



EFFECT OF INTERCROPPING OATS (*Avena sativa* L.) WITH ALFALFA (*Medicago sativa*) ON DRY MATTER YIELD, NUTRITIVE VALUE, *IN VITRO* RUMINAL FERMENTATION KINETICS AND SILAGE QUALITY

MSc THESIS

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EFFECT OF INTERCROPPING OATS (*Avena sativa* L.) WITH ALFALFA (*Medicago sativa*) ON DRY MATTER YIELD, NUTRITIVE VALUE, *IN VITRO* RUMINAL FERMENTATION KINETICS AND SILAGE QUALITY

By

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Submitted to Department of Animal Sciences, Jimma University College of Agriculture and Veterinary Medicine, in Partial Fulfillment of the Requirement for the Degree of Master of Science in Animal Nutrition

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SCHOOL OF GRADUATE STUDIES

JIMMA UNIVERSITY

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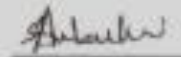
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DEDICATION

I dedicate this thesis to my son Baro and my daughter Urji Daniel, who were deprived of my love throughout my study. I also dedicate this thesis to my beloved wife, Diribe Tolina, for her moral support and full responsibility for my children during my research work for graduate studies at Jimma University.

AUTHOR'S STATEMENT

I hereby declare and confirm that this thesis is my own work and that all material sources used for this thesis have been acknowledged. I have followed all ethical and technical principles in the preparation, data collection, data analysis, and completion of this thesis. This thesis has been submitted in partial fulfillment of the requirements for an MSc degree at Jimma University and is deposited in the University Library to be made available to borrowers in accordance with the rules of the library. I declare that this thesis is not submitted to any other institution anywhere for the award of an academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author was born in June 1983 from his father, Wana Nemera, and his mother, Desta Waktol, in Kiltu Karra District, West Wollega Zone, Oromia Regional State, Ethiopia. He began his primary education at Kolati Jirma Primary School in the 1st and 2nd grades in 1990–1991. He then attended Wanki Gopho Elementary School for grades 3–8 from 1992–1998. He completed his high school studies in grades 9 and 10 at Kiltu Karra Secondary School in 1999 and 2000 and completed his preparatory school (11 and 12 grade) at Mene Sibu Preparatory School, Mene Sibu district, West Wallega Zone, Oromia, in 2001 and 2002. He joined Ambo University in 2003 and graduated with a Bachelor of Science in Animal Science in July 2005. After one year of his graduation in June 2006 up to June 2009, he was employed in the Livestock Development and Fisheries Office in Bora Woreda and worked as a livestock development expert for three years in East Shawa Zone, Oromia Regional State. After three years (2010–2014), he worked at the Oromia Agricultural Research Institute in the Adami Tulu Agricultural Research Center, Adami Tulu Jido Kombolcha District, East Shewa Zone, Oromia Regional State. In December 2014, he moved to Jimma University, College of Agriculture and Veterinary Medicine, to pursue his MSc study in animal nutrition program. The year indicated above is according to the Ethiopian calendar.

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LIST OF ABBREVIATIONS

ADF.....	Acid Detergent Fiber
ADL.....	Acid Detergent Lignin
AOAC.....	Association of Official Analytical Chemists
ARC.....	Agricultural Research Center
CEC.....	Cation Exchange Capacity
CECC.....	Cation Exchangeable Capacity
CH ₄	Methane
CO ₂	Carbon dioxide
CP.....	Crude Protein
CSA.....	Central of Statistical Agency
CV.....	Coefficient of Variation
DM.....	Dry Matter
DMI.....	Dry Matter Intake
EC.....	Electron Conductivity
GLM.....	General Linear Model
ILRI.....	International Livestock Research Institute
IVDMD.....	<i>In vitro</i> Dry Matter Digestibility
IVOMD.....	<i>In vitro</i> Organic Matter Digestibility
LER.....	Land Equivalent Ratio
MOA.....	Ministry of Agriculture
NDF.....	Neutral Detergent Fiber
NRC.....	National Research Center
OARI.....	Oromia Agricultural Research Institute
OC.....	Organic Carbon
OM.....	Organic Matter
PPM.....	Parts per million
RCBD.....	Randomized Complete Block Design
SAS.....	Statistical Analysis System
SCFA.....	Short Chain Fatty Acid
TN.....	Total Nitrogen
VFA.....	Volatile Fatty Acids

EFFECT OF INTER CROPPING OATS WITH ALFALFA ON DRY MATTER YIELD, NUTRITIVE VALUE, *IN VITRO* RUMINAL FERMENTATION KINETICS AND SILAGE QUALITY

ABSTRACT

There is limited information on planting density for oats and alfalfa when intercropped to achieve a mixture of high nutritional quality for use as animal feed. A field experiment was carried out to study the effect of seeding ratio on yield components (TDM and seed yield), chemical composition (CP, OM, NDF, ADF, ADL, and Ash), total gas, CH₄ and SCFA, silage (physical and chemical properties), and soil chemical composition (PH, OC, OM, EC, CEC, TN, K, and P). The experiment was conducted at the Adami Tulu Agricultural Research Center under irrigation conditions during February–June 2022/2023 in Oromia Regional State, Ethiopia. Treatments consisted of one adapted oats (bate) variety and one alfalfa (magna 788) variety in an additive design at three plant densities (25%, 50%, and 75% of their recommended sole seed rates of 80 and 15 kg per hectare for oats and alfalfa, respectively) and the recommended 100% plant density of sole oats and sole alfalfa. The experiment was laid out in a randomized complete block design (RCBD) with four replications. Data were organized and analyzed with excel and SAS software. Results showed statistically significant differences ($P < 0.05$) in all tested parameters of agronomic yield, chemical composition of forage and silage, soil chemical composition, total gas, methane (CH₄), and SCFA. The highest DMY (14.64 t/ha) was recorded from T4, followed by T3 (14.51 t/ha). The OM, CP, Ash, NDF, ADF, ADL, and IVDMD revealed significant variation ($p < 0.05$) among the treatments both in forage and silage. The OM and CP content of oats and alfalfa produced in intercrops, particularly from the T4 seed ratio (92.08 and 17.33), tend to be better due to the alfalfa component in the intercrop than that of sole oats (92.03 and 11.53), but lower than that of pure alfalfa (93.53 and 21.2), respectively. The physical score of silage, consisting of the sum of the smell, color, moldiness, and structure scores, ranged between 3.08 and 3.32, and the physical quality class of the silage ranged between the middle and very good classes. It was determined that silage DM, NDF, ADF, and ADL decreased, and the pH, OM, and CP values increased statistically ($P < 0.05$) in parallel with the increase in the alfalfa ratio in the mixture. The analysis of soil samples showed an increased level ($P < 0.05$) of OM, OC, EC, CEC, TN, K, and P soil nutrients based on treatment combinations. All treatment groups showed a slight change ($P > 0.05$) in pH values above the initial recorded average value of 4.43. The pH of the soil after forage harvest was statistically higher ($P < 0.05$) in sole oats (6.15) and sole alfalfa (6.3) than that of their mixtures. In vitro gas and methane production as well as short-chain fatty acids were all statistically ($P < 0.05$) affected by the seeding ratio of oats with alfalfa, with the highest values observed in sole alfalfa, followed by intercropped groups, and the lowest in sole oats. Total gas, CH₄, and SCFA production increased with an increasing proportion of alfalfa seeding ratios. While sole oats produced the lowest CH₄ among other treatments, Generally, the seeding ratio combinations of T4 and T3 performed the best in most agronomic practices, nutritional values, soil fertility, fermentation properties, and balancing total gas and methane production both in forage and silage. So they are recommended for fodder production in the studied area and related agro-ecologies. Further studies should be conducted to test by feeding forage produced by inter cropping of oats and alfalfa on animal performance.

