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BIOMASS YIELD DYNAMICS AND NUTRITIONAL QUALITY OF ALFALFA (*Medicago sativa)* CULTIVARS AT DEBRE ZEIT, ETHIOPIA

MSc. Thesis

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MSc Program in Tropical Animal Production and Health

June, 2014

Bishoftu, Ethiopia

BIOMASS YIELD DYNAMICS AND NUTRITIONAL QUALITY OF ALFALFA (*Medicago sativa)* CULTIVARS AT DEBRE ZEIT, ETHIOPIA

A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical Animal production and Health

By

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the MSc Thesis prepared by **Mekuanint Gashew Achenef** entitled in **biomass yield dynamics and nutritional quality of alfalfa (*Medicago sativa)* cultivars at Debre Zeit, Ethiopia.** And recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Tropical Animal Production and Health.

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DEDICATION

I dedicate this thesis to my beloved families who were beside me and for their unfailing encouragements throughout my life.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is the result of my own work and that all sources or materials used for this thesis have been duly acknowledged. This thesis is submitted in partial fulfillment of the requirements for an MSc degree at Addis Ababa University and to be made available at the University's Library under the rules of the Library. I confidently declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADL	Acid detergent lignin
AOAC	Association of Official Analytical Chemists
BW	Body weight
CP	Crude protein
DAP	Diamonium Phosphate
DM	Dry matter
DDM	Digestible Dry matter
DMI	Dry matter intake
DZARC	Debre Zeit Agriculture Research Center
EIAR	Ethiopia Institute of Agricultural Research
EARO	Ethiopian Agriculture Research Organization
FAO	Food and Agricultural Organization of United Nation
INRA	Institute of National Agricultural Research of America
IVDMD	*In vitro* dry matter digestibility
LWR	Leaf weight ratio
LY	Leaf yield
MP	Metabolizable protein
NDF	Neutral detergent fiber
NFE	Nitrogen free extract
NIRS	Near infrared reflectance spectroscopy
NRC	National Research Council
NSC	Non structural carbohydrate
RCBD	Randomized complet block design
RFQ	Relative feed quality
RFV	Relative feed value
SPSS	Stastical program for social science
SY	Stem yield
TDMY	Total dry matter yield

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ABSTRACT

The study was conducted at Debere Zeit Agricultural Research Center (DZARC) of Ethiopian Institute of Agricultural Research (EIAR)located 47.9 km from Addis Ababa. The objective was to determine biomass yield dynamics and nutritional quality of five alfalfa (Medicago Sativa) cultivars. The cultivars were planted on 12 m^2 plots (4 m long and 3 m wide) in randomized complete block design with four replications. The treatments were: FG10-09(F), FG9-09(F), Magna 801-FG(F), Magna 788 and Hairy Peruvian. The leaf and stem yield were determined by harvesting a central section of two adjacent middle rows with a sampling area of 0.2 m^2 (0.5 m length x 0.4 m width). For forage quality analysis, four randomly selected adjacent middle rows with a net area of 3.2 m^2 were harvested. The four replications were pooled into one and properly homogenized and one representative subsample was taken for each cultivar within each cutting cycle. The analysis of variance indicated that; interaction of cultivar and cutting cycle was not significant for leaf, stem and total biomass dry matter yield and chemical composition of the cultivars ($P>0.05$). Similarly, cultivars effect was not significant ($P>0.05$) for leaf, stem and total biomass DM yields. Magna 788, Hairy Peruvian and FG9-09(F) had higher leaf yield (1129 kg/ha), stem yield (1984 kg/ha) and herbage total DM yield (2861.5 kg/ha respectively other than the rest cultivars. Cutting cycles had significant effect ($P<0.05$) on leaf, stem and total DM yield. The 8th cycle had higher leaf, stem and total DM yields followed by 7th cycle, while the rest cycles had intermediate and comparable values. On the contrary the leaf to stem ratio was lowest. Cultivar and cutting cycles had significant effect ($P<0.05$) for leaf and stem proportion and leaf to stem ratio. The 6th cutting cycle had higher leaf to stem ratio comparable with second and fourth cycles. The effect of cultivar for chemical composition was not significant ($P \geq 0.05$). Though Magna 801-FG(F) had higher nutritional value other than the rest cultivars. Cutting cycles had significant effect ($P< 0.05$) for acid detergent fiber, digestible dry matter and cellulose content but not other chemical composition parameters. In general the cultivars had higher nutritional value and had lower biomass yield during drier season while higher during wetter season.

Key words; Alfalfa, Leaf and stem yield, chemical composition

1. INTRODUCTION

Livestock play a crucial role in Ethiopian agriculture. Currently, productivity per animal is very low, and the contribution of the sector to the overall economy is much lower than expected due, among others, to poor nutrition. The larger proportion of livestock feed comes from natural pastures and crop residues that are deficient in important nutrients like protein and energy (Tessema and Barras, 2006). Most of legume forages are a protein source in livestock nutrition and of which, those home grown feeds make farmers less dependent from the purchase of other protein source. This is an advantage for the farm economy and ecology, particularly because of restrictions concerning the environment since the prohibition of the use of animal protein in livestock nutrition. Moreover, the capacity of legumes to fix nitrogen from the air results in high protein contents, particularly in alfalfa (Gosselink, 2004).

Alfalfa is one of the most important forage crops worldwide due to its high forage quality and yield and adaptability to different climatic conditions (Turan *et al*., 2009). It can be used directly for grazing or conserved as silage or hay and is a reliable forage species that could represent a significant contribution to the livestock sector (Borreani and Tabacco, 2006). As a perennial legume, alfalfa may be used as a cover crop; its roots improve soil texture and its leaves add organic matter and nitrogen to the soil. The herbage DM yield and chemical composition of alfalfa depends on cutting cycles and cultivars, among others. Crude protein tends to be lower in aged alfalfa plants while the content of crude fibres increases (Stanaćev *et al*., 2008).

Forages provide 83% of the protein requirements of beef cattle and 90% of the protein requirement of sheep (Maheri *et al*., 2007). A major goal of alfalfa forage production is to provide a feed with sufficient protein and other nutrients to meet the requirements of livestock, particularly dairy animals. Alfalfa can produce around 25% more dry matter than pasture (Richard, 2011) and Yields of irrigated alfalfa have been shown to be up to 24,000 kg DM/ha/yr (Brown *et al*., 2000).

Alfalfa is commonly known as the “Queen of Forages” due to its ability to consistently produce high forage yields and forage quality (Kamalak *et al*., 2005). This forage legume is also known as an effective source of biological nitrogen fixation, an energy-efficient crop to grow, and an important source of protein yield. These qualities make it an excellent choice of feed for livestock producers (Barnes *et al*., 1988). The development of alfalfa hay quality standards, such as RFV and RFQ has provided alfalfa producers with an efficient method of comparing and marketing their crop to livestock producers. The stage of maturity at harvest, or harvest frequency and cultivar difference s are the main factor affecting alfalfa yield and quality (Collins and Fritz, 2003).

If harvested at the appropriate development stage, alfalfa can provide high yields of quality forage for 3 to 5 years. If it harvested at later stages (full flower) produces increased yields of green forage and DM whilst prolonging the productive life of the alfalfa forage (Lloveras *et al*., 1998). However, harvesting at earlier development stages (early flower) produces DM with improved quality, i.e., more crude protein and less crude cellulose (Katic *et al*., 2003). Its quality degradation with age is primarily a consequence of reduced leaf proportion and increased content of crude cellulose in the stem (Buxton *et al*., 1985). Alfalfa produces more protein per hectare than other legume and grasses; therefore, it is widely used for hay production and as pasture for livestock, especially to ruminants *(*Monteros and Bouton, 2009).

Tremblay *et al*. (2001) reported that, alfalfa is high quality forage that can produce superior animal performance. Energy levels and protein in alfalfa are generally higher than pasture particularly when pasture is not freshly growing. Correct management of alfalfa will provide top quality herbage. The author also indicated, Cultivars with the poorest plant digestibility had high leaf to stem ratios combined with poor stem digestibility, but the most digestible cultivars had high leaf to stem ratios combined with high stem digestibility. The other cultivars had either a high leaf to stem ratio or a high stem digestibility.

The intension in alfalfa forage production is on improving fodder yield and quality. This can be improved by increasing the leaf/stem ratio, which could be achieved by selecting genotypes (Cultivars) with having high leaf to stem ratio. On the other hand, to facilitate multiplication of the new cultivars, it is necessary to combine high fodder yield and quality (Bolanos *et al*., 2002). It has a high quality potential and ability to control factors that can affect the quality and will improve production quality. Factors affecting alfalfa hay quality are: soil fertility, cultivar, the presence of other species, the use of pesticides, climatic conditions, harvesting (season, time of day and stage of development at harvest) and the method of preservation (Stancheva *et al*., 2008).

Cutting frequency is critical factor influencing both productivity and persistence (Keoghan, 1982), which is generally associated with the flowering time. This represents an extremely important variable being a trade-off among quantity, and duration of the pasture (Teixeira *et al*., 2008). Moreover, these parameters are strongly linked to climatic conditions, management techniques, regrowth capability of the varieties and nutritional requirements for the livestock. Researchers had found that harvest time can affect forage yield and quality (Ghanbari and Lee, 2003). The aim to improve forage quality without reducing yields can be achieved by increasing the cutting frequency (Marten *et al*., 1988)

Evaluation of nutritional status is an important part of experimental assessment since inadequate nutrition increases the risk of health and performance problems (Becvarova *et al*., 2009). Nutritive value of alfalfa forages depends on their DM digestibility and voluntary DM intake. Relative feed value (RFV) is a widely accepted forage quality index in the marketing of hays. It combines the estimates for forage digestibility and intake into a single number. RFV value is calculated from estimation of acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Caddel, 2005). Hay producers and purchasers also use RFV in price discovery, especially in hay auctions (Undersander, 2001). The amount of protective substances residue obtained after boiling the sample feed with detergent solution is called ADF. ADF content is regularly higher than the crude fiber from forage, these features being closely related, since both are an estimate of the amount of cellulose and lignin (Jarrige *et al*., 1988).

The estimated livestock population of Ethiopia is 38.7 million cattle, 16.1 million sheep, 14.9 million goats, 5.8 million equine and 0.46 million camels (CSA, 2005), despite their productivity is low. The causes for low productivity of livestock in Ethiopia are multifaceted that include poor genetic makeup, poor veterinary services, inadequate quantity and quality of feed, and poor breeding strategy. Among these limiting factors, poor feed supply and feeding system is the most important as the feed resources in the highlands of Ethiopia are generally natural pasture and residues of different crops (Zegeye, 2003). McDonald *et al*. (2002) stated that all straws and related by-products are extremely fibrous, most of them have a high content of lignin and all are of low nutritive value. In connection to this, most dry forages and roughages found in Ethiopia have a crude protein (CP) content of less than 7% which indicates microbial requirement can hardly be satisfied unless supplemented with protein rich feeds (Van Soest *et al*., 1994).

Therefore to improve availability of livestock feed in terms of quality and quantity it is better to cultivate alfalfa forage that have better biomass yield and nutritional quality. Thus, it is better to see which cultivar can perform in terms of nutritive value and biomass yield across in different cutting cycles, before disseminating the cultivars to the livestock farming community. Therefore, this study was aimed to investigate biomass yield dynamics and nutritional quality of alfalfa (*Medicago sativa*) cultivars at Debre Zeit, Ethiopia with following specific objectives.

