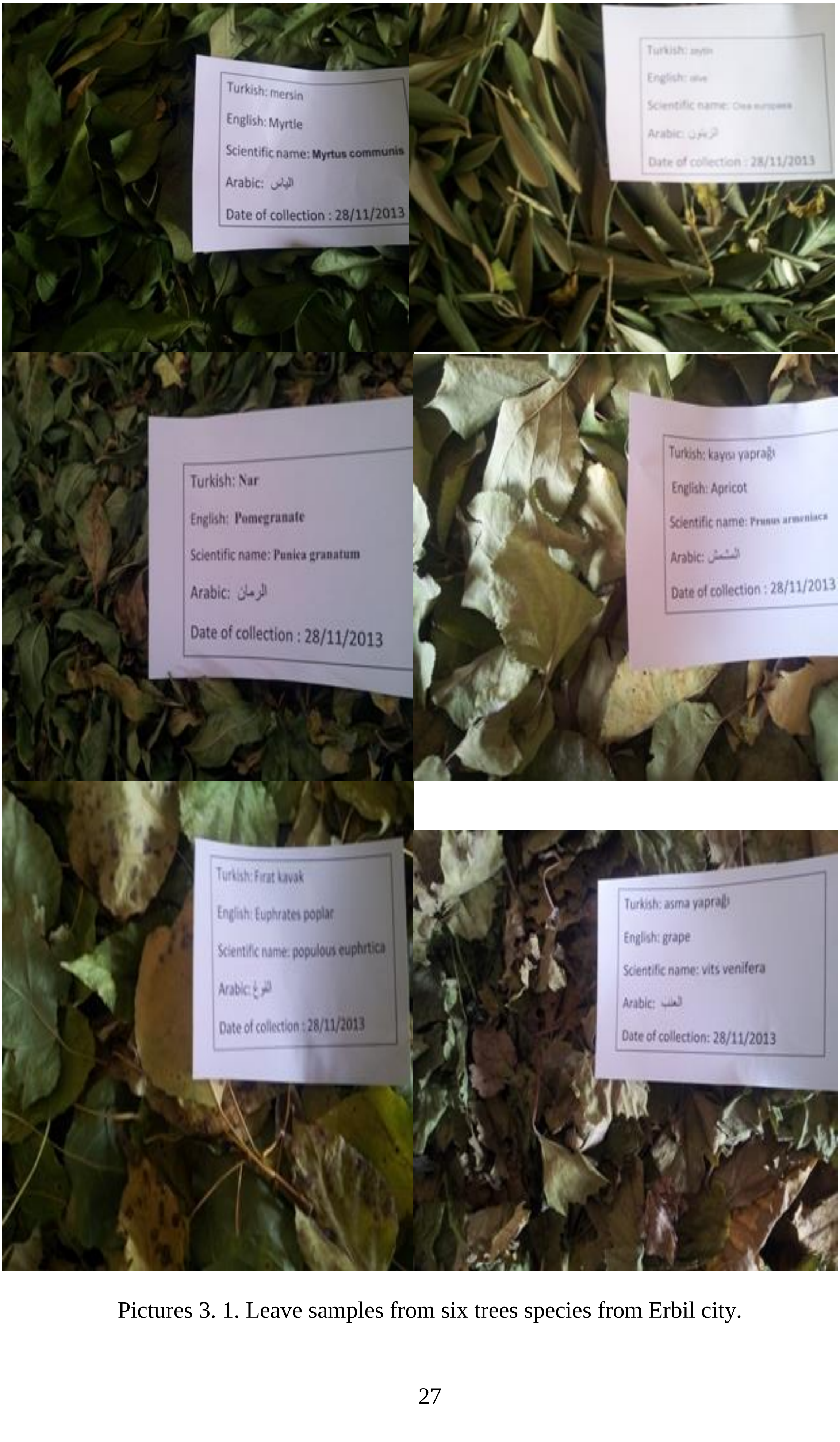
3. MATERIALS AND METHODS

3.1 Collection of Tree Leave Samples

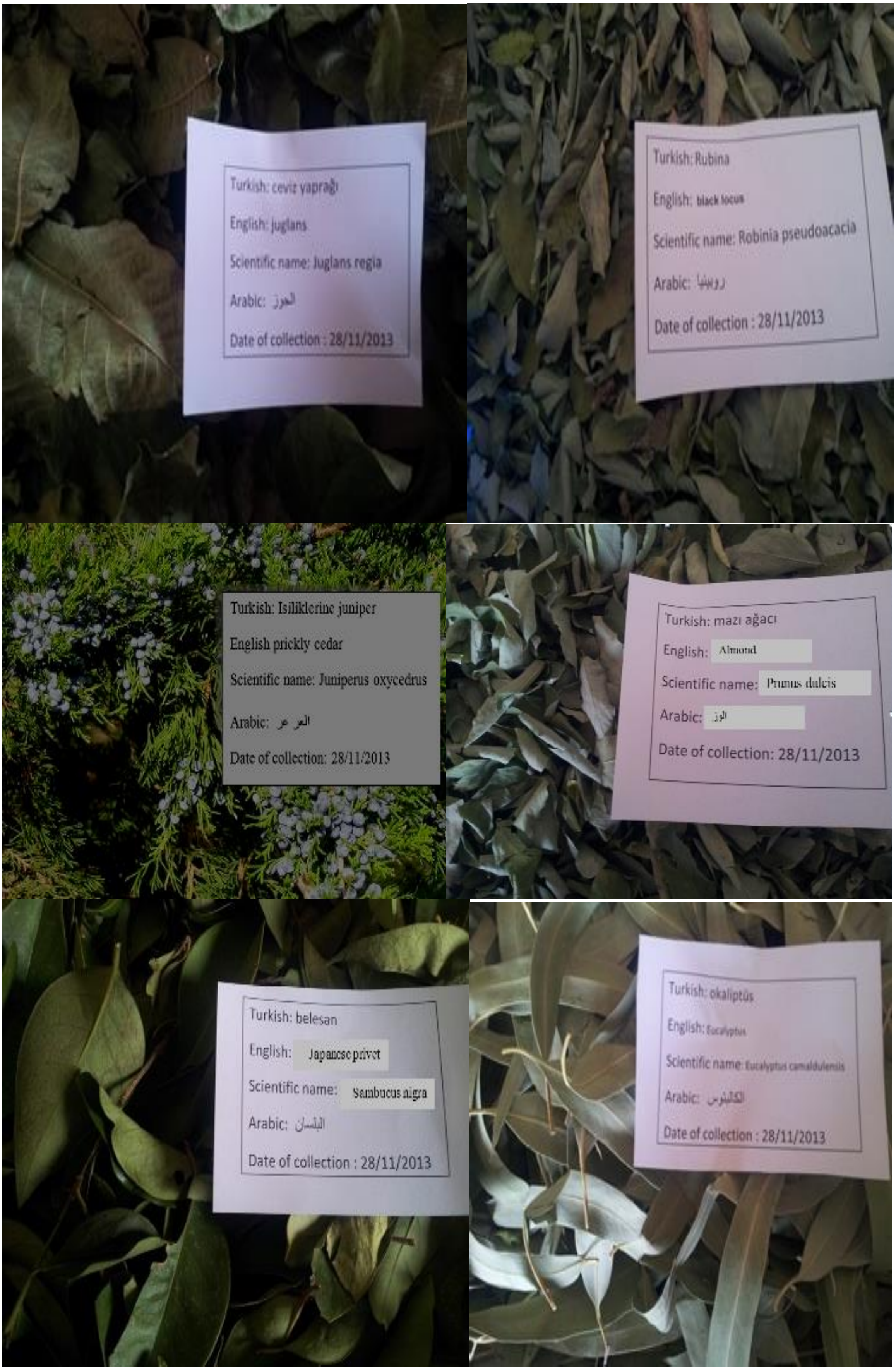
Twelve samples of tree leaves (Picture 3.1, 3.2 and Table 3.1) were collected around Erbil and Shaqlaw cities. Erbil is located in North of Federal Iraq. It lays between longitudes (43°, 20′, 35″ E) (44°, 04′, 55″ E) and the latitudes (35°, 25′, 33″ N) (37°, 19′, 13″ N). It is higher than the sea level 390 m. Also, Shaqlawa city located to the north of Erbil city it is 966m higher than the sea level at the situation Sefin Mountain. It is near 41km from Erbil it is a nice weather and temperature in summer reaches 30-35°C and 10°C below in winter. It is cover by rich trees and gardens. That collected samples during the months of December (28/11/2013).The mean monthly maximum and minimum temperatures of the Erbil site are 35.5 and 30.30°C, respectively (Abdullah, 2014).