Keywords: Alfalfa, Chemical Composition, Gas production, Methane, Oats, Seed ratio, Silage

1. INTRODUCTION

Livestock production remains an important industry for feeding the world's population (Andrea, 2021). Today's feed industry is working to meet the growing global demand and further enhance its sustainability (Spring *et al.*, 2019). Livestock farming represents an important livelihood for the agricultural, agro-pastoral, and pastoral societies that make up over 80% of the Ethiopian population. Despite the large livestock resource base economy and the sector is very important to millions of smallholder farmers, pastoral communities, and the wider national economy, livestock production remains underdeveloped, and the available potential has not been fully exploited. Under nutrition and malnutrition are major factors constraining animal production in Ethiopia. Nutritional stress leads to low growth rates, poor fertility, and high mortality, which is compounded by diseases.

In the highlands, more than half of the annual feed supply for livestock comes from the natural pasture, while the remaining comes from crop residues and stubble grazing. About 85% of feed intake is used to meet the animals' maintenance requirements, and only 15% is utilized for production (Alemayehu, 2002a; 2002b). Livestock feed in Ethiopia is derived primarily from natural pasture and crop residues, with agro-industrial by-products used as supplementary feeds by some urban and peri-urban market-oriented livestock producers. In the last three decades, natural pasture area accounted for about 53% of Ethiopia's total land area (Lulseged and Alemu, 1985). Currently, the human population has tripled, necessitating an expansion of arable crops at the expense of available grazing lands to meet food demand.

Moreover, increasing urbanization and the use of arable land for housing, recreation, and industrial development are displacing a significant portion of the grazing land. As a result, in the highlands alone, grazing lands have significantly dwindled to about 5.7 million hectares (Abera, 2006). These remaining grazing lands constitute marginal lands, highly fragmented and limited to areas where conditions for cropping are unfavorable due to topographical, edaphic, and climatic limitations. In general, rough estimates of the available feed in different parts of the country show a deficit by about 35% of the maintenance requirement of livestock, which further increases to 70% in bad years, such as prolonged drought.

Feed shortages in terms of both quantity and quality are the major problem affecting livestock productivity in Ethiopia (Adugna, 2007; Fekede *et al.*, 2015a). The traditional livestock production system is based primarily on poor pastures and crop residues, which are usually insufficient to support reasonable livestock production (Tsige, 2000). Towards the end of the dry season, livestock feed is usually scarce and of poor quality. Residues from cereals are the main source of feed, but these are low in protein and have poor digestibility. The production of adequate quantities of good-quality dry-season forages to overcome feed shortage and supplement crop residues with different cultivated nutritious feed stuffs to overcome the dry-season feed problem that affects livestock production in Ethiopia. Improved forages provide a reasonable source of nutrients for animal production while conserving soil fertility, water resources, and air quality (Chaudhry, 2008).

Improvement in livestock production and productivity requires a concomitant intensification in feed production using improved or cultivated forage crops in addition to efficient utilization of natural pasture and crop residues. The current understanding in the ongoing Economic Growth and Transformation Plan as regards the livestock sector is to transform the small-scale production system into a specialized and market-oriented production system that includes high quality cultivated pasture production. Improved forage crops have multiple functions and play an important role in sustaining the livelihoods of farmers, mainly as a result of their positive effects on livestock production and contribution to economic and environmental sustainability.

Improved forages not only provide better quantity and quality of forage but also have a number of other benefits in the farming system, including improvement in soil fertility through biological nitrogen fixation or when used as mulch (legumes), nutrient cycling and erosion control when established as conservation structures, firewood supply, bee forage, and control of weeds, pests, and diseases when integrated in crop rotation as break crops. Generally, the use of improved forage crops is an important step in supporting and improving livestock productivity while maintaining environmental sustainability. Oats (*Avena sativa* L.) are considered the most extensively used annual cool-season forage worldwide and are an important source of nutrition for ruminant livestock (Andrzejewska *et al.*, 2018).

Oats are a potential fodder crop for livestock feeding and have been growing in the central highlands of Ethiopia for about five decades. It has been well accepted by the farming community because of its hardy nature, which makes it perform better under stressful conditions with very minimal management inputs. It is used as livestock feed in the form of hay, silage, and grazing or green feed and provides an abundance of excellent forage at a time when other succulent, high-protein feeds are scarce. Similarly, Alfalfa (*Medicago sativa* L.) is one of the recommended forage legumes with high potential as a fodder crop. Alfalfa refers to the crucial perennial legume forage; it can be used to achieve symbiotic N₂ fixation (Dai & Jia, 2017). Alfalfa exhibits high yields, rapidly regenerating many new stems after it is harvested; it can also be harvested multiple times in the growing season (Brink *et al.*, 2015).

Alfalfa is one of the most commonly used species in arid and semi-arid regions. Alfalfa can grow in a mixture with most of the grasses, but a balance of the alfalfa with the grass is best maintained if only appropriate agronomic management is practiced. Alfalfa is also called the “green gold of forage crops” as it is rich in protein (18–22%), amino acids, fiber (20–30%), and vitamin ‘A’ content. It is considered the “Queen of the Forage Crop.” It is highly palatable, nutritious, rich in protein and mineral constituents, has an excellent growth habit, and fixes between 10 and 20 kg/ha of nitrogen for every ton of dry matter produced, increasing soil nitrogen levels for subsequent crops (Das *et al.*, 1964).