The specific objectives of this paper were:-

1. To determine the leaf and stem yield dynamics of five alfalfa cultivars across cutting cycles
2. To evaluate the chemical composition and DM digestibility of five alfalfa cultivars across cutting cycles

2. LITERATURE REVIEW

2.1 Overview of livestock Feed Resource in Ethiopia

The major feed resources in the country are crop residues and natural pasture, with agro industrial by-products and manufactured feed contributing much less. The importance of natural pasture is gradually declining because of the expansion of crop production into grazing lands, redistribution of common lands to the landless and land degradation. In the Ethiopian highlands crop residues are the major feed resources (Berhanu *et al.*, 2009). Zinash and Seyoum (1991) reported that about 70% of crop residues in the highlands are used as animal feed. In the lowlands of the country natural pasture is the major source of feed. EARO (2003) reported that there are no reliable estimates of the animal feed resources in Ethiopia. Also the author indicated, some estimates reported that there could be about 14 million tonne of crop residues and about 500,000 tonne of various types of agro industrial by-products produced annually in Ethiopia.

Despite the large livestock population in Ethiopia, the sector's contribution at the micro or the macro level is well below its potential due to various reasons, notably feed shortage and diseases (Gebremedhin *et al*., 2004). This author also indicated, introducing improved forage species, and cut and carry systems are other potential options that could contribute to the alleviation of the feed shortage problem, especially in the highlands of the country.

Forage legumes contribute significantly to livestock production in crop livestock systems. Low quality crop residues need nitrogen supplementation, often provided by forage legumes to become productive diets (Anderson, 1985). Legume forages generally lead to higher intakes and animal production than grass silages of comparable digestibility (Dewhurst *et al*., 2003). In livestock production one of the most important factors determining profitability is to achieve optimal level of feeding. Livestock farming communities are facing their biggest challenge during the dry season.

Producing supplementary feed on farm by establishing grass/legume pastures would reduce their problem. For instance mixed grass legume pasture produced higher DM yields of better nutritive value than sole grass swards (Oni-fade and Akinola, 1986).

According to (Alemayehu, 2006) finding, alfalfa Forage plays varying role in different livestock production systems. Even in the presence of abundant crop residues, which are often free fed to ruminants, forage crops especially legumes are needed to improve the utilization of crop residues, crop residues often provide energy while forage legumes provide proteins.

2.2 History of Alfalfa

Alfalfa yield and the nutritive value of dry matter make it a leading perennial leguminous forage crop (Dinić and Đorđević, 2005). It originated from the Mediterranean basin and southwest Asia (Iran, Afghanistan) and was one of the first forage crops to be domesticated (Cook *et al.*, 2005). However, the evolution of cultivated alfalfa, *Medicago sativa* ssp., has been greatly influenced by its winter hardy progenitor (Michaud *et al.*, 1988). It has a deep root that reaches down to 4 m, but can reach 7-9 m in well drained soils and stems are erect or decumbent, up to 1 m high, glabrous or hairy in the upper parts. Leaves are trifoliate, with obovate leaflets, 10-45 mm long and 3-10 mm broad (Ecoport, 2009). Aalfalfa is one of the few cultivated plants that can produce high level of biomass with minimum inputs. Sustainability of farming system under organic management may be increase by the introduction of alfalfa in the crop rotation *(*Annicchiarico *et al., 2006).*

There are numerous cultivars of alfalfa, selected for specific abilities, such as winter hardiness, drought resistance, tolerance to heavy grazing or tolerance to pests and diseases (Frame, 2005). Current breeding targets also include feeding value parameters such as digestibility and fiber content (Julier *et al.*, 2000). Due to its high nutritional quality, high yields and high adaptability, it is one of the most important legume forages of the world as major source of protein for livestock and it is a basic component in rations for dairy cattle, beef cattle, horses, sheep, goats and other classes of domestic animals.

It is cultivated in more than 80 countries in an area exceeding 35 million hactar (Radovic *et al*., 2009). World production of alfalfa was around 436 million tons (FAO, 2006).

The best conditions for its growth and development are in moderately warm or subtropical zones and in the uplands with the temperature of about 15-30 °C with a balanced amount of rainfall and plentiful sunshine (Mauriès 1991, 1994). This species tolerates short term shortage of moisture in the soil. Most often, it is associated with animal feed but also it is rarely used in human nutrition. Folk medicine uses this species because of its rich chemical composition and it is used it as an adjunct to pharmacological agents in the treatment of gastrointestinal, cardiovascular and immune system diseases (Zanin, 2009). Alfalfa contains features essential for organic farming because of its nutritional quality, nitrogen fixation and adaptive capacity (Torricelli, 2006). Specific varieties may be recommended depending on region-specific adaptation and management (Annicchiarico *et al*., 2010).

2.3 Alfalfa as a Feed for Animals

Alfalfa is used for livestock nutrition in different forms, most frequently as hay, but also dried/dehydrated in form of briquettes, as silage, haylage or for grazing. Alfalfa is harvested and stored primarily as hay or silage for use on the farm. The feeding value of harvested alfalfa may be changed by post-harvested factors as much as by precutting environment and history of plant (genetics). According to Radović *et al*. (2009) report, Conservation and storage system are designed to minimize the loss and deterioration of nutrients.

Alfalfa is widely used in ruminant livestock diets, impacting the performance of beef and dairy animals as well as the cost of production. The decline in forage nutritive value with increasing harvest interval is a consequence of progressing maturity, along with the associated effects of increasing stem growth and decreasing leaf proportion, and decreasing stem nutritive value. The implications of greater maturity for animal performance are generally negative (Brink *et al*., 2011).

It is a highly valued animal feed and a rich source of proteins, fibers, minerals and vitamins used in the diet of livestock. Alfalfa does not tolerate close grazing well, and some form of rotational grazing is necessary to maintain the persistence and production of plants, with rest intervals that replenish the crown and roots of plants in carbohydrates and nitrogen (Frame, 2005).

The duration of rest intervals depends on growth conditions, but 5 to 6 weeks are likely to be necessary. In a continuous grazing system, intensive defoliation can damage the plant crowns. In mixed pastures, stocking rates and grazing intensity should be controlled to prevent the selective overgrazing of alfalfa (Leach, 1983). Some cultivars are better adapted to grazing than others, including continuous grazing (Brummer *et al*., 1991). It's forage quality is determined by two main components: protein digestibility and protein content (Hill *et al.,* 1988) and Produce good nutrients with 15-22% protein content and high vitamin and mineral contents (Wu, 2004).

It is one of the most popular high-yielding plants harvested in 3–5 cuts per year and has high content of nutrients and digestibility in cattle. The content of nutrients in feedstuffs varies, however, depending on the vegetation stage (maturity), season of harvesting and plant origin (cultivars) (Elizalde *et al*., 1999). Ruminants benefit from two major characteristics of alfalfa. Firstly, its high protein content is readily digestible (protein digestibility varies from 81 % to 73 % in green alfalfa during the first cycle) and this digestibility surpasses that of competing forages. Secondly, alfalfa fiber is very valuable as it is rapidly digested in the rumen, which is beneficial to rumen activity due to its buffering effect (INRA, 2007).

Ruminants fed on alfalfa have higher nutrient intake and digestibility than when fed on other forage legumes and grasses (Frame, 2005). It may supply more than 30 % of the total digestible nutrients supplied by the same quantity of maize grain (Bruce *et al*., 2008). The value of high-quality alfalfa for dairy cows is that it reduces grain and protein needs by providing variable protein content and solubility, as well as relatively high energy.

But the unique value is that it promotes greater intake and milk production by containing low NDF, faster rates of digestion, particle size reduction (its coarse structural fiber that stimulates ruminative chewing and salivation which results in rumen buffering), and rate of passage (Robinson, 2003).

The beta-carotene content of alfalfa is much higher than in other forages and it has beneficial effects on the reproductive performance of dairy cows, as it increases calf weight at birth and reduces the interval between mating and calving. It also has a stimulatory effect on milk yield (Mauries, 2003). Recent studies showed that cattle, sheep, and goats preferred alfalfa harvested on a clear day at sunset compared with the same forage harvested at the following sunrise because of potential to accumulate carbohydrates during the daytime (Fisher *et al*., 2002).

The ability of alfalfa to provide approximately 25% more high quality feed than pasture, results in higher production potential. This is significant in economic terms (Moot, 2009). In addition to the nutritional components (proteins and carbohydrates) that are important in the use of alfalfa and other plants as animal feed or food supplements (Sylwia *et al., 2010*).

2.4 Biomass Yield of Alfalfa

Alfalfa is one of the highest yielding forage legumes. Under irrigation, it can produce 25 to 27 t/ha DM with a production reduced in the 3rd year to 8-15 t/ha DM. Production may be related to plant density, to disease resistance, cutting cycle and cultivar difference. Under rain-grown situations the production is also determined by the availability of soil moisture (Cook *et al*., 2005). There is a negative association between yield and nutritive value, which has greatest impact on timing of harvests made in spring and early summer in humid environments, and in early and late summer in more arid regions (Brink *et al*., 2010).

Best quality hay is obtained when cutting is done during a dry period so that the swathe dries quickly. Raking has to be done at 60 % DM, as carefully as possible so that the plant does not lose its leaves. Baling should be done at 82 % DM. These conditions are generally satisfied in small-scale farms (Suttie, 2000). Dehydration was found to be the best way as it dries and stabilizes alfalfa while preserving its high protein content, vitamins and overall nutritive value (Rwnaud, 2002). Moreover, dehydrated alfalfa is a good source of xanthophylls and beta-carotenes for poultry farmers (Coop de, 2008).

Dehydration requires pre-wilting and chopping in the field, transportation to the plant and drum-drying (between 250 °C and 800 °C) down to 10 % moisture (Coop de, 2010). After drying, long fiber dehydrated alfalfa may be compacted into big square bales. Alfalfa can also be ground to make alfalfa meal or ground and passed through a screw die to make pellets that can be included in big square bales (Pellets are often standardized to a certain protein content, such as 17 or 18 % (Desialis, 2013).

The high dry matter yield, protein and calcium content of alfalfa make it suitable forage for all classes of ruminants. Alfalfa can be grazed, fed as green forage, offered as hay and silage or dehydrated. Cattle highly relish it though there are palatability differences among cultivars that could result from different patterns of protein fractionation (Summers *and* Putnam, 2008).

Generally speaking, alfalfa forage quality declines as the summer progresses and tends to recover under autumn conditions. Alfalfa harvested in the spring or fall has a higher leaf and protein content than summer produced alfalfa, at the same maturity, due to the effects of temperature and photoperiod. High temperature, which increases the rate of plant maturation and cell wall lignification, has a dominant effect. Therefore, the rate of decline in digestibility with time is faster in summer when temperatures are higher than in spring or autumn when temperatures are low. Structural components rapidly form at the expense of metabolites in the cell contents when temperatures are high. This affects the quality of the forage because lignification of the cell wall is the primary factor limiting forage digestibility.

Increasing day length or light intensity has somewhat contrary effects to that of increasing temperature. As these two factors increase, amino acid and non-structural carbohydrate synthesis increases due to the greater photosynthetic rate. The cell wall content, relative to the total dry matter content, decreases (Shannon, 1990).

Effect of cultivar and cutting cycle on biomass yield of alfalfa: Growth stage, cut number, leaf to stem ratio, moisture conditions at harvest and processing method are the most important causes of variation for yield of alfalfa (Veronesi *et al*., 2010). Before flowering, alfalfa's nutrient uptake has been to promote vegetative growth. After flowering, nutrient absorption and photosynthates are diverted into seed production. This allows for the alfalfa yield to keep increasing, but quality decreases rapidly past this stage of development (Probst, 2008). The optimal harvest interval for alfalfa is between 30 to 35 days. However, this harvest interval is also based upon a compromise between yield, quality, regrowth, and persistence (Sheaffer, 2000). Maximum yield on alfalfa is achieved at reproductive maturity when the nutritive value of the forage is at a minimum (Collins and Fritz, 2003).