Table 3. 1. List of the selected tree leaves from Erbil city in Northen Iraq.

Scientific name	Common name	Place
Prunus dulcis	Almond	Shaqlaw- Erbil
Juniperus oxycedrus L	Prickly cedar	Hujran – Erbil
Robinia pseudoacacia	Black locus	Sami Abdurahman park -Erbil
Myrtus communis	Foxtail Myrtle	Shaqlaw- Erbil
Punica granatum	Pomegranate	Shaqlaw- Erbil
Eucalyptus camaldulensis	Eucalyptus	Sami Abdurahman park -Erbil
Sambucus nigra	Japanese privet	Sami Abdurahman park -Erbil
Olea europaea	Olive	Xanzad - Erbil
Prunus armeniaca	Apricot	Shaqlaw- Erbil
vitis vinifera	Grape, Wine grap	Shaqlaw- Erbil
populous euphrtica	Indian poplar	Shaqlaw- Erbil
Juglans regia	Walnut	Shaqlaw- Erbil



Pictures 3. 1. Leave samples from six trees species from Erbil city.



Pictures 3. 2. Leave samples from six trees species from Erbil city.

Picture 3. 3. Animal nutrition laboratory at faculty of Agricultutre, Kahramanmaraş Sütçü İmam Üniversite.

3.2 Drying and Grinding of Tree Leave Samples (AOAC, 1995)

The collected tree leaves were dried at 60 ^{o}C in oven for two days. Dried tree leaves were ground to pass through 1 mm size using Wiley mill (Picture 3.4).

Picture 3. 4. Wiley mill

3.3 Determination of Dry Matter Contents (AOAC, 1990)

Equipment:

1. Balance (Picture 3. 5)

2. Dry substance containers (Aluminum foil container), (Picture 3.6).

3. Oven (Picture 3.7).

4. Desiccators (Picture 3.8).

Picture 3. 5. Balance

Picture 3. 6. Aluminum foil container

Picture 3. 7. Oven

Picture 3. 8. Desiccators

Procedure:

Empty containers at the first are weighted and then recorded (W1). Approximately 20 g for the tree leaves samples were weighted (W) in containers. The containers with samples were put in the drying oven in 105 ^{0}C for 24 hours. After that, containers were removed and cooled it in the desiccators and reweighed (W2). After that dry matter content of the tree leaves samples were determined as follows:

$$\text{DM (g/kg DM)} = \frac{W2 - W1}{W} \times 100$$

W1=Weight of containers.

W2= Weight after 24 h drying containers with samples.

W= Samples weight.

3.4 Determination of Ash or Organic Matter Contents (AOAC, 1990)

Ash is an inorganic part of the dry matter, which carbon does not contribute in the composition, including minerals and inorganic salts in the dry matter and remain in feed material after placed in the burning oven temperature of 525 ° C for 8 hour. Organic matter is the non-metallic part of the dry matter which carbon contributes in chemical structure. However, it turns to carbon oxides and water vapor by burning at high temperatures of about 525 ^{0}C and completely lost it consists of proteins, fats and carbohydrates.

Equipment

1. Balance (Picture 3.5)
2. Desiccators (Picture 3.8)
3. Muffle furnace (525 °C), (Picture 3.9)
4. Porcelain crucibles (picture 3.10)

Picture 3. 9. Muffle furnace

Picture 3. 10. Porcelain crucibles

Procedure

The crucible containers were burn at 525 ^{0}C and cooled in desiccators then weighted; approximately 1-2g from dried leave samples were weighted in containers and placed in a burning oven then heated to 525 ^{0}C for 8 hours, and then the containers was removed to desiccators and cooled it to room temperature. After than removed the sample from the furnace, cooled in desiccators to a room temperature and reweighed immediately.

The weight of the residual ash was then calculated as:

$$\text{Percentage Ash} = \frac{\text{Weight of Ash}}{\text{Weight of original of sample}} \times 100$$

And the organic matter can calculated as follows:

Organic matter %=Dry matter% - Ash%

3.5 Determination of Cell Wall Contents (Van Soest., 1975)

Equipment

1. Balance (Picture 3.5)

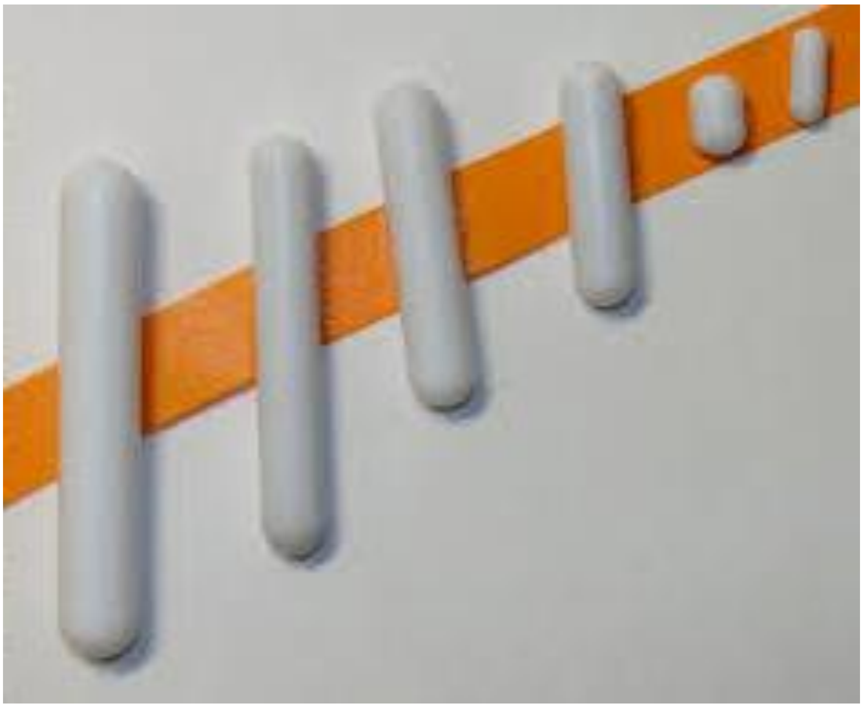
2. Oven (picture 3.7)