Alfalfa (*Medicago sativa* L.) is one of the best bet leguminous fodder crops for livestock, especially for dairy production in Ethiopia, due to its superior herbage yield, palatability, nutritional value, wide adaptability, and suitability for irrigated production systems. Its agronomic advantages, including fast growth, persistence under prolonged dry conditions, and versatility in its soil requirements, have made it a popular forage crop for intensive livestock production systems such as dairy production. In Ethiopia, alfalfa exhibits excellent performance over a wide range of altitudes, from cool temperate highlands up to 2400m above sea level to warm lowlands below 1000 m.a.s.l., and in a range of soil types, including heavy clay soils. It is often grown in pure stands as a source of protein supplement to supplement low-quality feed stuffs or in mixtures with grasses (Sisay, 1975; AStatke Haile, 1977). The chemical composition of forage grass varies with soil type, landscape, management practices, and other environmental factors that influence forage yield and quality (Gemiyo *et al.*, 2013).

Both oat and alfalfa were adapted in the study area before a long period of time, and their promising performance, especially under irrigation conditions, was reported by different researchers (Solomon *et al.*, 2018; Tesfaye, 2020; Nabi *et al.*, 2021). Oats and alfalfa produce green fodder, which plays an important role in reducing the scarcity of green fodder during the dry season, and they are used as multi-cutting forage (Solomon *et al.*, 2018). Rather than producing their pure stand, their mixture compensates for the CP reduction in oats (Salem *et al.*, 2015). Even though oats produce a large amount of biomass yield, their CP content is very low. Inversely, alfalfa is low in biomass yield but has a higher CP content (Maxin *et al.*, 2017). Different experiments were done on the effect of oats with alfalfa inter cropping, and significant variations in biomass yield and nutritional values were observed.

For instance, Befekadu and Yunus, 2015 reported that oats with different varieties of alfalfa revealed significant ($P < 0.05$) variation on dry matter yield and crude protein contents among the tested treatments. A similar report by Belayneh *et al.*, 2020 on the effect of oats inter cropped with alfalfa on the agronomic yield and nutritional values of the mixture. As indicated by different scholars, seed ratios significantly affect both dry matter yield and nutritional quality when oats are mixed with alfalfa and other legumes (Phelan *et al.*, 2015; Zhang *et al.*, 2015; Maxin *et al.*, 2017; Baruah *et al.*, 2018; Kulkarni *et al.*, 2018; Solomon *et al.*, 2018; Can *et al.*, 2020; Nabi *et al.*, 2021). However, until now, there is no information on the seeding ratio or density of both oats and alfalfa when they are inter cropping, so further investigation is needed to determine the optimum seed ratio to fill the gap.

Legume monocultures have a low risk of silage fermentation due to their low water-soluble carbohydrate and dry matter (DM) content and high buffering capacity (Kaiser *et al.*, 2007; Denek *et al.*, 2011). For this reason, grass-legume silage mixtures are preferred in the sense that cereals will provide ferment able carbohydrates and legumes will improve the protein content of silage. Inter cropping is the cultivation of two or more crop species on the same land. Compared to mono crops, inter cropping systems help to increase the overall yield and yield stability of cropping systems, decrease the negative impacts of environmental problems, and promote the coordinated use of light energy, nutrients, water, and other resources (Ai *et al.*, 2018).

Inter cropping systems play an important role in the sustainable intensification of agriculture and could help to improve the productivity of artificial grassland. Inter cropping compatible forage grasses with legumes improves and maintains soil fertility through nitrogen fixation and increases productivity and profitability (Yildirim and Guvenc, 2005). This is partly recognized due to the fact that legumes are able to fix atmospheric nitrogen through a symbiotic association with soil bacteria called rhizobium (Jensen *et al.*, 2010). Previous studies focused on rhizode position transfer, water use efficiency, nutrients (such as phosphorus), photo synthetically active radiation, the microbial properties of rhizospheres (Li *et al.*, 2016), and the control of weeds, pests, and wind erosion (Liang *et al.*, 2016). Species with different physiological and/or morphological characteristics can complement each other better and make better use of the feed resources. This study primarily aims to elucidate how inter cropping of alfalfa and oats impacts yield components, nutritional value, silage quality, gas production, and soil improvement.

Globally, the annual greenhouse gas (GHG) emissions attributed by livestock production are estimated to be 18%, or 7,516 million metric tons per year of CO₂ equivalents (CO₂ eq) (Steinfeld & FAO, 2006). Methane (CH₄) contributes to about 83.4% of the total GHG emissions in Ethiopia, of which 88% are from enteric fermentation (Berhe *et al.*, 2020). Energy loss from ruminant livestock through enteric methane emissions is a problem not only because of the impact on climate change but also because of its considerable effect on animal performance. Ruminant dietary energy loss due to enteric methane ranges from 2 to 12% of gross energy intake, depending on the feed type (Johnson & Johnson, 1995), so efforts to reduce emissions also present an opportunity to increase livestock productivity. Currently, there is growing interest in the use of forage legumes as a supplement to fiber-based diets of ruminants to compensate for nutrient deficiencies and mitigate enteric methane emissions in the tropics and subtropics (Baruah *et al.*, 2018; Delgado *et al.*, 2012).

However, management practices such as seeding rate ratio influence the yield and quality of these species as well as their compatibility when grown in mixtures (Atif *et al.*, 2012). The benefits of mixed forage species can be efficiently exploited only if proper management strategies, such as optimum proportions of seeding rates, are used. There is limited knowledge on the proportions of seeding rates that influence the yield and quality of most of the cereal-legume

mixture, including oats and alfalfa, under Rift Valley conditions in Oromia. Therefore, the aim of this study was to evaluate the effect of inter cropping of oats and alfalfa, which could optimize the dry matter yield and nutritional quality of the mixtures, *in vitro* fermentation kinetics, soil improvement, and silage quality.