Its leaves and stems contain different crude protein and crude fibre concentrations at different stages of growth. Herbage harvested at full bloom is expected to have a higher stem proportion than less mature herbage. The proportion of leaves in it's at the time of harvest is a major factor that determines the quality of the forage (Jung, 2005). The leaves/stems ratio varied depending on the number of cuts and harvest cycles and the leaves/stems ratio is an important quality indicator, because of this depend quality of alfalfa hay obtained. Percentage of leaves is desirable to be as high as possible, because in the leaves are found a crude protein content better than stem (Mihai *et al.*, 2012). Leaf to stem ratio higher than 1.8 was under estimated, but within the range of 0.5-1.8 prediction was accurate according to the report of (Julier, 1997).

With increasing alfalfa maturity in regrowth cycle, forage nutrient concentrations decrease while forage dry matter yield and root carbohydrates generally increase to about mid-flowering (Radović *et al.*, 2009). Alfalfa may be cut several times a year (up to 12 in warm regions). The best stage for cutting is at 25-50 % flowering as the nutritive value drops after that. After the first cut, it is advisable to wait for the young shoots to be 35 to 50 mm long before the next cut. The cutting height is important and to avoid damage to the plant 5-10 cm stubble should be left. This will help ensure good re-growth (Suttie, 2000). Pre-wilting is the best way to improve forage quality since wilting reduces water content and protein degradation. However, when moisture is above 50 %, leaf losses are very important and cause protein loss (Mauries, 2003).

2.5 Nutritive Value of Alfalfa

There are a number of chemical components of alfalfa hay that can be determined by currently available laboratory techniques. These allow the alfalfa hay to be divided into its ash (i.e., mineral) component (9 to 13% of hay dry matter (DM)), fat (2 to 3% of DM), protein (15 to 25% of DM), non-structural carbohydrate such as sugars, pectin and starches (20 to 35% of DM), and structural carbohydrates (30 to 50% of DM). Ash, protein, fat and structural carbohydrate (usually defined as fiber insoluble in a solution of boiling detergent at a neutral pH, or neutral detergent fiber (NDF)), are generally assayed directly, while the level of non-structural carbohydrate (NSC) is calculated by difference. Energetically, ash has no value while fat; NSC and proteins are generally almost fully digestible somewhere in the digestive tract. Thus the energy value of the hay, exclusive of the NDF, can be calculated with some accuracy. However it is the NDF portion of the hay, due to its relatively high contribution to the overall weight of the hay and its variable digestibility that makes it a key variable in estimating the energy value of alfalfa hay (Robinson, 1999).

Protein content in alfalfa dry matter varies from 18 to 25% depending on the growth stage (cutting cycle), cultivar difference and other factors. Alfalfa nutritive value is identified with protein content which depends on the share of leaves in dry matter yield which in its turn is positively correlated with protein content. The proportion of leaves and stems in alfalfa hay can vary greatly, depending on maturity at harvest, cultivars, handling, and rain damage (Katic *et al.*, 2006). The nutritive value of alfalfa may also be improved by increasing its DM digestibility. Digestibility of alfalfa decreases with maturity as a result of increased concentration of cell wall material in stems, decreased stem digestibility, and decreased leaf weight ratio (LWR) (Albrecht *et al*., 1987).

Decreasing protein content is a dilution effect related with the decreasing leaf to stem ratio; the leaves have stable protein content and their protein level is much higher than the protein content in stems. The decline of digestibility is the consequence of two processes: (a) the reduction of the highly digestible component (leaves) because of an increase of the less digestible component (stems) and (b) the decreasing average digestibility of the stem component, with more cell walls (NDF) and lignin *(*Veronesi *et al., 2010).*

When determining the nutritive value of alfalfa, ligneous cellulose content should be taken in account in addition to crude protein content. Neutral detergent fiber (NDF) content indicates the intake rate of alfalfa dry matter. The higher the NDF, the lower the alfalfa quality - the content of nutrients is reduced and livestock consumes such alfalfa less readily. In consequence, the livestock grows at a slower rate and the production of livestock products is proportionally reduced. ADF content indicates the potential production energy. Increase in ADF indicates a reduced energy, i.e., reduced quality (Katić *et al.,* 2008).

The term alfalfa forage quality is a broad term, referring to a number of factors that affect nutritive value of the forage. Among these factors, dry matter digestibility is considered to be the most important one (Posselt, 1994).

Digestibility of alfalfa organic matter depends on the contents of cellulose and lignin. As lignin is virtually indigestible, intensive lignifications of cell wall in late stages of alfalfa development tends to reduce the coefficient of digestibility. Since alfalfa leaf is preferably eaten by animals and has better nutritive value than stems, appropriate stage of maturity and cultivars having high leaf yield is important for livestock feed (Anacleto, 2004). Alfalfa has the potential to produce quality forage that is high in protein and carotene but low in fiber. Growing alfalfa is easy, but to produce a high yield of good quality forage and still maintain the stand demands attention to sound management practices namely: variety selection, insect and weed control, soil fertility and cutting management (James *et al*., 1984).

There is an optimum quality for alfalfa that should be fed to dairy cows. For forage that serves as the primary fibre source in the diet, NDF is the principal forage quality variable of concern. NDF is defined as the remnants of a feedstuff that is retained offer dissolving in a neutral detergent; consisting of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are wall carbohydrates and are available for degradation by rumen microbes, which in turn produce volatile fatty acids. Lignin is anti-nutritive phenolic compound that is indigestible by rumen microbes. The ideal NDF level in alfalfa hay for dairy cows is 40 % (of dry matter). NDF levels below 40% are to low and the hay have high rates of passage through the rumen; resulting inefficient dry matter conversion. NDF levels greater than 40 % begin to slow rate of passage down, creating a gut-fill effect. Higher gut-fill results in lower dry matter intake; and dry matter intake drives milk production (Găvan *et al*., 2013).

For forage trading (i.e., buying or selling), one number to describe different hays is more convenient rather than comparing their full nutrient analyses profiles. Such a Relative Feed Value (RFV) has been in place and proven very useful for livestock producers and hay farmers for long time to price hay and predict animal performance. Full bloom alfalfa hay containing 41% ADF and 53% NDF on a dry matter basis has an RFV of 100 and is considered the average score.

Forages with RFV greater than 100 are of higher quality than full bloom alfalfa hay, and forages with a value lower than 100 are of lower value than full bloom alfalfa (Moore and Undersander, 2002a, 2002b).

Alfalfa has one of the highest crude protein contents among forage crops, but it is rapidly and extensively degraded by rumen microorganisms. Synthesized microbial protein is subsequently used by the ruminal host as a source of amino acids for the production of animal protein. However, when the animal's protein requirements are high, microbial protein is insufficient to meet its nutritional needs. Furthermore, if protein is degraded too quickly in the rumen, more ammonia may be produced than can be used by the microbial population leading to inefficient conversion of feed N to microbial protein and excretion of excess ammonia as urea. Additionally, increasing the amount of protein resistant to rapid microbial degradation may reduce the bloat danger associated with grazing alfalfa (Dong *et al*., 2009).

Improvement of alfalfa with increased protein bypass will require not only variation for the trait but the ability to consistently separate genotypes for their rate of protein digestion. Previous research has demonstrated variability among alfalfa germplasm for ruminal degradation of total crude protein (Tremblay *et al*., 2003). The enzymatic degradability of forages declines significantly during plant maturation, due to decreasing degradability of cell walls in stems and their increasing contribution to plant dry matter. Declining cell wall degradability has been associated with the deposition of lignin and xylose-containing polysaccharides in maturing stems. These associations weaken, however, in forages of similar maturity, indicating that additional factors influence degradability (John *et al*., 2002).

Metabolizable protein (MP) is the combination of feed protein (actual protein not N) that escapes degradation in the rumen plus protein synthesized by ruminal microorganisms. The efficiency with which the forage crude protein is utilized as MP by ruminant livestock depends partly on its ruminal degradability (NRC, 2001).

Alfalfa leaves which are highest in nutritive value to livestock are most susceptible to loss resulting in an overall reduction of crop quality. Leaves of alfalfa hay that contain low moisture fall of in great numbers during the process of field curing, baling, transportation and storage (Huajia *et al*., 2012).

Digestibility is one of the most important characteristics of forage nutritional value. Differences among cultivars for IVOMD (in vitro organic matter digestibility) of stems were highly significant in both spring growth and summer regrowth. In spring, the stem IVOMD ranged from 547 to 579 g /kgDM, while in summer, it ranged from 536 to 563 g /kg DM. Differences in stem digestibility among alfalfa populations, cultivars, plant introduction accessions, and phenotypes have been reported (Tremblay, 2001).

Lignin levels increase with maturity in stems of forage crops such as alfalfa. Lignin concentration correlates negatively with forage digestibility for ruminant and small decreases in lignin content can improve digestibility and therefore profitability for the farmer or rancher (Dianging *et al*., 2001).

Feedstuffs quality is a very complex notion, which comprises data related to their raw chemical composition, digestibility and nutritional value. It also reveals feeds aptitude to fulfill animal’s nutritional requirements. The notion of quality itself, the diversity and complexity of assessments to be considered in order to qualitative depict feedstuffs became wider (Jarrige, 1995). Liming is an effective and dominant practice to raise soil pH and reduce acidity-related constraints to improve forage yields. The quantity of lime required depends on the soil type, quality of liming material, costs and crop species or cultivars (Fageria and Baligar, 2008).

Roles of cultivar and cutting cycle for alfalfa nutritive value: The main effect on alfalfa forage quality belongs to plant stage (maturity). Differences between alfalfa cultivars are not significant *(*Julier *et al., 2000;* Radović *et al., 2004).* The decline in alfalfa forage quality with advancing maturity is well documented *(*Marković *et al., 2008)*. This decline in quality is associated primarily with a decrease of crude protein content and to an increase in fibrous constituents of the steam. Digestibility and crude protein content of stem declined at a faster rate than those of leaves with increased maturity.

Organic matter digestibility ranges from 55 % to 77 % and depends on growth stage, cut number (leaf: stem ratio), and cutting frequency, harvesting conditions and processing (INRA, 2007). Factors affecting quality are represented by soil fertility, cultivar, the presence of other species in culture, the use of pesticides, climatic conditions, harvesting (season, time of day and stage of development at harvest) and the method of preservation (Stancheva *et al*., 2008). Advancing plant maturity is associated with a lowering of nutritive value by virtue of a decrease in leafiness and an increase in the stem: leaf ratio, change in the composition of the cell wall and loss of cell contents with maturity (Ballard *et al*., 1990).

Many factors affect the nutritive value of alfalfa hay: Variety selection, Harvest management, Harvest frequency. Cutting at earlier stages improves crude protein content and decreases fiber formation, but at the expense of yield (Dennis and Howard, 1993). Temperature and light are probably the most important environmental factors that affect nutritive value, both directly and indirectly. Higher temperature usually promote the accumulation of structural material (i.e. cell wall material) and also more rapid metabolic activity which decrease pool size of cell contents (Ford *et al*., 1979).

Alfalfa is an important source of protein for ruminants, but its protein is often poorly used because it is extensively degraded during ruminal fermentation. This degradation may be the most limiting factor of high-quality forage legumes. The nutritional quality of alfalfa could be greatly enhanced by increasing the amount of protein that escapes microbial degradation in the rumen. Significant genetic variation has been reported in alfalfa for ruminal in vitro protein degradability (Tremblay *et al*., 2002).