3. Desiccators (Picture 3.8)

4. Magnetic mixer (Picture 3.11)

6. Glass crucible (Picture 3.12)

7. NDF-boiling apparatus (Picture 3.13)



Picture 3. 11. Magnetic stirrer

Picture 3. 12. Glass crucible

Picture 3. 13. NDF-boiling apparatus

Chemicals

1. Cetyl-trimethylammoniumbromide (CTAB), ($C_{19}H_{42}BrN$).

2. Sodium dodecyl sulfate (SDS), ($C_{12}H_{25}O_4SNa$).

3. Ethylenedinitrilotetraacetic acid disodium salt dehydrates (EDTA), Titriplex III, ($C_{10}H_{14}N_2Na_2O_8 \times 2H_2o$).

4. Di-Sodium phosphate, ($NaHPO_4$).

5. Sodium tetraborate decahydrate, ($Na_2B_4O_7 \times 10H_2o$).

6. Triethylene glycol, ($C_4H_{10}O_2$).

7. Sodium metabisulfite, ($Na_2S_2O_5$).

8. Triethylene glycol, ($C_4H_{10}O_2$).

9. Sulfuric acid, (H_2SO_4).

3.5.1 Determination of acid detergent fiber (ADF)

Principle

Acid detergent fiber procedure provides a rapid method for determining lignocelluloses in tree leaves. The residue also includes silica, however. The difference between the cell walls (NDF) and acid detergent fiber is an estimate of hemicelluloses, although the difference does include some protein clinging to cell walls. The acid detergent fiber (ADF) is used to make ready step for lignin determination.

Procedure

For ADF analysis 20 g of Cetyl-trimethylammoniumbromide was dissolved in 1 lit 1 N (Normality) of sulfuric acid. Crucibles were dried at 105 ^{0}C for 2 hours and weighted (W1). Then approximately 0.5-1g of the leave sample were weighted (W). 100 ml of ADF solution was in addition to each sample, and then the crucibles with samples were boiled for 1 hour in ADF-boiling apparatus after heating. After boiling is completed, the crucibles were filtered then content was washed with acetone twice and distilled water. After that, crucibles were removed and dried at 80 ^{0}C for 10-12 hours and cooled in the desiccators and reweighed in sensitive balance (W2). Then ADF content of samples was determined as follows:

$$\text{ADF (g/kg DM)} = \frac{\text{W2} - \text{W1}}{\text{W}} \times 100$$

W1=Weight of crucibles

W2= Weight after drying crucibles with samples

W= Samples weight

3.5.2 Determination of neutral detergent fiber (NDF)

Principle

The neutral detergent procedure for cell walls is a fast method measuring the total fiber in fibrous plant tree leaves. It appears to divide the dry matter (DM) of feeds very near the point which separates the nutritive available and soluble constituents from those which are incompletely available or dependent on a microbial fermentation.

Procedure

For NDF analysis, 18.61g of ethylenedinitrilotetraacetic acid diazonium salt dehydrates (EDTA) and 6.81g of Sodium tetraborate decahydrate were weighted and put in a big beaker. Then 300 ml of distilled pure water was additional and the chemicals were dissolved after that, 30g of Sodium dodecyl sulfate and 10 ml of Triethylene glycol were dissolved in another beaker. Then this solution was added to the main solution. 4.56g of Di-Sodium phosphate was dissolved in a small beaker with distilled (pure) water by heating. After all the solutions were combined in a beaker and completed with distilled water to 1 lit, the pH of this solution was set to be 6.9-7.1 the crucibles were dried in an oven at 105 ^{0}C for 2 hours and weighted (W1). Approximately 0.5-1g (W) of dry leave samples were weighed into the crucibles than 100 ml of neutral detergent solution was added. After that, those crucibles with samples were boiled for 1 hour in NDF-boiling apparatus after heating. When boiling is finished the content was available. 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 were determined as follows:

$$\text{NDF (g/kg DM)} = \frac{\text{W2} - \text{W1}}{\text{W}} \times 100$$

W1= Weight of crucibles

W2= Weight after drying crucibles with samples

W= Samples weight

3.6 Determination of Crude Protein (AOAC, 1990)

Determination of total nitrogen (crude protein) is conducted using the Kjeldahl method.

This protocol consists of three necessary steps:

1. Digesting the feed sample in sulfuric acid in the presence of a catalyst, which results in the conversation of nitrogen to ammonia.
2. Ammonia distillation.
3. Determination of ammonia by titration with a standard solution (HCl).

Equipment

1. Sensitive (Picture 3.5)
2. Digestion block heater under fume hood, and install exhaust manifold (Picture 3.14)
3. Kjeldahl rack (Picture 3.15)
4. Kjeldahl distillation device (Picture 3.16)
5. Digital burette (Picture 3.17)
6. Kjeldahl flasks, 800 ml.
7. Conical flask 250-500 ml.

Picture 3. 14. Kjeldahl digestion unit for protein

Picture 3. 6. Kjeldahl rack

Picture 3. 7. Kjeldahl distillation device

Picture 3. 8. Digital burette

Chemicals

1. H $_2SO_4$, concentrated (98% w/w, d=l .84).

2. Sodium hydroxide (titrant), concentration (c = 0.1 mol/l).

3. H_3BO_3 (Boric acid) solution (w/w); 40 g pure boric acid weighed in 1000 ml beaker and add 800 ml of distilled water then slightly mixing than add 10 mL from metal red (0.1 gr From metal red powder in 100 mL Ethanol) and 7 mL from Bromisel Green (0.1 G from Bromisel Green in to 100 mL Ethanol).

4. Kjeldahl tablets composed of 10g anhydrous potassium sulfate (K_2SO_4), 0.3g copper sulfate ($CuSO_4$) anhydrous, 0.1g pumice.

5. Hydrochloric acid (HCl).

Digestion

1. Place approximately 0.5-1g sample, 2 Kjeldahl tablets and 20 ml sulfuric acid were add-ed to each Kjeldahl flask into labeled Kjeldahl flask.

2. Add 25 mL of concentrated H_2SO_4 for one gram samples or aqueous samples.

3. Turn blower on, and set burners on 370°C- 400°C for 2- 3 hours.

4. Digest until clear, and then set on 'Hi' for 30 more min. rotate flasks occasionally throughout digestion.

Distillation

1. Add 30 mL of boric acid solution to a 500 mL Erlenmeyer flask and label appropriately. Place the delivery tube from the condenser into the boric acid.