1.1. Objectives

- to evaluate the effect of inter cropping of oats and alfalfa on agronomic component, biomass yield and nutritive value
- to evaluate the effect of inter cropping of oats and alfalfa on *in vitro* fermentation kinetics
- to evaluate the silage quality of oats and alfalfa obtained by intercropping

2. LITERATURE REVIEW

2.1. Oats (*Avena sativa* L.)

Oats are one of the most important cultivated winter fodder crops in Ethiopia. It has been adapted to a wide range of soil types and climatic conditions. It is promising for multi-cutting and fits well in farming systems for quality and quantity fodder supply during the winter feed scarcity period (December to April). In an estimation by FAO (2010/011), oats ranked sixth in the global context of wheat, maize, rice, barley, and sorghum as a food crop and ranked first in the world within the fodder production statistics. Oats are mainly grown below 1600 m.a.s.l. but can be cultivated at higher altitudes (Pariyar, 2002). They are mainly used as green feed for the winter gap, but some are already being made into hay, mainly at higher altitudes. It is well known that fodder oats have the potential to produce nutritious fodder during the dry winter. The productivity of oats might depend on environmental and management factors such as altitude, soil type, rainfall, fertilizer, etc. The oats may be grazed, cut-and-carried, or could be used in the form of hay and silage. The oats may be used as a winter cover to protect soil erosion and to trap N that would otherwise be alleviated during the winter (Sare, 2008).

2.1.1. Distribution of oats

Oats are mostly found at 45–65° N and 20–46° S (Stevens *et al.*, 2004). In temperate regions, they are grown as a spring- or autumn-growing crop and as a cool-season crop in the Mediterranean and tropical areas (Heuze *et al.*, 2016). Oats grow on a wide range of soils at temperatures ranging from 5 to 26°C and rainfall over 500 mm (Eco Port, 2013). Further, oats perform better in loam soils but tolerate acidic and low-fertile soils with pH ranging from 4.5 to 8.6 (Heuze *et al.*, 2016). Oats are grown in more than 50 countries, but statistical information is mainly concerned with the food grain (Heuze *et al.*, 2016). Although oats are grown as a dual crop (forage and grain), their introduction into the commercial market is rare. However, there is no reliable estimation of worldwide oat forage acreage or production in general (Suttie *et al.*, 2004). Oats remain an important grain crop for people in marginal ecologies throughout the developing world and also in developed countries (Suttie & Reynolds, 2004).

Livestock grain feed is still the primary use of oat crops, accounting for an average of around 74 percent of the world's total usage from 1991 to 1992 (Welch, 1995). Stevens *et al.*, 2004 found that oats are well adapted to a wide range of soil types but perform better on acid soils. They are mostly grown in cool, moist climates and can be sensitive to hot, dry weather from head emergence through to maturity (Suttie & Reynolds, 2004). Stevens *et al.*, 2004 noted that Russia, the United States, Canada, Germany, and Poland account for about 75 percent of the world's supply of grain, seed, and industrial-grade oats. Since the 1960s, the proportion of oats used for feed has declined in the US and Canada, remained unchanged in the former Soviet Union countries and Poland, and increased slightly in Germany (Stevens *et al.*, 2004). The leading export countries of oat grain are Canada, Finland, Sweden, Australia, and Argentina.

2.1.2. Utilization of oats

Animal feed: It is the preferred feed of all animals, and grain is also valuable feed for horses, dairy cows, poultry, and young breeding animals. Oats are grown for use as grain as well as forage and fodder. It is an important winter fodder, mostly fed as green, but excess is converted into silage or hay for animal feeding during fodder deficit periods (Suttie & Reynolds, 2004). Interest in oat hay for the dairy, feedlot, and horse industries has grown in recent years.

Oats as an intercrop with legumes: inter cropping is a traditional and extensive agricultural practice used in low-input cropping systems around the world (Anil *et al.*, 1998). During the 20th century, there was a shift from mainly labor-intensive systems to more optimized cropping through the use of external inputs, especially synthetic fertilizers and pesticides (Crews & Peoples, 2004). There has been a growing interest in inter cropping systems in developed countries due to the increasing awareness of environmental degradation arising from the heavy use of non-renewable resources (Fujita *et al.*, 1992). Inter cropping systems, especially cereals with legumes, have several major benefits, such as higher total yield and better land use efficiency (Dhima *et al.*, 2007), yield stability of the cropping system, better utilization of light, water, and nutrients (Javanmard *et al.*, 2009), improved soil conservation (Anil *et al.*, 1998), and better control of pests and weeds (Banik *et al.*, 2006; Vasilakoglou *et al.*, 2008).

2.2. Alfalfa (*Medicago sativa* L.)

Alfalfa (*Medicago sativa* L.) is one of the most prevalent forage legumes grown worldwide. Known as ‘the king of forage’ (Ma *et al.*, 2016). Alfalfa is widely used for beef and dairy production because it is high in yield, palatable, and rich in nutrients (Phelan *et al.*, 2015; Zhang *et al.*, 2015; Kulkarni *et al.*, 2018). However, the consumption of Alfalfa is associated with a few challenges. For example, ruminants feeding on large quantities of Alfalfa in a short time are likely to suffer from gastric distension (bloats) due to the rapid digestion of Alfalfa, which produces a lot of gas. When the gas mixes with soluble proteins from digested plant cells, it forms a stable, frothy structure in the rumen (Makoni *et al.*, 1994; Min *et al.*, 2005), which is difficult to discharge and may even result in ruminant death. Therefore, ruminants are allowed to graze on forage legumes such as clover or alfalfa only as components of grass-legume mixtures to avoid the above-mentioned issue (Phelan *et al.*, 2015).

Alfalfa (*Medicago sativa* L.) is characterized by its ability to produce forage every 35–40 days by storing energy in the crown to support re-growth after cutting (Undersander *et al.*, 2011). It adds nitrogen to the soil through symbiotic nitrogen fixation in bacterial nodules on the roots and can also withstand long periods of water deficiency by halting its vegetative growth and accessing water from greater depths through its deep root system (Annicchiarico & Pecetti, 2010). These properties make alfalfa among some of the most widely grown forage crops in the world. The crop can produce 24 t/h dry matter annually, and it is estimated to be 25% more than pasture (Richard, 2011). Evidence indicated that feeding alfalfa improves the productivity of animals; particularly, milking cows supplemented with alfalfa could produce over 50–100% additional yield.