Protein content diminishes with maturity while fiber content increases, due to a decreasing leaf: stem ratio. Leaves have a stable protein content that is much higher than that of the stems. Stem develops at the expense of leaves and their cell walls and lignin content increases with maturity, resulting in higher fiber content for the whole plant (Veronesi *et al*., 2010). In Ethiopia, cell wall constituents increased by 0.16 % of dry matter per day with advancing maturity (Keftasa and Tuvession, 1993). Likewise, the mineral content is affected by the stage of maturity and the leaf: stem ratio, since alfalfa leaves contain more P, Ca, Mg, Cu, Zn, Fe and Mn while stems contain more K (Markovic *et al*., 2009).

Table 1 Forage quality values of some forages at different growth stages.

Forage type	*CP*	*ADF*	*NDF*	*RFV*
Alfalfa-prebud	22	28	38	164
Alfalfa-bud	20	30	40	152
Alfalfa-early bloom	18	33	43	138
Alfalfa-full bloom	16	41	53	100
Alfalfa + grass	13	39	54	101
Bromegrass,				
Bromegrass-late bloom	7	49	81	58
Corn silage-well eared	10	28	48	133
Sorghum silage	8	32	52	114

Source: (Dunham 1998 and INR, 2007)

Earlier maturity harvests are higher in quality, but repeated harvests at an immature stage will reduce stand longevity, vigor, and yield (Sheaffer, 1990). In addition to high forage yield, the economics of alfalfa production requires that the herbage possess high nutritional value. Since alfalfa leaves have significantly higher nutritive value than stems, one approach to improve forage quality has been to develop cultivars which possess a greater proportion of leaves in their forage (i.e. a greater leaf-to-stem ratio). Some evidence suggests, however, that alfalfa populations possessing greater leaf to stem ratio may be more sensitive to deficit irrigation management (Ray *et al*., 1999).

Cultivars and their genetic characteristics crucially determine the volume and stability of yield, as well as the quality of alfalfa forage (Stanisavljević, 2006). Significant differences were registered in the contents of crude fiber, ADF and NDF that were caused by genetic factors *(*Katić *et al., 2008)*. The proportion of leaves in the alfalfa forage might be considered as an indirect indicator of alfalfa quality (Rotili *et al*., 2001). The optimum NDF content of alfalfa forage, 40.13 % of DM was in stage 5 in 2011, and in 2012 in stage 4 (40.56 % of DM). Cool and wet conditions caw delay the flower to open while NDF continues to increase (Găvan *et al*., 2013).

Table 2 Classification of alfalfa hay quality

Quality Standard	CP (%DM)	NDF (%DM)	ADF (%DM)	RFV
0-Prime	>19	<40	<31	>151
1	17-19	40-46	31-40	125-151
2	14-16	47-53	36-40	103-124
3	11-13	54-60	41-42	87-102
4	8-10	61-65	43-45	75-86
5	<8	>65	>55	<75

Source: (Redfearn and Zhang, 2011).

Relative feed value is calculated by estimating the digestibility of the forage dry matter, and how much the cow can eat based on its “filling” capacity. However, cows sometimes perform differently even when fed forages of identical RFV. Variations in the digestibility of the NDF fraction can probably account for these differences.

Feed quality of alfalfa harvested as haylage or hay depends, to a great extent, on the maturity of the stand. With increasing maturity, plant structural carbohydrates, as measured by the ADF and NDF fractions, increase. These fiber fractions represent the more indigestible parts of the plant. As a result, digestibility and energy obtained through fermentation decrease with maturity. Relative feed value (RFV) has been used for years to compare the quality of legume and legume/grass hays and silages (Peter and Alvaro, 2004).

3. MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted at Debere Zeit Agricultural Research Center (DZARC) located 47.9 kilometers south east of Addis Ababa and East Shewa Zone of the Oromia Region. It has an elevation of 1,920 meters. Debere Zeit Research Center, founded in 1953, is run by the Ethiopian Institute of Agricultural Research; at a Latitude of 08044' N, Longitude of 380, 38'E, Altitude (m):1900, Temperature (^{0}C) have Max: 28.3 ^{0}C Min: 8.9 ^{0}C, Rainfall (mm) 1100 mm having bimodal pattern , Agro-ecology of the area is : Tepid to cool sub moist, Major soil types: light soil (alfisols/Holisols)&Black soil (vertisols) (http://www.eiar.gov.et/index.php?option=com).

Fig. 1 Map of Debere Zeit

3.2 Treatments and Experimental Design

Five selected alfalfa cultivars were grown at forage and pasture research site of the DZARC on finely prepared seed beds. The cultivars were: FG10-09 (F), FG9-09 (F), Magna 801-FG (F), Magna 788 and Hairy Peruvian.

The cultivars were planted on 4 July, 2012 on 12 m^2 plots (4 m long and 3 m wide). Each plot consisted of 15 rows arranged length-wise in an east-west direction, with intra-row spacing of 0.2m. A seeding rate of 20 kg/ha was used and diammonium phosphate (DAP) fertilizer was applied at the rate of 100 kg/ha at planting. The plots were laid out in randomized complete block design (RCBD) with four replications. At early stages of seedling development, weeds were controlled through manual weeding followed by hoeing. Subsequent weed and other plot management practices were undertaken when deemed necessary.

3.3 Data Collection and Chemical Analysis

3.3.1 Data collection

The sample was collected to determine DM yield (leaf & stem), leaf proportion, stem proportion & leaf to stem ratio. The leaf and stem yield was determined by harvesting a central section of two adjacent middle rows with a sampling area of 0.2 m^2 (0.5 m length x 0.4 m width). The harvested biomass separating in to leaf and stem and packed to paper bag followed by partitioning the harvested biomass in to leaf and stem fractions, and drying the fractions at 65 ^oC for 72 hrs for determination of partial DM yield. Finally the recorded DM yield data on the plot was changed to per hectare.

For leaf and stem yield and evaluation of chemical composition of selected alfalfa cultivars as influenced by cutting cycle and cultivar difference, required data (1-8th cutting cycles) were extracted from an ongoing project from a forage stand established at DZARC. The harvesting dates were: Cycle 1, Oct. 19, 2012; Cycle 2, Dec. 26, 2012; Cycle 3, Feb. 26, 2013; Cycle 4, April 2, 2013; Cycle 5, May 20, 2013; Cycle 6, June 25, 2013; Cycle 7, Aug. 20, 2013; Cycle 8, Oct. 10, 2013.

3.3.2 Chemical composition, and relative feed value (RFV) index determination

At full bloom stage, described as a stage when open flowers emerge on average of 2 or more nodes and no seed pods present (Ball, 1998), four randomly selected adjacent middle rows with a net area of 3.2 m^2 were harvested. For forage quality analysis, chopped herbage of the four replications were pooled into one and properly homogenized and one representative subsample was taken for each cultivar within each cutting cycle. The harvested biomass was manually chopped into small pieces using sickle and a subsample of 500 g was taken. The forage sample (0.5 kg) in the experiments was ground to pass through a 1 mm screen using a Wiley mill for chemical composition analysis. The sample was assessed for DM and Ash by the methods of the Association of Official Analytical Chemists (A.O.A.C, 1990). Nitrogen was determined by the micro-Kjeldahl method and CP was calculated as N×6.25.

The NDF, ADF and ADL as well IVOMD values were determined by Using Near Infrared Reflectance Spectroscopy (NIRS) facilities at Holetta Agricultural Research Center, Ethiopia. NIRS was performed on 3g of ground sample using Foss NIRS 5000 in the 1108 – 2492 range with an 8 nanometer step. The samples were pre-dried at 60°C over night in an oven to standardize moisture conditions before scanning. The spectra of each sample were taken by scanning for three consecutive times (Win Scan 1.5, 2000 Intra soft International).

The IVOMD was determined by the methods of Tilley and Terry (1963) as modified by Van Soest and Robertson (1985) as well Near-infrared reflectance spectroscopy.

The hemicelluloses and cellulose content was calculated as:

Hemi cellulose = NDF – ADF.

Cellulose= ADF-ADL

The index ranks forages relative to the digestible DMI of full bloom alfalfa were calculated according to (Undersander *et al*., 1993).

DDM = Digestible Dry Matter = 88.9 - (0.779 x % ADF)

DMI = Dry Matter Intake (% of BW) = 120 / (% NDF)

RFV = (DDM x DMI) / 1.29

3.4. Data Analysis

Analysis of variance procedures was used to analyze the quantitative data to be generated under experimental sets. The General Linear Model procedure of SPSS version 20 was used for data analysis and significant mean differences were declared at $P \leq 0.05$ using Tukey. In herbage yield dynamics study, the model used include, the effect of cutting cycle, cultivar and replication. For herbage quality data, the effect of cutting cycle (n = 2) and cultivar (n = 5) was used.

For herbage yield dynamics data, the following model was used:

$Y_{ijk} = \mu + T_i + \beta_j + CC_k + \varepsilon_{ijk}$ $\quad$ i = 1,...,a; j = 1,...,b

Where: $\quad$ k=1,…..c

Y_{ijk} = an observation in treatment i , block j and cutting cycle k; μ = the overall mean

T_i = the effect of treatment i (n = 5); β_j = the effect of block j (n = 4)

CC_k= the effect of cutting cycle (n = 8); ε_{ijk} = random error with mean 0 and variance $\sigma 2$; a = the number of treatments; b = the number of blocks; c= cutting cycles

For herbage quality, the following model was used:

$Y_{ik} = \mu + T_i + CC_k + \varepsilon_{ik}$ $\quad$ i = 1,...,a; k= 1,...,c

Where:

Y_{ik} = an observation in treatment i, and cutting cycle k; μ = the overall mean

T_i = the effect of treatment i (n = 5); CC_k= the effect of cutting cycle k (n = 2)

ε_{ik} = random error with mean 0 and variance $\sigma 2$; a = the number of treatments; c = the cutting cycles

4. RESULTS

4.1 Effect of Cultivar and Cutting Cycle on Leaf, Stem and Total Biomass Dry Matter Yields

The combined analysis of variance showed that the effect of cultivar and the interaction of cultivar by cutting cycle was not significant (P>0.05) for the three measured herbage traits. Thus, the average effects of the cultivars and cutting cycles were presented separately. Though cultivar effect was not significant as presented in Table 3, Magna 788 had the highest leaf yield (1129 kg/ha) while FG10-09(F) exhibited lowest leaf yield (670.6 kg/ha). Hairy Peruvian had higher stem yield (1984 kg/ha), whereas Magna 801-FG(F) had lower stem yield (1516.1 kg/ha). Correspondingly, FG9-09(F) obtained higher total biomass DM yield (2861.5 kg/ha) and Magna 801-FG(F) had lower total biomass DM yield (2411.8 kg/ha).

Table 3 Effect of cultivar on leaf, stem and total biomass DM yields of selected alfalfa cultivars

Cultivar	LY (kg/ha)	SY (kg/ha)	TDMY (kg/ha)
FG10-09(F)	670.6	1785.6	2456.3
FG9-09(F)	959.6	1901.8	2861.5
Magna 801-FG(F)	895.7	1516.1	2411.8
Magna 788	1129.1	1541.6	2670.8
Hairy Peruvian	753.6	1984.0	2737.6
SE	139.6	289.8	381.5

Note: SE, Standard error; LY, Leaf yield; SY, Stem yield; TDMY, Total dry matter yield
Means in a column are not significantly different (P>0.05)

The effect of cutting cycle on the three agronomic parameters was presented in Table 4. Cutting cycles had significant effect on leaf, stem and total biomass DM yields of five alfalfa cultivars (P≤0.05). The 8th cutting cycle had highest leaf yield (2500.7 kg/ha) followed by 7th cutting cycle, while first cutting cycle obtained lowest and comparable leaf yield (288 kg/ha) with the rest cutting cycles. Stem yield was highest (6805.6 kg/ha) on 8th cutting cycle followed by 7th cutting cycle and 6th cutting cycle had lowest and intermediate stem yield (271.4 kg/ha) with the rest cutting cycles. In the same way, total biomass DM yield was highest in the 7th cutting cycle next to 8th cutting cycle, even as the 6th cutting cycle exhibited lowest and equivalent total biomass DM yield (585.9 kg/ha) with the rest cutting cycles.