2. Turn water on to distillation system and set burners on '4'.

3. Add NaOH slowly to Kjeldahl flask.

4. The samples were distillated for 3 minutes and the color of boric acid was changed from the red to blue.

5. Remove receiving flask and replace with beaker containing 400 mL of deionized water.

7. Titrate distillate with 0.1N HCl, recording amount of acid used

8. Clean glassware and area thoroughly

$$\text{Crude Protein \%} = \frac{N \times ME \times 6.25\,(\text{Acid used titration} - \text{Acid ml used in Blank}}{W} \times 100$$

W=Sample weight

ME= The milligram equivalent weight of nitrogen which is 0.014

N= The normality of the acid used in titration of distillate (use 0.1 N)

Acid = Usually HCl or H_2So_4 used for titration.

3.7 Determination of Ether Extract (AOAC, 1995)

Equipment

1. Balance (Picture 3.5)
2. Oven (Picture 3.7)
3. Soxhlet appar(Picture 3.18)
4. Extraction beakers (Picture 3.19)
5. Extraction thimbles (Picture 3.20)

Picture 3. 9. Soxhlet apparatus with control unit

Picture 3. 19. Soxhlet extraction beaker

Picture 3. 20. Extraction thimbles

Materials:

Anhydrous ethyl ether

Extraction thimbles

100-mL tall-form beakers

2 g samples in filter paper envelopes

Procedure: (AOAC, 2002)

Two grams of the leave samples were transferred into the extraction thimbles. Then thimbles were extracted with ethyl ether using Soxhlet apparatus for 3 hours. Ether extract in flask was calculated as follow:

$$\%\text{Ether Extract} = \frac{W2 - W1}{W} \times 100$$

W1= Mass of flask before extraction (gram)

W2= Mass of flask after fat extraction (gram)

W= Leave sample (gram)

3.8 Determination of Condensed Tannins (Makkar, 2003)

Equipment

- Balance (Picture 3.5)
- Soveril Test tubes (Picture 3.21)
- Transferring the soveril tubes into ice to stop the reaction(Picture 3.22)

0.01 gram of leave samples was weighed in triplicate tubes and 6 ml butanol-HCl reagent (95 ml butanol +5 ml HCl + 0.05 gram $Fe_2SO_4.7H2O$) added. The tubes were then placed into boiling water (100 ^oC) and heated for 1 h after which they were removed, cooled and centrifuged at 3000 X g for 100 minutes. The supernatant was decanted into vials and absorbance read at 550 nm using a CE 2030 single beam spectrophotometer (Cecil Instruments, England). Blank samples containing the reagent were only included in the measurements.

Picture 3. 21. Soveril tubes

Picture 3. 22. Transferring the soveril tubes into ice to stop the reaction.

3.9 Determination of *In Vitro* Gas Production (Makkar et al. 1979)

Equipment

- Balance (Picture 3.5)
- Circulating water bath with holder for syringes (Picture 3.23)
- Transferring the buffered rumen fluid into syringes (Picture 3.24)
- CO_2 cylinder (Picture 3.25)
- Syringe special for methane measurement (Picture 3.26)
- Fistulated Awassi sheep (Picture 3.27)

Procedure

Approximately 0.200-0.209 mg of leave samples were weighed carefully in 100 ml calibrated glass syringe with pistons oiled with Vaseline. All incubation was carried out in quadruplicate. Four syringes without leave samples were also incubated as blank. 30 ml of buffered rumen fluid was transferred into syringes containin leave 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.

Micromineral solution

13.2 g $CaCl_2.2H_2O$ + 10.0 g $MnCl_2.4H_2O$ + 1.0 g $CoCl_2.6H_2O$ + 0.8 g $FeCl_2.6H_2O$ were dissolved in 100 ml distilled water.

Bicarbonate buffer

35 g $NaHCO_3$ + 4 g (NH_4) HCO3 were dissolved in 1 liter distilled water

Resazurine

100ml resazurine 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.

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 than 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 complete all removal of gas emission. The clip was closed carefully. The initial volume was recorded. Then syringes were placed in a water bath set at 39 °C. The gas measurement was carried out after 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) (Goel et al. 2008). After measuring gas produced at 24 h incubation, gas samples was transferred into inlet of the infrared methane analyzer using the plastics syringe. The infrared methane analyzer displays methane as percent of total gas. Methane production (mL) was calculated as follows:

Methane production (mL) = Total gas production (mL) × Percentage of Methane (%)

3.10 Determination of Metabolizable Energy Contents (Menke et al., 1979)

Metabolizable energy (ME) (MJ/kg DM) content of tree leave were calculated by using equation of Menke et al. (1979) as follows:

$$ME\ (MJ/kg\ DM) = 1.68+0.1418GP+0.073CP+0.217EE-0.028ASH$$

GP: Gas production (ml) at 24 h

CP: Crude protein (%)

EE: Ether extract (%)

3.11 Determination of Organic Matter Digestibility (Menke et al., 1979)

Organic matter digestibility (OMD) (%) of tree leaves was calculated using equation of Menke et al. (1979) as follows:

$$OMD\ (\%) = 14.88+0.889GP+0.45CP+0.0651Ash$$

OMD: Organic matter digestibility (%)

GP = Net gas production at 24 h (ml/200 mg)

CP = Crude protein (%)

Picture 3. 23. Water bath with holder for syringes

Picture 3. 24. Transferring the buffered rumen fluid into syringes

Picture 3. 25. CO_2 cylinder

Picture 3. 26. Syringe containing feed samples

Picture 3. 27. Fistulated Awassi sheep

3.12 Statistical Analysis

The effect of species on the chemical composition, *in vitro* gas production, methane production and estimated parameters of tree leaves were tested using ANOVA. Significance between individual means was identified using Tukey's multiple range tests (Pearse and Hartley 1966). Standard errors (SE) of means were calculated from the residual mean square in the analysis of variance. A simple correlation analysis was used to find the relationship between chemical compositions, *in vitro* gas production, metabolize energy and organic dray mater parameters.

4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 The effect of species on the chemical composition of tree leaves

The large variations in chemical constituents among tree leaves from Erbil in Iraq were given in Table 4.1. Dry matter (DM) contents of tree leaves ranged from 90.47% in *Prunus dulcis* to 97.95% in *Prunus armeniaca*. The crude ash contents of tree leaves samples ranged from 5.49% in *Juniperus oxycedrus* to 18.83% in *Prunus armeniaca.* Crude protein contents ranged widely from 9.23% to 16.23 %. The highest value was found for *Robinia pseudoacacia,* but the lower value was found for *Oleaeuropaea*.

Neutral detergent fiber contents of tree leaves varied from17.93 % for *Punica granatum* to 59.32% for *Juniperus oxycedrusL*. Acid detergent fiber contents of tree leaves ranged widely from 10.43 % in *Punica granatum* to 38.19% in *Juniperus oxycedrus.* Ether extracts content of tree leaves samples ranged from 4.35% in *Robinia pseudoacacia* to 16.3% in *Olea European*. Condensed tannin contents of tree leaves samples ranged from 1.04 % in *Sambucus nigra* to 14.45% in *Juniperus oxycedrus*.

Table 4. 1. The effect of species on the chemical compositions of tree leaves from Erbil-Iraq.