There are various genotype of alfalfa selected for winter hardiness, drought resistance, tolerance to heavy grazing, pests, and diseases (Frame, 2005). While selecting the alfalfa genotype in Ethiopia, it is important to focus on the environmental adaptation, herbage dry matter yield potential, and seed-bearing ability of the genotype. Five alfalfa varieties, namely, FG10-09 (F), FG9-09 (F), Magna 801-FG (F), Magna 788, and Hairy Peruvian, were evaluated in seven federal and regional research centers representing diverse agro-ecological areas of Ethiopia. The locations were: Debre Zeit, Holetta, Werer, Melkassa, Wondo, Adami Tulu, and Hawassa

research centers. For instance, at the Adami Tulum Agricultural Research Center, five alfalfa varieties were evaluated and their dry matter yield recorded accordingly: FG-10-09 (F) (10.57 t/ha), Magna788 (10.4 t/ha), Magna 801 (FG) (10.51), FG-09-09 (F) (9.6 t/ha), and Altarivera (12.18 t/ha) under irrigation conditions (Solomon *et al.*, 2018).

2.2.1. Adaptability of alfalfa in Ethiopia

Alfalfa (*Medicago sativa* L.) is one of the best bet leguminous fodder crops for livestock, especially for dairy production in Ethiopia, owing to its superior herbage yield, palatability, nutritional value, wide range of adaptation, and suitability for irrigated production systems. Its agronomic merits, including fast growth, persistence under prolonged dry conditions, and versatility in its soil requirements, have made it a popular fodder crop for intensive livestock production systems such as dairy production. In Ethiopia, Alfalfa has excellent performance in a wide range of altitudes, from cool temperate highlands up to 2400 to warm lowlands below 1000 m.a.s.l., and a range of soil types, including heavy clay soils. It has been popularly grown in pure stands (Astatke Haile, 1977) as a source of protein supplement for low-quality feed stuffs or in mixtures with grasses (Sisay, 1975).

2.2.2. Factors affecting alfalfa production

2.2.3. Effect of alfalfa on silage and total gas production

Ensiling has been increasingly used for forage preservation across the world, especially where precipitation patterns limit opportunities for dependable hay production. In contrast, the sole use of legumes (e.g., alfalfa (*Medicago sativa*) and clover) can increase the protein content of silage but cause undesirable fermentation and proteolysis due to the high buffering capacity, low WSC, and poor utilization of nitrogen (N) by ruminants (McDonald *et al.*, 1991; Winters *et al.*, 2000). Additionally, previous studies noted that the high degradable nutrients in alfalfa provided a large number of substrates for methanogens, resulting in relatively high methane (CH₄) and carbon dioxide (CO₂) emissions (Cronje, 2000; Humphry *et al.*, 2012). To overcome the imbalance of nutrient supply, inter cropping of grass and legumes has been increasingly accepted as a good practice for high-quality forage production in terms of either mixed hay or mixed silage

(Adesogan *et al.*, 2004; Maxin *et al.*, 2017). It is important to identify the suitable ratio of grass-legume that well coordinates with the needs in the field and during the ensiling process, quickly increasing acidity and reducing proteolysis. However, it is presently not clear whether the inter crop mixtures of oats and alfalfa at appropriate ratios would make superior-quality silage with high nutritive values.

2.3. Advantages of Grass-Legume Inter Cropping

2.3.1. Increased production

Production is more than a pure cropping of the same land area (Caballero & Goicoechea, 1995). Ghanbari and Lee, 2002 reported that dry matter production in wheat and bean inter crops had been greater than their pure cropping. Similarly, Martin and Snaydon, 1982 reported that grain and dry matter yields in bean and barley inter crops were higher than their pure cropping. Odhiambo and Ariga, 2001 found that there was increased production with maize and bean inter crops in different ratios due to reduced competition between species compared to competition. Within species. Willey, 1990 considers inter cropping as an economic method for higher production with lower levels of external inputs. More production in inter cropping can be attributed to the higher growth rate, reduction of weeds, reduced pests and diseases, and more effective use of resources due to differences in resource consumption (Eskandari, 2012b; Willey, 1990). In addition, the production increases in inter cropping if there are complementary effects between the components of inter cropping that have been achieved by inter cropping, which reduces the competition between them (Mahapatra, 2011).

2.3.2. Greater use of environmental resources

The benefits of inter cropping in crop production in comparison with pure stands are due to the interaction between components in intercrops and the differences in competition for the use of environmental resources (Mahapatra, 2011). If the inter crop components have a difference together in the use of environmental resources, so that is complementary in the use of these resources, then resource use is more effective than a pure stand, and the resultant increase in yield (Jensen, 1996).

2.3.3. Reduction of pests, diseases and weeds damage.

The other benefit of inter cropping is its ability to reduce pest and disease damage. In a review by Francis, 1989 on inter cropping, in 53% of the experiments, inter cropping reduced the pest, and in 18%, it increased the pest compared to the pure stand. Increasing pests can be due to several reasons, such as the second crop being a host for pests in inter cropping or increasing the shade in the canopy, which provides favorable conditions for pest and pathogen activity. In addition, plant residues can be a source for pathogens inoculated (Anil *et al.*, 1998). In mono cropping systems, available resources such as soil moisture, nutrients, and light are rarely used by the plant, and thus the released niches are occupied by the weeds. In inter cropping, there is better utilization of nutrients, soil moisture, and light, which fills the empty niche, which leads to weed suppression (Altieri, 1995; Saady and El-Metwally, 2009).

2.3.4. Improve soil fertility

Inter cropping improves soil fertility and increases the nitrogen content. And the resulting Inter cropping improves soil fertility and increases the nitrogen content. And the resulting nitrogen is an essential element for soil fertility and plant growth. Several reports indicate that the increase in nitrogen content of non-legume plants is due to the inter cropping of these plants with legumes (Eskandari *et al.*, 2009a; Anil *et al.*, 1998; Fujita *et al.*, 1992). Rhizobium bacteria have a symbiotic relationship with plants of the leguminous family and thereby can fix atmospheric nitrogen into available nitrogen for plant uptake. Nitrogen is an essential element for soil fertility and plant growth. Several reports indicate that the increase in nitrogen content of non-legume plants is due to the inter cropping of these plants with legumes (Eskandari *et al.*, 2009a; Anil *et al.*, 1998; Fujita *et al.*, 1992). Rhizobium bacteria have a symbiotic relationship with plants of the leguminous family and thereby can fix atmospheric nitrogen into available nitrogen for plant uptake.

2.4. Disadvantages of Grass-Legume Inter Cropping

The grass-legume association is particularly complex because the components intermingle competitively, and the transfer of N from the legume to the grass contributes to the complex interaction between them. Further, the ideal environment for the growth of both the grass and

legumes may vary, and the rhizobium-legume association also has particular requirements. Management, especially the grazing behavior of animals, may also influence the association, and in turn, the composition of the sward may influence the animal in terms of the amount of nutritive value (Laidlaw and Teuber, 2001). The compatibility of grasses with legumes depends on the morphology and physiological characteristics of the grass and legume, the response of each to management and the climate, and the soil and biotic factors under which the crop is growing. The disadvantages of grass-legume mixtures, including difficulty in management and harvesting of the mixtures, higher seed costs, etc., have been reported by many workers.