Table 4 Effect of harvest cycle on leaf, stem and total DM yield of selected alfalfa cultivars

Cutting cycle	LY(kg/ha)	SY (kg/ha)	TDMY (kg/ha)
First	288.0^{b}	330.2^{c}	618.2^{c}
Second	417.5^{b}	471.0^{c}	888.5^{c}
Third	480.0^{b}	565.0^{c}	1045.0^{c}
Fourth	523.2^{b}	537.9^{c}	1061.1^{c}
Fifth	410.0^{b}	460.8^{c}	870.8^{c}
Sixth	314.4^{b}	271.4^{c}	585.9^{c}
Seventh	2120.0^{a*}	4525.0^{b}	6645.0^{b}
Eighth	2500.7^{a}	6805.6^{a}	9306.4^{a}
SE	176.6	366.6	482.6

Note: SE, Standard error; LY, Leaf yield; SY, Stem yield; TDMY, Total dry matter yield

*, Means in a column followed by different superscripts are significantly different (P<0.05)

4.2 Effect of Cultivar and Cutting Cycle on Leaf Proportion, Stem Proportion and Leaf to Stem Ratio

Cultivar effect was significant ($P \leq 0.05$) for leaf, stem proportion and leaf to stem ratio as illustrated in Table 5. Magna 788, Magna 801-FG(F) and FG10-09(F) had higher and comparable leaf proportion with FG9-09(F) though Hairy Peruvian had lower and comparable leaf proportion with FG9-09(F).

Hairy Peruvian had higher and comparable stem proportion with FG9-09(F) than other cultivars. Magna 788, Magna 801-FG(F) and FG10-09(F) exhibited lower and comparable stem proportion with FG9-09(F). Likewise leaf proportion, Magna 788, Magna 801-FG(F) and FG10-09(F) had higher and comparable leaf to stem ratio with FG9-09(F) however Hairy Peruvian had lower and comparable leaf to stem ratio with FG9-09(F).

Table 5 Effect of cultivar on leaf and stem proportion and leaf to stem ratio

cultivar	Leaf proportion % (TDMY)	Stem proportion % (TDMY)	Leaf to stem ratio
FG10-09(F)	44.3^{a*}	55.7^{b}	0.91^{a}
FG9-09(F)	43.2^{ab}	56.8^{ab}	0.8^{ab}
Magna 801-FG(F)	47.2^{a}	52.8^{b}	0.96^{a}
Magna 788	47.1^{a}	52.9^{b}	0.96^{a}
Hairy Peruvian	38.8^{b}	61.3^{a}	0.70^{b}
SE	1.3	1.3	0.49

Note: SE, standard error; TDMY, total dry matter yield; *, Means in a column followed by different superscripts are significantly different ($P<0.05$)

The effect of cutting cycle on leaf, stem proportion and leaf to stem ratio were significant ($P \leq 0.05$) and presented on Table 6. The 6th cutting cycle had higher and comparable leaf proportion with 2nd and 4th cutting cycles; in spite the 7th and 8th cutting cycle obtained lower leaf proportion than the rest cutting cycles. Higher stem proportion was documented in the 7th and 8th cutting cycles, regardless the 6th cutting cycle had lower and comparable stem proportion with the 2nd and 4th cycles. Cycle 1, 3 and 5 had intermediate and comparable leaf proportion, stem proportion and leaf to stem ratio values with 2nd and 4th cutting cycles.

Table 6 Effect of harvest cycle on the leaf, stem proportion and leaf to stem ratio

Harvesting cycle	Leaf proportion (%TDMY)	Stem proportion(%TDMY)	Leaf to stem ratio
First	46.8^{b*}	53.2^{b}	0.95^{b}
Second	49.4^{ab}	50.6^{bc}	1.2^{ab}
Third	45.7^{b}	54.3^{b}	0.86^{b}
Fourth	49.0^{ab}	51.0^{bc}	0.99^{ab}
Fifth	47.1^{b}	52.9^{b}	0.92^{b}
Sixth	54.5^{a}	45.5^{c}	1.3^{a}
Seventh	32.1^{c}	67.9^{a}	0.48^{c}
Eighth	28.4^{c}	71.6^{a}	0.40^{c}
SE	1.6	1	0.60

Note: SE, standard error; *, Means in a column followed by different superscripts are significantly different ($P<0.05$)

4.3 Chemical Compositions of Alfalfa Cultivars for the 7th and 8th Cutting Cycles

The effect of cultivar for chemical composition was not significant ($P \geq 0.05$) as appeared in Table 7. Thus, all cultivars had comparable value for all the chemical entities assessed. Though not statically significant, all cultivars had greater than 90% and 18% DM and crude protein content respectively. FG10-09(F) had higher ash content (10.47%) than the rest cultivars. Magna 801-FG(F) had higher nutritive value compared with the rest cultivars containing; lower ash (9.07%), NDF, ADF,ADL and higher crude protein (20.3%), IVOMD, DDM ,DMI and RFV contents. Hairy Peruvian had lower RFV (163.31) than the rest cultivars.

Table 7 Chemical composition of the five alfalfa cultivars for 7th and 8th cutting cycles

	Herbage quality traits (%DM)											
Cultivar	DM	Ash	CP	NDF	ADF	ADL	IVOMD	DDM	DMI	RFV	HCL	CEL
FG10-09(F)	91.08	10.47	19.29	37.96	30.94	4.44	69.37	64.79	3.16	181.05	7.02	26.12
FG9-09(F)	90.95	9.525	18.48	36.47	28.59	3.9	68.97	66.62	3.30	170.86	7.87	24.69
Magna801-FG(F)	90.83	9.07	20.30	33.70	26.57	3.43	71.08	68.19	3.57	189.21	7.13	23.14
Magna 788	91.02	9.43	18.51	37.02	28.54	4.02	69.02	66.66	3.24	167.49	8.48	24.52
Hairy Peruvian	91.01	9.73	19.19	37.16	29.97	4.23	69.22	65.55	3.21	163.31	7.19	25.74
SE	0.074	0.606	1.38	1.06	1.770	0.22	0.66	1.37	0.10	14.20	1.23	1.55

Note: SE, Standard error; Means in a column are not significantly different (P>0.05)

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Cutting cycles did significantly ($P \leq 0.05$) affect Acid detergent fiber, dry matter digestibility and cellulose content as shown in Table 8. The ADF and cellulose content were higher in the 7th cutting cycle despite the fact that DDM was higher in the 8th cutting cycle. The rest chemical compositions are not significant ($P > 0.05$) and had comparable values. Though the 8th cutting cycle had lower crude protein and higher ash content, but had higher RFV (178.36) than the 7th cutting cycle.

Table 8 Chemical composition of cultivars across two cutting cycles

Chemical composition	Seventh	Eighth	SE
DM	90.99	90.97	0.047
Ash	9.31	9.98	0.383
CP	19.44	18.86	0.877
NDF	37.35	35.58	0.670
ADF	30.81^{b}	27.03^{a}	1.119
ADL	4.25	3.76	0.145
IVOMD	69.13	69.93	0.419
DDM	64.89^{b*}	67.83^{a}	0.872
DMI	3.20	3.38	0.064
RFV	170.41	178.36	8.982
HC	6.53	8.54	0.782
Cellulose	26.41^{a}	23.27^{b}	0.983

Note: SE, standard error; *, means in a row followed by different superscripts are significantly different ($P<0.05$)

5. DISCUSSION

5.1 Leaf, Stem and Total Biomass Dry Matte Yield of Five Alfalfa Cultivars

The non-significant effect of the interaction between cultivars and cutting cycles for the leaf, stem and total biomass yield traits suggests that the phenotypic performance of the cultivars was independent of harvesting cycles, with genetics of cultivars**.** The non-significant effect of cultivar for leaf, stem and total biomass DM yield in the present work concurs with the reports of (Ji-shan *et al*., 2012 and Afsharmanesh, (2009); "Ranger" alfalfa cultivar had herbage forage yield (2.81 t/ha)). The cultivars in the present study had lowest herbage yield when compared with reported literature values, even under drought and unfavorable environmental condition. In the first year of vegetation, in the severe drought conditions Maria *et al*. (2007) reported that a mean yield values of alfalfa was 3 t/ha DM.

Cutting cycles had significant effect for leaf, stem and total biomass DM yield and this in agreement with the finding of (Julier and Huyghe, 1997). According to the study of Neal *et al*. (2006) cutting interval, which directly impacts maturity, had a stronger influence on herbage yield and quality than did cultivars thus, this is in accordance with the present report. Totally, eight harvests were taken at an average cutting interval of 54.6±12.4 days during October 2012 and October 2013, which is in disagreement with work of Sheaffer (2000) that the optimal harvest interval for alfalfa is between 30 to 35 days. The interval between harvests was observed to be longer for wetter months of the year compared to months of low or no rainfall. It was evident that harvests taken during or following the long and short rainy months had comparatively higher leaf yield, stem yield and total dry matter yield, while those taken during months of low or no rainfall had lower herbage yields.

The leaf, stem and total biomass DM yields of alfalfa cultivars increase from the first cycles to the eighth cutting cycles, except decreasing in the sixth cycle. It was due to season effect. When the age of alfalfa increase; its tiller, crown, shoot and root are more developed and have many branches to produce more herbage yields. The other reason might be when moisture and rain is available; alfalfa's root, tiller, shoot and crown are ready to continue its growth and the herbage yield of alfalfa is increased.

In agreement with the present work, Lloveras (2001) and Afsharmanes (2009) reported that, alfalfa forages in the first and second cut did not develop well root, crown, tiller and shoot and they did not had higher herbage yields. The 7th and 8th cutting cycle were harvested at wetter season, the 8th cycle had higher leaf, stem and total biomass DM yield followed by 7th cycle than the other cutting cycles. The 6th cutting cycle harvested at the drier season had lower dry matter yield but have high leaf to stem ratio and this correlated with the study of Davodi *et al*. (2011) that dry matter yield was negatively correlated with leaf to stem ratio.

5.2 Leaf Proportion, Stem Proportion and Leaf to Stem Ratio of Alfalfa Cultivars

Cultivars had significant effect on leaf proportion, stem proportion and leaf to stem ratio of five selected alfalfa cultivars and this in contrast with that of (Afsharmanesh , 2009) and concur with the report of ((Mousa *et al*., (1996) and Hamd *et al*.,(2013); evaluated six alfalfa varieties and found significant differences for leaf to stem ratio). Similarly, Abdel-Galil and Hamed (2008) found a significant difference among alfalfa cultivars which concur with the present study. Leaf to stem ratio is an important trait in the selection of appropriate forage cultivar as it is strongly related to forage quality (Julier *et al.*, 2000; Sheaffer *et al.,* 2000). Among cultivars evaluated FG10-09(F), Magna 801(FG) and Magna 788 had higher and comparable leaf proportion and leaf to stem ratio with FG9-09 (F).