Scientific name	DM	CA	CP	NDF	ADF	EE	CT
Prunus dulcis	90.47^{g}	15.61^{cd}	14.84^{b}	33.46^{c}	19.00^{e}	5.60^{fg}	8.76^{b}
Juniperus oxycedrus	96.90^{bcd}	5.49^{i}	10.90^{f}	59.32^{a}	38.19^{a}	5.93^{ef}	14.45^{a}
Robinia pseudoacacia	90.64^{g}	14.09^{ef}	16.23^{a}	29.97^{de}	19.51^{e}	4.35^{h}	6.11^{c}
Myrtus communis	95.72^{ef}	14.71^{de}	12.04^{de}	18.82^{i}	16.00^{f}	10.13^{c}	1.22^{e}
Punica granatum	96.06^{de}	16.69^{g}	11.73^{def}	17.93^{i}	10.43^{g}	6.82^{de}	1.43^{e}
Eucalyptus camaldulensis	94.89^{f}	17.43^{b}	11.08^{ef}	28.73^{ef}	24.31^{c}	10.82^{bc}	1.65^{e}
Sambucus nigra	96.69^{cde}	10.54^{g}	11.06^{ef}	31.17^{d}	24.11^{c}	4.47^{gh}	1.04^{e}
Olea europaea	97.83^{ab}	7.01^{h}	9.23^{g}	40.67^{b}	29.69^{b}	16.30^{a}	1.17^{e}
Prunus armeniaca	97.95^{a}	18.83^{a}	13.37^{c}	21.88^{h}	15.03^{f}	11.86^{b}	4.55^{d}
Vitis vinifera	96.21^{de}	13.38^{f}	11.26^{ef}	27.36^{f}	18.32^{e}	7.39^{d}	7.71^{b}
Populous euphrtica	97.25^{abc}	15.31^{de}	12.80^{cd}	26.07^{g}	22.14^{d}	9.92^{c}	3.43^{d}
Juglans regia	97.42^{abc}	16.86^{bc}	12.84^{cd}	23.49^{h}	14.70^{f}	7.68^{d}	4.41^{d}
SEM	0.273	0.365	0.310	0.566	0.440	0.338	0.359
Significance	***	***	***	***	***	***	***

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

4.1.2 The effect of species on the gas and methane production of tree leaves

The effect of species on the gas production and methane production of some tree leaves is given in Table 4.2. As can be seen, the species had a significant effect on the gas production and methane production of some tree leaves. Total gas production of tree leaves ranged from 23.00 ml in *Eucalyptus camaldulensis* to 44.50 ml in *populous euphrtica*. Total methane production of tree leaves ranged from 4.52 ml in *Eucalyptus camaldulensis* to 8.70 ml in *populous euphrtica*. The percentage of methane gas ranged from 17.54 % in *Punica granatum to 22.44* % *in Prunus dulcis.*

4.1.3 The effect of species on the metabolizable energy and organic matter digestibility of tree leaves

The effect of species on the OMD and ME contents of tree leaves Erbil in Iraq is given in Table 4.2. Species had also significant effect on the OMD and ME contents of tree leaves. The ME contents of tree leaves ranged from 7.40 in *Juniperus oxycedrus* to 10.76 MJ/kg DM in *Prunus armeniaca.* The OMD values of tree leaves ranged from 47.12 % in *Juniperus oxycedrus* to 71.14 % in *Prunus armeniaca.*

Table 4. 2. The effect of species on the *in vitro* gas production, methane production, metabolisable energy and organic matter digestibility of tree leaves.

Scientific name	TGP	CH_4 (ml)	CH_4 (%)	ME	OMD
Prunus dulcis	28.75^{ef}	6.44^{bcde}	22.44^{a}	7.61^{e}	57.25^{cd}
Juniperus oxycedrus	26.75^{ef}	5.56^{de}	20.81^{ab}	7.40^{e}	47.12^{e}
Robinia pseudoacacia	30.25^{de}	6.21^{cde}	20.60^{abc}	7.70^{de}	58.22^{c}
Myrtus communis	40.25^{ab}	7.99^{abc}	19.87^{abc}	10.05^{ab}	65.64^{ab}
Punica granatum	36.75^{bcd}	6.45^{bcd}	17.54^{c}	8.92^{c}	59.78^{bc}
Eucalyptus camaldulensis	23.00^{f}	4.52^{e}	19.44^{abc}	7.61^{e}	51.64^{de}
Sambucus nigra	39.00^{abc}	8.49^{a}	21.78^{a}	8.69^{cd}	61.37^{bc}
Olea europaea	30.00^{def}	6.19^{cde}	20.43^{abc}	9.94^{ab}	50.26^{e}
Prunus armeniaca	42.75^{ab}	8.45^{a}	19.78^{abc}	10.76^{a}	71.14^{a}
Vitis vinifera	32.75^{cde}	5.92^{de}	18.11^{ab}	8.37^{cde}	57.75^{cd}
Populous euphrtica	44.50^{a}	8.70^{a}	19.54^{abc}	10.64^{a}	70.15^{a}
Juglans regia	38.00^{abc}	8.26^{ab}	21.70^{a}	9.20^{bc}	65.40^{ab}
SEM	2.012	0.553	0.892	0.285	1.790
Significance	***	***	***	***	***

$^{a\ b\ c}$ *Column means with common superscripts do not differ (P>0.05);* ***S.E.M****: standard error mean;* ***TG****: Total gas production at 24 h incubation,* ***CH_4****: Methane production (ml),* ***CH_4****: Methane production (%),* ***ME*** *:Metabolisable energy(MJ/kg DM) ,* ***OMD*** *:Organic matter digestibility(%), *** P<0.001.*

4.1.4 Correlations between chemical composition and gas production parameters

There were significant correlations between chemical composition and estimated of some tree leaves from Erbil in Iraq given in Table 4.3. The contents of DM negatively correlated with CP and CH_4 (%) but positively correlated with EE, total gas production, methane production and ME vontents of tree leaves ($p<0.001$). The NDF contents of tree leaves were positively correlated with ADF and CT but negatively correlated with total gas production, CH_4 (ml), ME and OMD of tree leaves. The ADF contents of tree leaves were negatively correlated with CP, gas production, methane production, ME and OMD values of tree leaves. The EE contents of tree leaves were positively correlated with CT but positively correlated with ME contents. The ash contents of tree leaves were negatively correlated with NDF and ADF but positively correlated with OMD of leaves. Condensed tannin of tree leaves were negatively correlated with CP, gas production, methane production, ME and OMD of tree leaves. Crude protein negatively correlated with EE but positively correlated with OMD contents of tree leaves.

Table 4. 3. Correlation coefficient (r) between chemical composition and gas production parameters of some tree leaves.