2.5. The Crude Protein of Forage Species and Their Mixture

The crude protein content of forage is one of the most important criteria for forage quality evaluation (Caballero *et al.*, 1995; Assefa & Ledin, 2001). The use of a grass-legume mixture can increase the fodder yield and quality (Sturludottir *et al.*, 2013). Jannink *et al.*, 1996 found that the vetch mixture had a much higher CP content than pea and oats alone. Research has shown that oats grown with peas can provide excellent tonnage and high-quality forage. Including peas in the mixture generally increases crude protein (CP) by 2 to 4 percentage units (Owens *et al.*, 2007). Herbage nutritive value of forage grasses and legumes is negatively related to DM accumulation (Belanger *et al.*, 2001). Thus, increases in herbage DM yield are expected to result in a decrease in the nutritive value of the mixture compared to legume monoculture.

Haq *et al.*, 2018, reported that the CP content of the oats-vetch mixture and the oats-pea mixture was higher than the oats grown alone, while it was lower than legume mono crop pea and vetch. Daniel Keftasa, 1989, based on a study over a period of three years, reported that the herbage yield, crude protein concentration, and crude protein yield of the oats-alfalfa mixture were enhanced by an average of 22.61 and 104 percent, respectively, compared to the pure stand of oats, indicating its value in mixed pasture production. Legume-cereal inter crops may produce higher grain and protein yields as compared to the respective cereal sole crops (Jensen, 1996; Hauggaard-Nielsen *et al.*, 2001; Lauk, 2005) and show greater yield stability across years than when growing legumes and cereals as sole crops (Willey, 1979a; Ofori & Stern, 1987).

2.6. Forage Yield and Quality

An important measure in grassland resources is the yield of forage; this defines the volume of dry matter obtainable to livestock (Shi *et al.*, 2013). Thus, legume-cereal configuration is considered a management approach in producing both quality and quantity forage (Hamdollah, 2012). Inter cropping cereals and legumes is important due to some potential benefits, including the enhancement of forage quality through the complementary outcome of two or more crops grown instantaneously on the same part of land (Hamdollah, 2012). As forage quality increases, crude protein and ether extracts from grasses increase, while crude fiber declines. Hence, a negative association exists between forage quality and above-ground biomass (Shi *et al.*, 2013). Legume cultivars were reported to have a significant effect on plant heights, leaf fresh yield, stem fresh yield, total fresh yield, leaf dry yield, and entire proteins in plants (Ali & Mohammad, 2012). Shi *et al.*, 2013 also found that environmental features such as temperature and soil fertility disturb physiological developments and therefore impact forage quality. A high forage harvest means more crude fiber but fewer ether extracts and crude proteins. It is the measure of dry matter made available to be consumed by livestock (Shi *et al.*, 2013).

Many scholars have reported that plant mass completely affects forage yield and the maximum of its quality mechanisms (Emine *et al.*, 2010). Although cereals are extensively used in livestock nutrition for their high dry material production and low price (Ghanbari-Bonjar, 2000), they have a low sustenance value due to their low forage value (Hamdollah, 2012). The forage quality of legumes is high but has low dry matter production (Ross *et al.*, 2005). The NDF and ADF contents are important in ration formulation because they reflect the amount of forage that can be used up by animals (Lithourgidis *et al.*, 2006). Protein content in forage may likely decrease as dry matter yield increases (Foster & Malhi, 2013). The protein content in forage tended to be superior for the cereals with the early planting date and lowest duration to harvest. Acid Detergent Fiber (ADF) content of forage retorted inversely to protein content of forage but has a tendency to increase as dry matter yield increased with increased days to harvest (Foster & Malhi, 2013).

2.7. Land Equivalent Ratio (LER)

This is the relative area of land under mono cropping that is needed to obtain the yield produced in inter cropping (Wiley, 1979). Rao and Willey, 1980 showed a clear variation in the duration of maturity of component crops was due to largely the advantage in yield, which clearly allowed in this combination for a good resource use with time. Yield advantages in maize-based inter cropping were also reported in Ethiopia (Fininsa, 1997), where the LER for inter cropping was far above that of mono cropping, with a maximal relative yield advantage of 28%. For instance, Ganvit *et al.*, 2018 reported that the significantly highest mean LER (1.44) was recorded in inter cropping of oats and alfalfa planted in a row ratio of 2:1, followed by oats + alfalfa in a 1:2 row ratio (LER = 1.31).

2.8. Gas Production Potentials of Fiber Based-Diets

Ruminants fed on poor-quality fiber-based diets produce more CH₄ per unit product (e.g., 9 kg CO₂-eq./kg of milk in sub-Saharan Africa) than those fed on high-quality forage diets (e.g., 1.6 kg CO₂-eq./kg of milk in Eastern and Western Europe) (Opio *et al.*, 2012). The average CH₄ emissions by individual dairy cattle in the tropics and temperate regions are 319.1 and 283 g/day, respectively (Castelan-Ortega *et al.*, 2014). This could be attributed to the elevated proportion of cellulose in tropical forages, which is reported to produce three times more CH₄ than hemicellulose. Appropriate forage supplementation and feed choices to improve forage digestibility mitigate enteric methane emissions (de Souza Filho *et al.*, 2019; Gerber *et al.*, 2013; Herrero *et al.*, 2016).

Fodder of legumes is a cheap forage resource that can complement the nutrient scarcity and digestibility of poor-quality fiber-based diets. Studies substantiated that the incorporation of fodder legumes in poor-quality fiber-based diets significantly enhanced digestibility and mitigated enteric methane emissions. The secondary metabolites in fodder legumes reduce enteric methane emission via reducing methanogens, shifting the metabolic pathway to less hydrogen synthesis, and increasing rumen bypass proteins and carbohydrates (Bhat *et al.*, 2013).