Julier (1997) report indicated that, Leaf to stem ratios' higher than 1.8 was under estimated, but within the range of 0.5-1.8 prediction was accurate. In the current study, leaf to stem ratio was different across cutting cycles, which means cutting cycle seventh and eighth had lower leaf to stem ratio which is below 0.5, While the rest cutting cycle was in range of 0.5-1.8 (Julier, 1997). In this work, harvests taken during the drier season had a higher leaf to stem ratio in the range of 0.858 -1.251 and this concur with the finding of (Saeed and Nadi, 1997) and Martin *et al*., (1988) that water deficit stress had significant effects on leaf to stem ratio. Also Petil *et al*. (1992) and Bonner (1997) support the result of this study, regarded the further decrease in stem growth as the main reason for the 20% increase in alfalfa leaf to stem ratio under the increased intensity of drought stress.

The varieties with lower stem to leaf ratio have good palatability and quality. Therefore, a large amount of leaves in the forage indicates its good quality and the stem to leaf ratio directly reflects the nutritional value of the forage according to the work of (Li and Yan, 1997).

The sixth harvest cycle had better leaf proportion and leaf to stem ratio but had lower stem proportion. This result is in consistent with the report of (Shannon, 1990) that alfalfa harvested in the spring has a higher leaf and protein content than summer produced alfalfa, at the same maturity, due to the effects of temperature and photoperiod. High temperature, which increases the rate of plant maturation and cell wall lignification, has a dominant effect on the proportion of leaf and stem and quality of herbage. Harvests taken during the drier season had higher leaf proportion and higher leaf to stem ratio. This is due to that leaf growth is higher than stem. The increase in alfalfa quality, i.e. the increase in leaf to stem ratio under water deficit stress has been reported by Buxton (2004) and Martens (2007) that alfalfa leaf to stem ratio increased by 20% under water deficit stress compared with wetter season plant, being due to the decrease in stem growth rate.

Even though, at the wetter season the yield of alfalfa cultivars was increased than the drier seasons, they had less leaf to stem ratio and had high stem proportion. Thus, this study supported by the study of (Jung, 2005) that alfalfa harvested at full bloom is expected to have a higher stem proportion. The proportion of leaves in it's at the time of harvest is a major factor that determines the quality of the forage. During wetter season, tiller and roots of alfalfa was well developed and produce better stem than leaf yield. Since moisture is not available in dry season alfalfa cultivars was produce less yield of stem and leaf than wetter season but had better leaf proportion and leaf to stem ratio. Harvests taken in the rainy season had higher biomass yields but had lower leaf to stem ratio and this study coincide with the outcome of (Julier and Huyghe, 1997). The proportion of leaves and stems in alfalfa hay can vary greatly, depending on maturity at harvest, cutting cycle, when maturity of alfalfa increase, leaf to stem ratio was decrease (Sheaffer *et al*., 2000).

5.3. Dynamics of Biomass across Cutting Cycles

A total of eight harvests (October 2012 – October 2013) obtained per year in the current study was low in view of what was usually attainable for alfalfa stands managed under Debre Zeit condition. The dynamics of herbage DM yield trend was increase until eight cutting cycle because moisture availability trigger development of root, tiller and shoot of alfalfa. In this study the herbage DM yield was gradually increase until fourth cutting cycle and This was consistent with the report of Lloveras (2001) who reported the increase in alfalfa dry forage yield from the first cutting (2.77 t/ha) to the second cutting (3.52 t/ha) and decreased in fifth and six cutting cycle. When the wetter season comes the yield increase dramatically in seventh and eighth cutting cycles and it assumed to be season and cutting cycle effect.

Evidence shows that alfalfa could be harvested at shorter intervals, around 30 days, with higher number of cuts achieved during the dry months of the year under irrigated conditions, which indeed is lower than an interval of around 54.6±12.4 days recorded in the present study.

The interval between harvests in the current study was longer during wetter months compared to dry months, and this could be explained by the fact that when light conditions do not trigger transition from vegetative to reproductive growth, shoots remain in the vegetative stages of development (Gramshaw *et al.*, 1981; Sheaffer *et al.,* 1988; Gramshaw *et al.,* 1993) thereby delaying the predetermined stage of biomass removal which in here was full bloom stage (Ball, 1998). Găvan (2013) reported that, cool and wet conditions could delay the flower to open, hereby delay full bloom stage and on the other side NDF content of the forage is continues to increase and it is in disagreement with this study.

Low leaf, stem and total DM yield for the dry months of the year in this study clearly suggests the significant role of moisture availability in growth and development of alfalfa crop which concurs with the claims of (Sammis, 1981) and the water deficit was high, affecting the yield level (Maria, *et al*., 2007).

In this regard, water deficiency was reported to diminish shoot growth rate through a variety of mechanisms, among which the following were reported in the literature: reduced shoot elongation rate, decreased inter node length, slow rates of leaf development and reduced leaf area expansion (Durand *et al.*, 1989; Grimes *et al*., 1992 and Brown *et al.*, 2009). In the present study, leaf to stem ratio was higher during the drier months of the year and this in line with what was documented by other works (Carter and Sheaffer 1983; Halim *et al.,* 1989), who indicated a negative effect of water deficiency on stem growth than on leaf area, leading to higher leaf to stem ratios for stands grown under water stress. During rainy and at the end of rainy season the leaf, stem and total DM yield of alfalfa cultivars was very high comparing with dry season, but had lower leaf to stem ratio.

During the wetter season (7^{th} and 8^{th} cutting cycles), the temperature and moisture was moderate and so the herbage DM yield was higher compared to the rest of cycles as appeared in Table 4. Therefore, an increasing trend was observed up to the eighth cutting cycle with the increase in plants age and availability of moisture as well temperature.

At the sixth cutting cycle, the increase in leaf area (as the photosynthesizing organ) and leaf to stem ratio increased but forage yield was decrease. The seventh and eighth cutting cycles had better forage yield but had low leaf to stem ratio which in agreement with the finding of Sengul (2002) that alfalfa stem number increase due to ageing and moisture availability.

5.4 Herbage Nutritive Value

Cultivars effect was not significant for chemical composition of alfalfa (P >0.05) which in agreement with the findings of (Ji-shan *et al*., 2012) and in disparity with that of others (Katić *et al.*, 2008 , Milić *et al*., 2011 and Diriba *et al*., 2014*).* High quality alfalfa was reported to contain >19 % CP, <31% ADF, <40 % NDF and > 151 % RFV (Redfearn and Zhang H, 2011). Moreover, alfalfa forage quality values at full bloom stage contain CP >16, ADF < 41, NDF <53 and RFV >100 (Dunham, 1998).

In the current study, the DM content of alfalfa cultivars had higher yields (above 91%), greater than what was reported by Martin *et al*. (1988), however, Kamalak *et al*. (2005) reported alfalfa hay contain 93.2% DM and that had higher DM doesn't mean had higher crude protein content. The present work revealed ash content ranges from 9.07-10.47 which in agreement with the study of (Giger-Riverdin, 2000; Kamalak *et al*., 2005; Preston, 2010).

Currently, FG10-09(F), Magna 801-FG (F) and Hairy Peruvain had higher CP content which is above 19%, but FG9-09(F) and Magna 788 had below threshold level according to the report of (Redfearn and Zhang, 2011) and Dunham (1998). According to the report of Collins (1988) and NAS (1978)*;* corn silage has 10% CP, 4% lignin, 51% NDF and 28% ADL but this study revealed that, alfalfa had higher crude protein content than corn silage; on the contrary had higher lignin content. .

Hairy Peruvain had lower leaf to stem ratio other than the rest cultivars, despite higher and comparable CP content with FG10-09(F) and Magna 801-FG (F) as appreciated in Table 7. This result differed from with the report of (Julier *et al.,* 2001 and Katic *et al.,* 2005*)* that alfalfa nutritive value is identified with protein content which depends on the share of leaves in DM yield which in its turn is positively correlated with protein content. Had low leaf to stem ratio does not necessarily mean the cultivar had less CP content.

The Magna 788 had higher leaf to stem ratio but had less than threshold level of CP according to the report of (Redfearn and Zhang, 2011, CP>19%). However, all cultivars were in range of good quality hay according to *(*Katic *et al.*, 2006 and Stanisavljevic *et al*., 2008*)* that Protein content in alfalfa DM varies from 18 to 25% depending on the growth stage, cutting cycle and cultivar difference.

According to the results reported currently, cultivar difference was not significant for lignin and cellulose content which agrees with findings of Milić *et al*. (2011) and Diriba *et al* (2014) where the cultivars evaluated did not significantly differ in their ADL content. This work revealed the alfalfa cultivars had lower NDF, ADF and ADL content compared with the finding of (Dien *et al*., 2006, Yu *et al.*, 2003, INRA, 2006 and Homolka *et al*., 2008). The ideal NDF level in alfalfa hay for dairy cows is 40 % (%DM). Since NDF levels below 40% are to low and the hay have high rates of passage through the rumen; resulting in inefficient dry matter conversion. The NDF levels greater than 40 % begin to slow rate of passage, creating a gut-fill effect. Higher gut-fill results in lower DM intake; and DM intake drives milk production (Găvan *et al*., 2013).

All cultivars had lower ADF value less than 31%; it indicates that the cultivar had better nutritive value compared with the result of Mustafa *et al*. (2010) and lower NDF compared with the report of Canbolat *et al. (2006)*. When maturity increases the ash and lignin content tend to increase, however in this study it did not hold true. The 8^{th} cutting cycle had lower ADF and cellulose and higher DMD than 7^{th} cycle.

Had lower ADF content increase DMD and this was related to what was documented by Wilson *et al* (1991) that the decrease in DMD, DMI, RFV parameters are possibly associated with increased NDF and ADF contents.

Even if the effect of cutting cycle was not- significant on nutritive value of alfalfa except for ADF, DDM and cellulose, numerically they had different values. The crude protein content was lower in the 8th cutting cycle accordance with report of (Redfearn and Zhang, 2011, good nutritive alfalfa is >19), but had higher crude protein according to finding of (Dunham, 1998; alfalfa at full bloom stage called as good quality >16%), in spite of lower NDF, ADF and lignin content. This is due to, the 8th cutting cycle had higher stem yield than 7th cutting cycle. It indicated that those alfalfa cultivars leaf had high crude protein and high lignin content than stem and which concur with the work of (Petkova and Panayotova, 2007) that, Percentage of leaves is desirable to be as high as possible because in the leaves are found higher crude protein content at least twice than in the stems, as shown by many authors.

According to the discovered of Kallenbach *et al*. (2002); Canbolat *et al.* (2006) and Mustafa *et al*. (2010), this finding had higher DDM and and DMI value ranging from 64.79-68.19% and 3.16-3.57 respectively. Digestibility of organic matter had a negative correlation with NDF, ADF and hemicelluloses (Čerešňáková *et al*., 1996). Furthermore, in this study Organic matter digestibility of selected alfalfa cultivars ranges from 68.97-71.08% and, which in accordance with the finding of (INRA, 2007) that, Organic matter digestibility of alfalfa ranges from 55 % to 77 % and it depends on growth stage, cutting frequency, harvesting season.

In the present work Magna 801-FG(F) had higher DM and organic matter digestibility and had lower cell wall constituents than the rest cultivars in accordance with the report of Abdulrazzak *et al.* (2000) that there was a negative correlation between cell wall constituents and digestibility. Anacleto (2004) work supports this study; Digestibility of alfalfa organic matter depends on the contents of cellulose and lignin.

As lignin is virtually indigestible, intensive lignification of cell wall in late stages of alfalfa development tends to reduce the coefficient of digestibility. Since alfalfa leaf is preferably eaten by animals and has better nutritive value than stems, appropriate stage of maturity and cultivars having high leaf yield is important for livestock feed. The IVOMD is lower in the 7th and 8th cutting cycles compared with cutting cycles of 1-6th done by Diriba *et al*., (2014), this is due to. ADF content was lower in cutting cycle 1-6 compared to 7 and 8. If the fiber content is less, on contrary digestibility is increase.