	DM	NDF	ADF	EE	ASH	CT	CP	TGP	CH_4	CH_4%	ME
NDF	0.000ns										
ADF	0.140ns	0.930***									
EE	0.516***	-0.076ns	0.103ns								
ASH	-0.203ns	-0.697***	-0.650***	0.044ns							
CT	-0.241ns	0.671***	0.447*	-0.427*	-0.250ns						
CP	-0.708***	-0.281ns	-0.433*	-0.490***	0.546***	0.229ns					
TGP	0.453*	-0.586***	-0.511***	0.079ns	0.300*	-0.388*	0.115ns				
CH_4	0.365*	-0.448**	-0.388**	0.008ns	0.299*	-0.324**	0.187ns	0.934***			
CH_4%	-0.287*	0.382*	0.314**	-0.238ns	-0.004ns	0.236ns	0.275ns	-0.127ns	0.231ns		
ME	0.617***	-0.475*	-0.327*	0.624***	0.232ns	-0.524***	-0.155ns	0.823***	0.734***	-0.218ns	
OMD	0206ns	-0.473***	-0.685***	0.023ns	0.651***	-0.370*	0.394*	0.913***	0.869***	-0.070ns	0.717***

DM*: dry matter;* ***CP****: crudee protein;* ***ADF****: acid detergent fiber;* ***NDF****: neutral detergent fiber;* ***CT****: condensed tannin.* ***TG****: Total gas production at 24 h incubation,* ***CH_4****: Methane production (ml),* ***CH_4****: Methane production (%),* ***ME:****Metabolisable energy (MJ/kg DM),* ***OMD****: Organic matter digestibility (%), ***P < 0.001; NS: nonsignificant (P > 0.05).*

4.2 Discussion

4.2.1 The effect of species on the chemical composition of tree leaves

It is well established that food contents play the most important role in the health and longevity of organisms as well as the environmental concerns. Various methods have so far been suggested to estimate the proportion of good and bad contents of feeds. One of the most common ways partially describing and estimating of chemical compositions is the wet chemical analysis. This method is widely employed to predict the digestibility and nutritional values in tree leaves due to the rapidity and low cost advantages (Van Soest, 1994).

It was reported that the chemical compositions of tree leaves can be affected by many factors such as species, stage of growth (Von Kyerserlingk et al., 1996; Agbagla-Dohnani et al., 2001), growth environment, drying method (Mupangwa et al., 1997) and type of soils (Thu and Preston, 1999) however most of these factors may stand for partial variations in the chemical composition between these results and previous studies .Rubanza et al. (2005) suggest that plant genetic structures can be a potential source of variations among the nutrient contents of their leaves.

The current study clearly showed that therae are significant differences among tree species in terms of chemical compositions (Table 4.1). The CP value of *Prunus dulcis* were considerably higher than that reported by Getachew et al. (2002). Although, CP content of *Robinia pseudoacacia* had low CP when compared to some of Acacia species reported by Rubanza et al. (2005). As can be seen from Figure 4.1, CP contents of tree leaves in the present study was found in all above the recommended level for optimum maintenance and growth performance. Minson (1990) and Norton (2003) reported that CP more than 7% is required for optimum growth of ruminant animals to maintane the sufficient ammonia level.

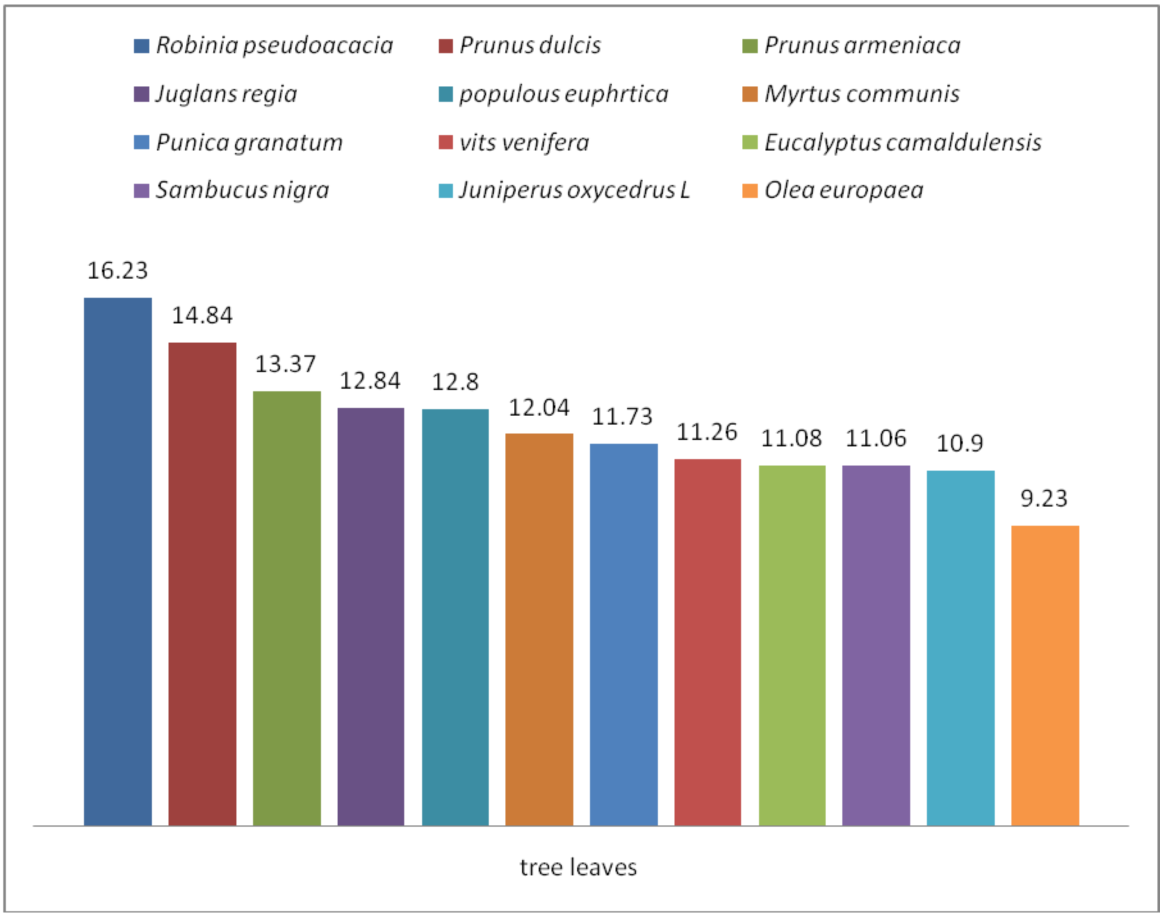


Figure 4. 1. Tree leaves with crude protein more than 7%.

The increased leaf surface area increses growing rate, DM and protein content of leaves due to increased number of photosynthetic organ per unit of leaf surface (Hattab and Harb, 1990).

The chemical composition of tree leaves studied in the current experiment was considerably different from those previously investigated. The amount of CP in the *Myrtus communis* was slightly lower than that reported by Gasmi et al. (2005) who indicated that CP of *Myrtus communis* was 13%. The crude protein contents of tree from *Eucalyptus camaldu-lensis* was also lower than that repoted by Sallam et al. (2009) who obtained that CP of levaes of *Eucalyptus camaldulensis* was (9.5%). The CP content of tree leaf of *Olea europaea* was considerably higher than that reported by Garcia et al. (2006). The crude protein (CP) of leaves from *Punica granatum* was lower than that reported by Abarghuei and Salem (2014).

The CP content of the tree leaves namely *Robinia pseudoacacia*, *Prunus dulcisand* and *Prunus armeniaca* is higher than 13 %, which is indicating that these tree leaves can be used to improve the low crude protein contents of diets during the shortage time.