Chemical composition, IVOMD, and SCFA production are useful feed evaluation indicators for ruminant livestock production ration balancing. More degradable by-products have higher IVOMD values and a wider range of ruminal fermentability (Garcia-Rodriguez *et al.*, 2019) for improved animal production. In this case, using *in vitro* gas production to evaluate forage digestibility is a quick and low-cost method of determining ruminant feed nutritive value (Fievez *et al.*, 2005). It is easy to use, fast, and can handle a large number of samples at once. In tropical forage grasses, Bezabih *et al.*, 2014 discovered a link between forage chemical composition and *in vitro* fermentation parameters. In contrast, nitrogen (N) fertilization improved biomass yield, crude protein, cellulose, and lignin content by decreasing the energy content available for metabolism and slowing the fermentation rate, as well as decreasing *in vitro* dry matter disappearance and cumulative gas production (Cui *et al.*, 2016).

2.9. Effect of Inter Cropping on Silage Quality

Mixing different species in silage is a method used to obtain fodder with high nutritional value. Legumes and cereals are generally used for this purpose (Can *et al.*, 2020). Legume mono cultures have a low risk of silage fermentation due to their low water-soluble carbohydrate and dry matter (DM) content and high buffering capacity (Kaiser *et al.*, 2007; Denek *et al.*, 2011). For this reason, grass-legume silage mixtures are preferred in the sense that cereals will provide fermentable carbohydrates and legumes will improve the protein content of silage. Mixing legumes with cereals for ensiling will not only improve the quality of silage but also diversify forage production with multifunctional crops, produce rich feeds with functional photochemical (β -glucan, etc.), help breed more healthy and disease-tolerant farm animals, and produce more nutritious feeds. Therefore, it is also important to determine the best legume-grass combination ratio during the ensiling process.

3. MATERIALS AND METHODS

3.1. Description of the Study Sites

The study was conducted at the Adami Tulu Agricultural Research Center (on-site) under irrigation conditions. Adami Tulu Agricultural Research Center is located in the Adami Tulu Jido Kombolcha District, which is located in the Middle Rift Valley of Oromia, Ethiopia, 167 kilometers from the capital city of the country (Addis Ababa), in the southeastern part of Oromia between 38°20' and 38.5°5' E and 7°35' and 8°05' N (Figure 1). It lies in an altitude range of 1500 to 2000 m.a.s.l. The average annual rainfall is 760mm. It has bimodal rainfall from March to April (short rains) and July to September (long rains), with a dry period from May to June that separates short rains from long rains (Figure 2). The average annual minimum and maximum temperature of the area were 11.8 °C and 28.3 °C (metrology station of Adami Tulu Agricultural Research Center). The soil consists of clay with a proportion of 44%, 34%, and 22% sand, silt, and clay, and the soil pH is 7.88 (Teshome *et al.*, 2012). The chemical properties of EC (0.08 mm/kg), Na (0.21 cmol/kg), K (4.45 cmol/kg), Ca (15.2 cmol/kg), Mg (2.14 cmol/kg), and P (0.2 mg/kg) are reported (Nabi *et al.*, 2021).