The CP, cell wall components, and IVOMD and indices like RFV are commonly used to assess the potential of a feed (El-Waziry, 2007 and Pinkerton, 2005). In the present study, even if they didn’t have significant effect; variation among the cultivars was observed for CP, detergent fibers, hemicellulose and cellulose implying the possibility of selecting suitable plant protein sources for designing appropriate feed supplementation strategies. Relative forage quality (RFV) is an index used for legumes based on potential intake and fiber digestibility (Undersander and Moore, 2002). The index is used to price forage and to allocate forage to appropriate ruminant livestock performance levels. Accordingly, feeds with RFV index higher than 100 are considered to be of higher quality compared to full bloom alfalfa hay and those with a value lower than 100 are of lower value (Dunham, 1998). In the current study alfalfa cultivars evaluated had a RFV ranging from 163-189 and this was apparently above the threshold level of 151 according to the revealed of (Redfearn and Zhang, 2011).

6. CONCLUSION AND RECOMMENDATIONS

Alfalfa is the most essential nutritive forage for livestock feed. It can be harvested up to eight cycles per year under Debre Zeit environmental condition. The current result indicated that, yield was low as compared with other researches and documents. The effect of cultivar and cutting cycle interaction was not significant on leaf, stem and total biomass DM yields. Thus it is concluded that, genetics of cultivars did not affect by cutting cycles.

Moreover, in this study, the effect of cultivars had not significant effect on leaf, stem and total biomass DM yield of selected alfalfa cultivars. Among cultivars Magna 788, Hairy Peruvian and FG9-09(F) had higher leaf yield, stem yield and TDMY respectively. Cutting cycles had significant effect on leaf and stem DM yields and herbage total DM yields of selected five alfalfa cultivars. Harvest taken during wetter season; the 8^{th} cutting cycles had higher herbage yields followed by 7^{th} cutting cycle than the other cutting cycles.

This study revealed that, cultivar and cutting cycles had significant effect on leaf and stem proportion and leaf to stem ratio. FG10-09(F), Magna 801-FG (F) and Magna 788 cultivars had higher leaf proportion and leaf to stem ratio among other cultivars. Harvest taken during rainy season, had lower leaf proportion and leaf to stem ratio, where as harvest taken during drier season had higher leaf proportion and leaf to stem ratio.

Cultivar effect was not significant for chemical composition of selected alfalfa cultivars. All cultivars had higher chemical composition compared with the other researches and documents; had above 18% crude protein content, less than 10.47% Ash content, above 90% dry matter yield and above 163 RFV.

Even though the cultivars had not significant effect on chemical composition of selected alfalfa forages, numerically they had different values. Magna 801-FG (F) had higher CP, IVOMD, RFV, and lower fiber content.

Cutting cycles had significant effect, for DDM, ADF and cellulose components. The Eighth cutting cycle had lower ADF and cellulose but had higher DDM than seventh cutting cycle. It is true that forages have lower ADF mean, it has higher DM digestibility.

Therefore, based on the study results, the following recommendations are forwarded:

- These alfalfa cultivars can be maintained productivity up to 8th cutting cycle
- Magna 801-FG (F) give prioritize for dissemination to the livestock farming community because it has higher nutritional value than the rest cultivars
- For a sustainable alfalfa based feeding system use of irrigation scheme is advisable because it increase the biomass yields.
- It needs further research to know at which cutting cycle will decrease the yield and quality of cultivars after the 8th cutting cycle.

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8. APPENDICES

Appendix 1 Leaf yield of each cultivar for each harvest cycles

cultivar	Harvest cycle	Mean yield (kg/ha)	Std. Deviation	N
FG10-09(F)	first harvest	268.750000	73.8664335	4
	second harvest	395.000000	134.2261773	4
	third harvest	575.000000	193.6491673	4
	fourth harvest	562.500000	201.5564437	4
	fifth harvest	345.750000	167.0715914	4
	sixth harvest	303.250000	82.8467461	4
	seventh harvest	2075.00000	379.6928583	4
	eighth harvest	840.000000	1087.0108862	4
	Total	670.656250	681.2108105	32
FG9-09(F)	first harvest	182.500000	41.9324854	4
	second harvest	380.000000	191.8766965	4
	third harvest	525.000000	221.7355783	4
	fourth harvest	525.000000	150.0000000	4
	fifth harvest	436.250000	49.6344974	4
	sixth harvest	333.250000	79.4748388	4
	seventh harvest	2050.000000	456.4354646	4
	eighth harvest	3245.250000	854.1465038	4
	Total	959.656250	1088.9262723	32

Magna 801-FG(F)	first harvest	357.500000	122.9159604	4
	second harvest	438.750000	170.1653608	4
	third harvest	450.000000	267.7063067	4
	fourth harvest	525.000000	132.2875656	4
	fifth harvest	439.000000	224.1234779	4
	sixth harvest	381.750000	155.1072210	4
	seventh harvest	2450.00000	1501.665741 8	4
	eighth harvest	2123.50000	1388.292356 3	4
	Total	895.687500	1048.685673 2	32
Magna 788	first harvest	405.000000	61.7791766	4
	second harvest	315.000000	214.3206321	4
	third harvest	412.500000	62.9152870	4
	fourth harvest	400.000000	177.9513042	4
	fifth harvest	495.000000	146.7583047	4
	sixth harvest	304.750000	38.2044936	4
	seventh harvest	2337.50000 0	1002.808556 0	4
	eighth harvest	4363.25000 0	3810.794613 1	4
	Total	1129.12500 0	1865.160699 6	32
Hairy peruvian	first harvest	226.250000	110.7831967	4
	second harvest	558.750000	209.1799465	4
	third harvest	437.500000	209.6624271	4
	fourth harvest	603.750000	180.4335058	4
	fifth harvest	334.000000	75.3967285	4
	sixth harvest	249.250000	5.5000000	4

	seventh harvest	1687.500000	278.0137886	4
	eighth harvest	1931.750000	1487.9530403	4
	Total	753.593750	799.2935937	32
Total	first harvest	288.000000	114.8385595	20
	second harvest	417.500000	185.2061440	20
	third harvest	480.000000	190.8430052	20
	fourth harvest	523.250000	166.6091787	20
	fifth harvest	410.000000	144.7553290	20
	sixth harvest	314.450000	89.9113452	20
	seventh harvest	2120.000000	810.1981564	20
	eighth harvest	2500.750000	2185.0381877	20
	Total	881.743750	1168.2238211	160

Appendix 2 ANOVA table on the effect of cultivar and cutting cycle of leaf yield

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	142070839.244[a]	39	3642842.032	5.834	.000
Intercept	124395526.506	1	124395526.506	199.235	.000
Cultivars	4110163.963	4	1027540.991	1.646	.167
Harvest cycle	111135877.044	7	15876553.863	25.428	.000
Cultivars * Harvest cycle	26824798.237	28	958028.508	1.534	.059
Error	74923917.250	120	624365.977		
Total	341390283.000	160			
Corrected Total	216994756.494	159			

Appendix 3 Stem yield of each cultivar across cutting cycles

cultivar	Harvest cycle	Mean yield (kg/ha)	Std. Deviation	N
FG10-09(F)	first harvest	276.250000	138.8869444	4
	second harvest	358.750000	152.1717779	4
	third harvest	625.000000	175.5942292	4
	fourth harvest	612.500000	193.1105038	4
	fifth harvest	372.000000	190.4678451	4
	sixth harvest	238.500000	102.3995443	4
	seventh harvest	4775.000000	905.9985283	4
	eighth harvest	7027.250000	5165.1752068	4
	Total	1785.656250	2974.8035838	32
FG9-09(F)	first harvest	216.250000	61.5595917	4
	second harvest	388.750000	168.4426213	4
	third harvest	612.500000	165.2018967	4
	fourth harvest	575.000000	170.7825128	4
	fifth harvest	512.750000	81.3321380	4
	sixth harvest	321.750000	139.5907709	4
	seventh harvest	4250.000000	843.6033823	4
	eighth harvest	8338.000000	1491.4898592	4
	Total	1901.875000	2833.1225421	32
Magna 801-FG(F)	first harvest	347.500000	177.8341924	4
	second harvest	411.250000	166.1011238	4

	third harvest	500.000000	308.2207001	4
	fourth harvest	537.500000	179.6988221	4
	fifth harvest	462.000000	244.3699381	4
	sixth harvest	261.750000	113.2648077	4
	seventh harvest	5125.000000	3405.9996085	4
	eighth harvest	4484.250000	4281.8284548	4
	Total	1516.156250	2583.8327634	32
Magna 788	first harvest	391.250000	14.3614066	4
	second harvest	323.750000	336.8574130	4
	third harvest	562.500000	221.2653008	4
	fourth harvest	375.000000	64.5497224	4
	fifth harvest	537.250000	102.8441377	4
	sixth harvest	260.250000	87.7890464	4
	seventh harvest	4150.000000	1902.6297590	4
	eighth harvest	5733.500000	2539.4920096	4
	Total	1541.687500	2267.3841605	32
Hairy peruvian	first harvest	420.000000	146.4581852	4
	second harvest	872.500000	382.8511460	4
	third harvest	525.000000	86.6025404	4
	fourth harvest	589.500000	162.1963830	4
	fifth harvest	420.250000	129.2449225	4
	sixth harvest	275.000000	104.6422477	4
	seventh harvest	4325.000000	485.6267428	4

	eighth harvest	8445.000000	5992.4862954	4
	Total	1984.031250	3362.5459967	32
Total	first harvest	330.250000	133.9724540	20
	second harvest	471.000000	311.2605948	20
	third harvest	565.000000	188.5540548	20
	fourth harvest	537.900000	167.3690847	20
	fifth harvest	460.850000	155.9302391	20
	sixth harvest	271.450000	102.5370151	20
	seventh harvest	4525.000000	1680.8910544	20
	eighth harvest	6805.600000	4073.5470823	20
	Total	1745.881250	2798.9963641	160

Appendix 4 ANOVA table on the effect of cultivar and cutting cycle of stem yield of the cultivars

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	923072344.494[a]	39	23668521.654	8.804	.000
Intercept	487696214.256	1	487696214.256	181.415	.000
Cultivars	5667205.962	4	1416801.491	.527	.716
Harvestcycle	872650818.894	7	124664402.699	46.373	.000
Cultivars * Harvestcycle	44754319.638	28	1598368.558	.595	.944
Error	322594178.250	120	2688284.819		
Total	1733362737.000	160			
Corrected Total	1245666522.744	159			