Althogh crude ash content is used to estimate the quantity and quality of inorganic contents in foods, crude ash contents is not a good parameter for this purpose because during ignition some volatile substances such as potassium, sodium, chloride, sulphur and phosphorus could be lost (Rosales, 1996). Crude ash content of *Prunus dulcis* was considerably more than that obtained by Getachew et al. (2002).

Cell wall content consists of cellulose, hemi cellulose and lignin. Neutral detergent fiber consists of cellulose, hemicelluse and lignin. Acid detergent fiber consists of cellulose and lignin (Van Soest, 1975).

According to Allen (2000), higher levels of NDF in forage are related to a lower dry matter (DM) intake. This limitation was caused by the rumen fill, with diets with NDF content above 60% (Berchielli et al., 2006).

The ADF and NDF contents for *Prunus dulcis* were lower than those reported for by Getachew et al. (2002). The NDF and ADF values for *Myrtus communis* were lower to those reported by Gasmi et al. (2005). The NDF and ADF values for *Punica granatum* were lower than those reported by Abarghuei and Salem (2014).

Fat in tree leaves consists mainly of mono- di-and triacylglycerides, free fatty acids and phospholipids. Forages usually harbor animal fats and further waste products. It is suggested that processes of rendering for example heating and/or storing may cause unsaponifiable issues, oxidizing and polymerizing of fatty acids and these can contribute to crude fat values, but not be of nutritional value (Edmunds, 1990).

The ether extracts (EE) content of tree leaf sample *Olea europaea* were considerably lower than that reported by Garcia et al. (2006). Although EE content of *Punica granatum* in the current research was higher EE content that reported by Bhowmik et al. (2013), but lower than that reported by Abarghuei and Salem (2014).

In the current study the tree leaves had a significant amount of tannins. This high level of tannin may have some possible effects on the nutritive value of tree leaves. However the effect of tannin depends on the amount of tannin in tree leaves. The condensed tannin in feedstuffs may combine with feed protein and bacteria resulting in decrease in digestibility of feedstuffs. This process can lead to reduction of bacterial attachments to plant molecules (McAllister et al., 1994). On the other hand, low level condensed tannin in feedstuffs may have beneficial effect of nutritive value of feedstuffs. Tannin combines with protein and decresed the degradation of protein in the rumen and increase the bypass protein which can be digested post-ruminally (Barry, 1989; Reed, 1995; Silanikove et al., 1996)

Acording to the results of the current study tree leaves from *Sambucus nigra* seems to be promising for ruminant animals to obtain the beneficial effect of low level tannin since due to high protein content and low level of tannin.

Tannins can make poor digestible complexes with nutritional proteins and internal proteins like digestive juice in stomach and intestines (Kumar and Singh, 1984). Hence, tannin can strongly influence the activity of enzymes and microbes (Singleton, 1981; Lohan et al., 1983; Barry and Duncan, 1984; Makkar et al., 1988; Silanikove et al., 1994). The effects of tannins on rumen fungi, protozoa, bacteria, and methanogens are variable and usually depend on the type of tannins, their supplementation levels and origin (Patra and Saxena, 2011).

As can be seen from figure 4.2, tannin contents of five tree leaves (*Sambucus nigra*, *Olea europaea* , *Myrtus communis* , *Punica granatum* and *Eucalyptus camaldulensis*) were lower than 2%. Therefore these tree leaves can be used to obtain the beneficial effect of low level tannin. On the other hand, Tannin content of leaves from *Juniperus oxycedrus* was very high, approximately 14.45%. This amount of condensed tannin may have detrimental effect on the animal.

The condensed tannin content of leaves of *Myrtus communis* was considerably lower than that reported by Gasmi et al. (2005), the condensed tannin content of leaves of of *Eucalyptus camaldulens* is was lower than that found by Sallam et al. (2009) while CT content of tree leaves of *Robinia pseudoacacia* lower than that reported by Sallam et al. (2009).

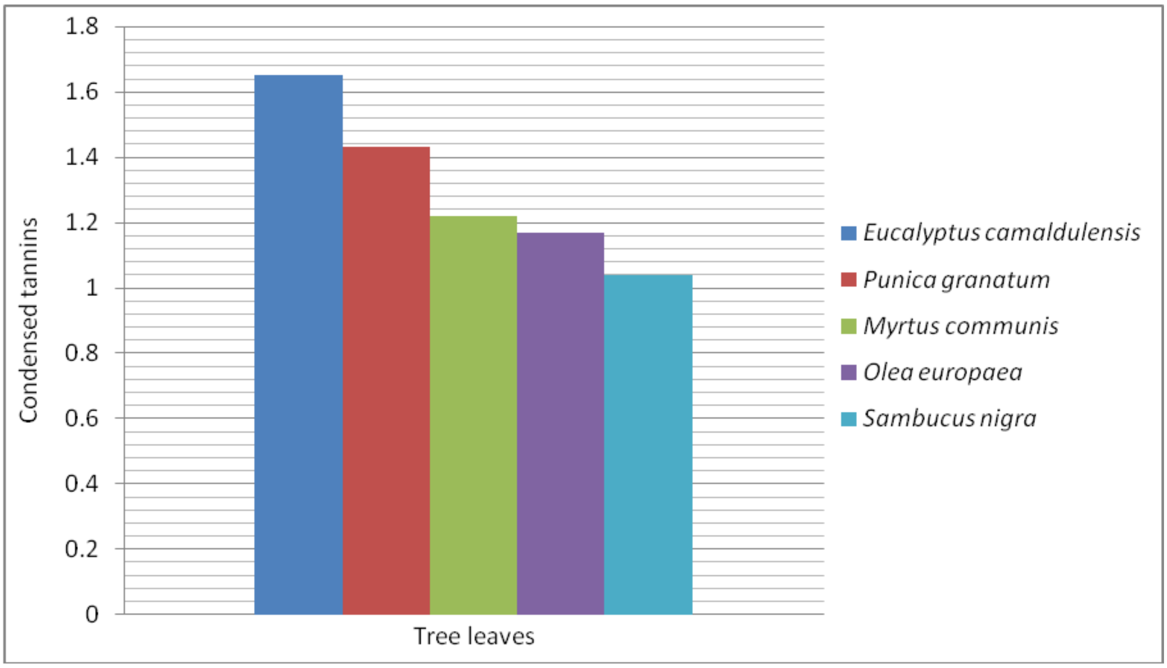


Figure 4. 2. Tree leaves with Condensed tanin lower than 2 %.

4.2.2 The effect of species on the metabolizable energy, organic matter digestibility and gas production of tree leaves

Metabolizable energy (ME) is estimated from digestible energy minus urine and gas energy. The principal components of urinary energy are incompletely oxidized nitrogenous compounds (primarily urea) and endogenous nitrogen constituents (primarily creatinine). Urine energy amounts ranges from 7 to 10% of fat contents in ruminants. Gaseous products arise from fermentation of food in the alimentary tract, and, as such, are really a digestive loss. However, it has been convention to group this loss with metabolic losses.

The ME content of leaf sample of *Prunus dulcis* was considerably higher than that reported by Getachew et al. (2004). Likewise the ME content estimated in *Robinia pseudo-acacia* was slightly lower than that reported by Ladipo and Akinfemi (2014). Also, ME content of leaves of *Punica granatum* was lower than that reported by Abarghuei and Salem (2014).