Appendix 5 Total DM yield of cultivar across cutting cycles

cultivar	Harvest cycle	Mean yield (kg/ha)	Std. Deviation	N
FG10-09(F)	first harvest	545.000000	193.9501654	4
	second harvest	753.750000	280.6651326	4
	third harvest	1200.000000	341.5650255	4
	fourth harvest	1175.000000	392.6406330	4
	fifth harvest	717.750000	350.2526469	4
	sixth harvest	541.750000	180.6310697	4
	seventh harvest	6850.000000	1225.4250963	4
	eighth harvest	7867.387500	5372.1447812	4
	Total	2456.329688	3373.9493317	32
FG9-09(F)	first harvest	398.750000	75.3187670	4
	second harvest	768.750000	359.3367733	4
	third harvest	1137.500000	383.7859647	4
	fourth harvest	1100.000000	294.3920289	4
	fifth harvest	949.000000	124.0618126	4
	sixth harvest	655.000000	216.5979378	4
	seventh harvest	6300.000000	872.7351641	4

	eighth harvest	11583.250000	2300.1443976	4
	Total	2861.531250	3898.7317291	32
Magna 801-FG(F)	first harvest	705.000000	300.3608940	4
	second harvest	850.000000	326.4965543	4
	third harvest	950.000000	575.9050848	4
	fourth harvest	1062.500000	298.2588361	4
	fifth harvest	901.000000	460.8246232	4
	sixth harvest	643.500000	249.8833061	4
	seventh harvest	7575.000000	4878.9513901	4
	eighth harvest	6607.750000	5468.3536599	4
	Total	2411.843750	3590.9090085	32
Magna 788	first harvest	796.250000	72.6148516	4
	second harvest	638.750000	549.7328897	4
	third harvest	975.000000	259.8076211	4
	fourth harvest	775.000000	232.7373341	4
	fifth harvest	1032.250000	235.5396287	4
	sixth harvest	565.000000	104.3647450	4
	seventh harvest	6487.500000	2904.4147431	4
	eighth harvest	10096.750000	5831.7210367	4
	Total	2670.812500	3986.2540621	32
Hairy peruvian	first harvest	646.250000	219.3693613	4
	second harvest	1431.250000	591.0072617	4
	third harvest	962.500000	295.4516317	4

	fourth harvest	1193.250000	281.1563918	4
	fifth harvest	754.250000	175.8548170	4
	sixth harvest	524.250000	110.0465810	4
	seventh harvest	6012.500000	708.7253817	4
	eighth harvest	10376.875000	7096.1047105	4
	Total	2737.640625	4066.0707280	32
Total	first harvest	618.250000	221.8182933	20
	second harvest	888.500000	484.5890826	20
	third harvest	1045.000000	360.1534761	20
	fourth harvest	1061.150000	311.5532015	20
	fifth harvest	870.850000	289.3641441	20
	sixth harvest	585.900000	170.4600277	20
	seventh harvest	6645.000000	2415.0787281	20
	eighth harvest	9306.402500	5183.3937362	20
	Total	2627.631563	3748.0148391	160

Appendix 6 ANOVA table the effect of cultivar and cutting cycle on the TDMY

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1674398662.84[a]	39	42933299.04	9.214	.000
Intercept	1104711620.51	1	1104711620.	237.074	.000
Cultivars	4626699.629	4	1156674.907	.248	.910
Harvestcycle	1600419192.67	7	228631313.2	49.065	.000
Cultivars * Harvestcycle	69352770.544	28	2476884.662	.532	.973
Error	559172159.339	120	4659767.994		
Total	3338282442.70	160			
Corrected Total	2233570822.18	159			

Appendix 7 leaf proportion of each cultivar across cutting cycles

cultivar	Harvest cycle	Mean yield (kg/ha)	Std. Deviation	N
FG10-09(F)	first harvest	.512344	.1297102	4
	second harvest	.528275	.0478129	4
	third harvest	.477387	.0566162	4
	fourth harvest	.475961	.0236042	4
	fifth harvest	.485536	.0867118	4
	sixth harvest	.570521	.0558851	4
	seventh harvest	.303888	.0267412	4
	eighth harvest	.192625	.1828776	4
	Total	.443317	.1460072	32
FG9-09(F)	first harvest	.460753	.0801128	4
	second harvest	.485185	.0324331	4
	third harvest	.449264	.0528501	4
	fourth harvest	.475649	.0574409	4
	fifth harvest	.461007	.0239241	4
	sixth harvest	.520189	.0527420	4
	seventh harvest	.327392	.0745607	4
	eighth harvest	.277500	.0221736	4
	Total	.432118	.0926430	32
Magna 801-FG(F)	first harvest	.518538	.0395417	4
	second harvest	.520816	.0545071	4
	third harvest	.478715	.0142647	4
	fourth harvest	.497631	.0501336	4
	fifth harvest	.486980	.0695580	4

	sixth harvest	.595867	.0641247	4
	seventh harvest	.330482	.0347492	4
	eighth harvest	.347500	.0877021	4
	Total	.472066	.0984864	32
Magna 788	first harvest	.506514	.0321472	4
	second harvest	.541590	.0950654	4
	third harvest	.437500	.0842999	4
	fourth harvest	.498146	.0954183	4
	fifth harvest	.475186	.0480733	4
	sixth harvest	.546814	.0793595	4
	seventh harvest	.362395	.0086463	4
	eighth harvest	.400000	.1140175	4
	Total	.471018	.0927564	32
Hairy peruvian	first harvest	.343285	.0956287	4
	second harvest	.394867	.0174319	4
	third harvest	.439811	.0641757	4
	fourth harvest	.503374	.0763130	4
	fifth harvest	.447109	.0762403	4
	sixth harvest	.490147	.0936485	4
	seventh harvest	.279943	.0244307	4
	eighth harvest	.202500	.0665207	4
	Total	.387630	.1176666	32
Total	first harvest	.468287	.1003659	20
	second harvest	.494146	.0737013	20
	third harvest	.456536	.0556151	20
	fourth harvest	.490152	.0592588	20
	fifth harvest	.471164	.0597268	20

	sixth harvest	.544708	.0735272	20
	seventh harvest	.320820	.0457402	20
	eighth harvest	.284025	.1271647	20
	Total	.441230	.1142955	160

Appendix 8 ANOVA table of the effect of cultivar and cutting cycle on leaf proportion (%)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.469[a]	39	.038	7.433	.000
Intercept	31.149	1	31.149	6146.761	.000
Cultivars	.154	4	.038	7.575	.000
Harvest cycle	1.140	7	.163	32.123	.000
Cultivars * Harvest cycle	.176	28	.006	1.240	.212
Error	.608	120	.005		
Total	33.226	160			
Corrected Total	2.077	159			

Appendix 9 Stem proportion of each cultivar across cutting cycles

cultivar	Harvest cycle	Mean yield (kg/ha)	Std. Deviation	N
FG10-09(F)	first harvest	.487656	.1297102	4
	second harvest	.471725	.0478129	4
	third harvest	.522613	.0566162	4
	fourth harvest	.524039	.0236042	4
	fifth harvest	.514464	.0867118	4
	sixth harvest	.429479	.0558851	4
	seventh harvest	.696112	.0267412	4
	eighth harvest	.806750	.1823246	4
	Total	.556605	.1458018	32
FG9-09(F)	first harvest	.539247	.0801128	4
	second harvest	.514815	.0324331	4
	third harvest	.550736	.0528501	4
	fourth harvest	.524351	.0574409	4
	fifth harvest	.538993	.0239241	4
	sixth harvest	.479811	.0527420	4
	seventh harvest	.672608	.0745607	4
	eighth harvest	.720000	.0244949	4
	Total	.567570	.0921637	32
Magna 801-FG(F)	first harvest	.481462	.0395417	4
	second harvest	.479184	.0545071	4

	third harvest	.521285	.0142647	4
	fourth harvest	.502369	.0501336	4
	fifth harvest	.513020	.0695580	4
	sixth harvest	.404133	.0641247	4
	seventh harvest	.669518	.0347492	4
	eighth harvest	.652500	.0877021	4
	Total	.527934	.0984864	32
Magna 788	first harvest	.493486	.0321472	4
	second harvest	.458410	.0950654	4
	third harvest	.562500	.0842999	4
	fourth harvest	.501854	.0954183	4
	fifth harvest	.524814	.0480733	4
	sixth harvest	.453186	.0793595	4
	seventh harvest	.637605	.0086463	4
	eighth harvest	.600000	.1140175	4
	Total	.528982	.0927564	32
Hairy peruvian	first harvest	.656715	.0956287	4
	second harvest	.605133	.0174319	4
	third harvest	.560189	.0641757	4
	fourth harvest	.496626	.0763130	4
	fifth harvest	.552891	.0762403	4
	sixth harvest	.509853	.0936485	4
	seventh harvest	.720057	.0244307	4
	eighth harvest	.802500	.0713559	4
	Total	.612995	.1189607	32
Total	frst harvest	.531713	.1003659	20
	second harvest	.505854	.0737013	20
	third harvest	.543464	.0556151	20

	fourth harvest	.509848	.0592588	20
	fifth harvest	.528836	.0597268	20
	sixth harvest	.455292	.0735272	20
	seventh harvest	.679180	.0457402	20
	eighth harvest	.716350	.1280959	20
	Total	.558817	.1144846	160

Appendix 10 ANOVA table the effect of cultivar and cutting cycle on stem proportion (%)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.474[a]	39	.038	7.438	.000
Intercept	49.964	1	49.964	9831.737	.000
Cultivars	.156	4	.039	7.652	.000
Harvestcycle	1.142	7	.163	32.099	.000
Cultivars * Harvestcycle	.177	28	.006	1.242	.210
Error	.610	120	.005		
Total	52.048	160			
Corrected Total	2.084	159			

Appendix 11 Leaf to stem ratio of each cultivar across cutting cycles

cultivar	Harvest cycle	Mean yield (kg/ha)	Std. Deviation	N
FG10-09(F)	first harvest	1.210099	.7996125	4
	second harvest	1.136315	.2163671	4
	third harvest	.929514	.1978972	4
	fourth harvest	.911111	.0846197	4
	fifth harvest	.989094	.3618121	4
	sixth harvest	1.359061	.3159123	4
	seventh harvest	.438146	.0553872	4
	eighth harvest	.290997	.3110981	4
	Total	.908042	.4755518	32
FG9-09(F)	first harvest	.884209	.2680397	4
	second harvest	.948111	.1200886	4
	third harvest	.828125	.1721116	4
	fourth harvest	.925000	.2179449	4
	fifth harvest	.858034	.0817434	4
	sixth harvest	1.103422	.2345017	4
	seventh harvest	.502117	.1849933	4
	eighth harvest	.386513	.0433802	4

	Total	.804441	.2773840	32
Magna 801-FG(F)	frst harvest	1.087551	.1715874	4
	second harvest	1.106492	.2303826	4
	third harvest	.919444	.0539585	4
	fourth harvest	1.005556	.2004239	4
	fifth harvest	.975421	.2586619	4
	sixth harvest	1.516168	.3513941	4
	seventh harvest	.496662	.0783905	4
	eighth harvest	.554568	.2194220	4
	Total	.957733	.3605966	32
Magna 788	first harvest	1.033020	.1356406	4
	second harvest	1.252613	.4585916	4
	third harvest	.808036	.2709641	4
	fourth harvest	1.042163	.3495571	4
	fifth harvest	.916787	.1654056	4
	sixth harvest	1.264479	.4439534	4
	seventh harvest	.568586	.0213299	4
	eighth harvest	.720346	.3793189	4
	Total	.950754	.3628132	32
Hairy peruvian	first harvest	.549697	.2479639	4
	second harvest	.653548	.0472268	4
	third harvest	.805128	.2330091	4
	fourth harvest	1.055795	.3668593	4
	fifth harvest	.837269	.2775644	4
	sixth harvest	1.013239	.3774038	4
	seventh harvest	.390005	.0481860	4
	eighth harvest	.259069	.1010083	4
	Total	.695469	.3469967	32

Total	first harvest	.952915	.4288067	20
	second harvest	1.019416	.3110961	20
	third harvest	.858049	.1861708	20
	fourth harvest	.987925	.2434071	20
	fifth harvest	.915321	.2294631	20
	sixth harvest	1.251274	.3623985	20
	seventh harvest	.479103	.1057507	20
	eighth harvest	.442299	.2810369	20
	Total	.863288	.3790619	160

Appendix 12 ANOVA table the effect of cultivar and cutting cycle on leaf to stem ratio

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	13.930[a]	39	.357	4.807	.000
Intercept	119.243	1	119.243	1604.760	.000
Cultivars	1.606	4	.402	5.405	.000
Harvest cycle	10.521	7	1.503	20.227	.000
Cultivars * Harvest cycle	1.803	28	.064	.866	.660
Error	8.917	120	.074		
Total	142.089	160			
Corrected Total	22.846	159			