Since the estimated OMD value ranged between 40 and 67%, this was nevertheless appreciable since a digestibility value of 40 to 50% was recommended for high performance of ruminants in pasture (McDowell, 1985).

The OMD values of tree leave of *Eucalyptus camaldulensis* (51.64%) in the current study was comparable to that reported by Ladipo and Akinfemi (2014) who reported that

OMD values of tree leave of *Eucalyptus camaldulensis* was (52.66%).

Methane is most widely available gas in our biosphere and there is evidence that its concentrations have recently been increasing globally at a rate between 0.7% and 1.0 % year (Crutzen, 1995). Domestic ruminants are responsible for about 12.5% of global CH_4 emissions (Crutzen, 1995) Methane emissions are influenced by the size of the animal, the consuming quantity of feeds and the metabolic effectiveness of animals that converts feed to other products.

Gas production is basically the result of fermentation of carbohydrates to volatile fatty acids i.e., propionate, acetate and butyrate, and total gas production (TGP) from protein fermentation is proportionately lesser as compared fermentation of carbohydrate (Makkar et al., 1995).

Higher NDF decreases methane production by shifting short chain fatty acid proportion in the direction of acetate which produces more hydrogen (Carulla et al., 2005; Puchala et al., 2005; Tavendale et al., 2005). Taking these parameters into account, we can infer in this study the higher value of NDF in *Juniperus oxycedrus* is 59.32% affected the lower emission in total gas production is 26.75% in same sample (Table 4.2).

Lower concentrations of ADF and NDF, both characteristic of higher quality forages, are generally related to a lower proportion of dietary energy converted to methane (Machado et al., 2011). However, in the current study, the volume of methane produced by *Punica granatum* reflect their better chemical composition because low level of ADF 10.43%, NDF, 17.93% and tannin 1.4%. Similar results were reported by Canesin et al. (2010), who found higher values of methane production per gram of dry matter degraded in a diet with lower concentrations of ADF and NDF using the *in vitro* semi automated total gas production technique to evaluate the methane production from marandu grass the supplemented with different percentages of concentrate, and linked this result to higher fermentation rates (Oliveira et al., 2014).

Jayanegara et al. (2009) observed that substrates with higher ADF contents produced less total gas production and lower organic matter digestibility (OMD) as shown by the correlation coefficients between the respective parameters. Further, addition of concentrate may stimulate rumen microbial activity and population and, in turn, contributes to the overall fermentation and digestion process.

Tomich et al. (2003) indicated that the gas production reflects the degradation of the tested sample, the total gas production are the main factors for evaluating the quality of tree leaves. Taking these parameters into account, we can infer in this study that the *populous euphrtica* presented better fermentative quality since its high gas production in both tree leaves were higher than in other sample.

Ahmed et al. (2007) suggest a negative association between total gas productions and CT this is likely to be the consequences of decreasing activity of microbes by the adverse conditions. It is believed that tannins can inhibit bacterial cell growth, but cellulose digestion is not simply inhibited by tannins. Similar in current study maximum rate total gas production (TGP) in sample *populous euphrtica* 44.50 ml may be effect to decrease the rate of CT to 3.43 %.

4.2.3 Correlation between chemical compositions, total gas production, organic matter digestibility and metabolize energy

The highest gas production was recorded for *populous euphrtica*, *Prunusarmeniaca* and *Myrtus communis* which may be due to the tree leave cell contents (ADF and NDF). In the present study there was a strong negative correlation between gas productions. The gas productions of *Prunus dulcis* leaves were considerably higher than that reported by Getachew et al. (2004). The gas production of *Eucalyptus camaldulensis* leaves studied in this experiment was lower than that was reported by (Ladipo and Akinfemi, 2014).

There was a negative correlation between the contents of DM and CP which is in disagreement with the earlier findings of (Larbi et al., 1988). However, some studies suggested positive correlations between NDF and ADF indicating that as NDF concentration increased the ADF. A significant negative correlation ($P < 0.001$) was found between NDF and ADF with total gas production (TGP) in the present study and this is in agreement with findings of Kulivand and Kafilzadeh (2015), Bhatta et al.(2012), Nsahlai et al. (1994), Larbi et al. (1998), Getachew et al. (2004) and Seresinhe et al. (2012). The negative correlation between gas production and ADF may be due to the reduction of microbial activity from increasingly adverse environmental conditions.

As can be seen from Table 4.3, in thee current study, there is no significant correlation between EE and CH_4 although it is well indicated that the role of lipids in decreasing the total gas production (Beauchemin et al., 2008).

Although the EE contents of most of the samples in the current study were tree low the EE contents of levaes of *Olea europaea* was high, which approximately was 16.3%. The nature of lipid and its form are also determining factors in their effect on methane production (Beauchemin et al., 2008; Zeitz et al., 2013).

Several studies reported a negative influence of tannins on feed digestibility (Grainger et al., 2010; Jayanegara et al., 2009). Makkar (2003) showed that tannins are negatively correlated with *in vitro* rumen protein degradability, but the correlation in the present study was not statistically significant.

In the current study, tannin was negatively correlated with methane emission. The feedstuff with high CT produces less methane this is in agreement with findings of Njidda and Ikhimioyza (2010), Mueller (2004) and Getachew et al. (2004). A strong positive correlation was found between TGP and OMD ($P< 0.001$) and this is consistent with findings of Mauricio et al. (1999).

The total gas production (TGP) was positively correlated with ME of tree leaves measured in the current study and this is consistent with observations by Menke and Steingass (1988), Adu (2012) and Njidda and Nasiru (2010). In the current study strong positive correlations ($P < 0.001$) were observed among *in vitro* total gas production with CH_4, OMD this result agrees with findings of Bezabih et al. (2014). There was also a strong positive correlation between ME and OMD.

5. CONSLUSION

Species had a significant effect on the chemical composition, *in vitro* gas production, metabolisable energy and organic matter digestibility of tree leaves from Erbil province in Northern Iraq. The most of tree leaves studied in the current study have considerable amount of crude protein and metabılisable energy which are enough to meet the requirement of the ruminant animals for maintanence and production. However leaves from *Juniperus oxycedrus* have higher condensed tannin which would be detrimental to ruminant animals. Therefore condensed tannin content of leaves from *Juniperus oxycedrus* should be taken into consideration when leaves from *Juniperus oxycedrus* were included into ruminant diets. Condensed tannin contents of most of tree leaves studied in the current study was lower than 2%, which is suggesting that these tree leaves could be used as alternative forage to reduce the risk of bloating and methane emission in ruminants.

There were significant correlations between chemical composition and *in vitro* gas production or metabolisable energy contents of tree leaves. Cell wall contents were negatively correlated with *in vitro* gas production or metabolisable energy contents of tree leaves. Methane emission was negatively correlated with cell wall contents such as NDF and ADF. These results also suggest that analyzing of chemical components of plant species can potentially help in identifying the best sources of organic food for commercial farm animals.

These findings may offer good knowledge on the chemical composition and potential nutritive value of twelve plant species for farmers in Erbil province in northern Iraq to identify the best sources for their ruminant animals.

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