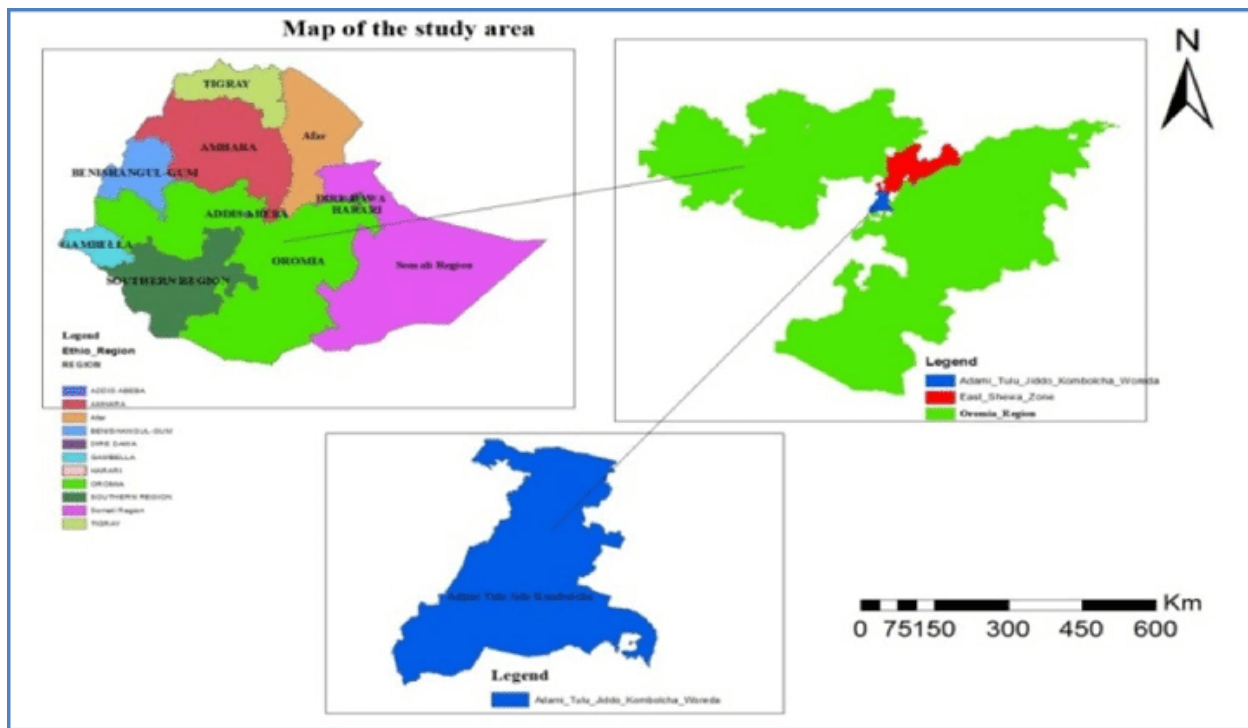


Figure 1. Map of the study area. Source: Ethiopian GIS, 2019

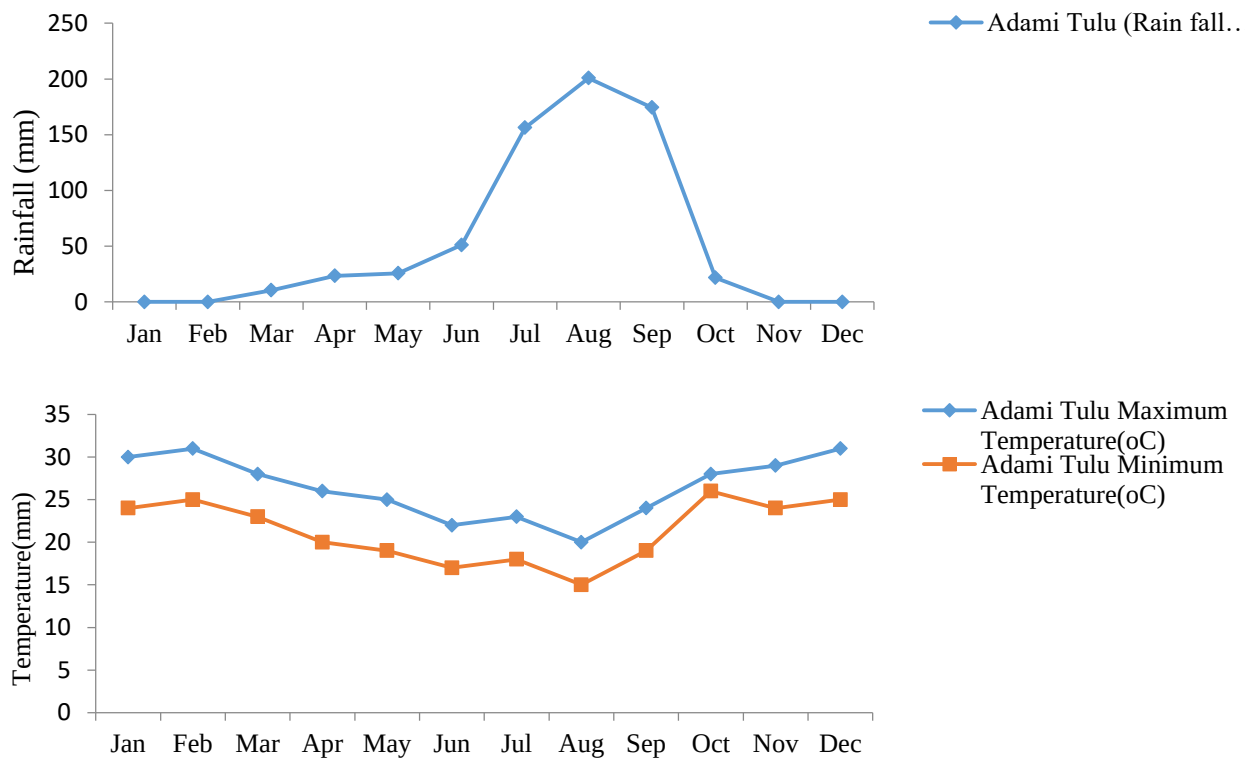


Figure 2. Monthly rainfall distribution and average maximum and minimum temperature variations over the months in the months in the study sites during the experimental (2022/2023).

3.2. Planting Material

An adapted variety of oats (*Avena sativa* .L) *bate* variety and one variety of alfalfa (*Medicago sativa*) *magna 788*) were used.

Table 1. Descriptions of oats and alfalfa varieties used for the trial.

SN	species	Variety	DMY (t/ha)	SY (q/ha)	Altitude(m)	Year release	Breeder/Institute	source
1	<i>Avena sativa</i>	Designation	8.56	31.99	1200-3000	2018	Bako ARC/OARI	MoA, 2018
2	<i>Medicago sativa</i>	Bate(ILRI-5453)	9.8		1000-2600	2006	USA	

3.3. Treatments and Experimental Design

The experiment was undertaken in a randomized complete block design with four replications. The alfalfa and oat varieties adapted to the study area were arranged according to the following treatments:

- T1** 100% oats (sole oats of *bate* variety)
- T2** 100% alfalfa (sole alfalfa of *magna 788*)
- T3** 75% oats + 25% alfalfa (*magna 788*)
- T4** 50% oats + 50% alfalfa (*magna 788*)
- T5** 25% oats + 75% alfalfa (*magna 788*)

The seed proportions were calculated based on the recommended sole seed rates of 80 and 15 kg per hectare for oats and alfalfa, respectively (Dawit *et al.*, 2015). The plot size was 4 m x 4 m (16 m²). A total of 15 rows per plot with a row spacing of 30 cm apart from each other was alternatively used (Atif *et al.*, 2012). The spacing between replications and plots was 1m. A germination test was done for both forages before sowing in order to adjust the seeding rates. The seeds were established in rows on a well-prepared seedbed and covered with soil. The forage was watered two times per week. All other cultural practices, including weeding, were kept normal and uniform across all treatments.

3.4. Data Collection

Data on yield and yield-related traits such as date of emergence, plot coverage (%), plant height (cm), fresh weight, sample weight, leaf to stem ratio, number of tillers per plant, flowering date,

biomass yield and Leaf to stem ratio is an important trait in the selection of appropriate forage cultivar as it is strongly related to forage quality (Juan *et al.*, 1993; Kratchunov and Naydenov, 1995; Julier *et al.*, 2000; Sheaffer *et al.*, 2000). yield was collected from each treatment. The forage was harvested for biomass yield determination when 50% of the oats reached heading and alfalfa reached flowering stage. Days to forage harvest were recorded from the planting date to the date when alfalfa plants reached the 50% flowering stage and oats reached the heading stage (Aklilu and Alemayehu, 2007). Biomass yield determination was made when 50% of the oats had reached heading and 50% of alfalfa had reached flowering stage.

Land equivalent ratio (LER)

The land equivalent ratio (LER) was used to assess the advantage of forage production in the mixture. LER was defined as the relative area of a mono crop plant required for the same yield obtained from its inter cropping. The LER was calculated using the formula given below.

$$\text{LER} = \frac{\text{Yield of alfalfa in mixture}}{\text{Yield of alfalfa in pure stand}} + \frac{\text{Yield of oats in mixture}}{\text{Yield of oats in pure stand}}$$

When LER is greater than 1, mixed growing favors the growth and yield of the mixture species. In contrast, when LER is lower than 1, mixed growing negatively affects the growth and yield of plants grown in mixtures (Dhima *et al.*, 2007).

3.5. Dry Matter Yield Determination

The DM yield of each botanical component in each plot was determined by drying a representative sample in an oven at 60 °C for 72 hours for partial DM determination. DM yields were determined at the Adami Tulu Agricultural Research Center, Animal Feed Laboratory. The DM yield of each leaf and stem of both *oats* and alfalfa were calculated separately and added together to provide the total DM yield of the plot, and the final DM yields were reported in tons per hectare. The DM production (ton/ha) was calculated as

$$(10 \times \text{TotFW} \times (\text{DWss}/\text{FWss} \times \text{Ha}))$$

Where TotFW = total fresh weight, DWss = dry weight sub sample, FWss = fresh weight sub sample, and HA = harvesting area (Tarawali *et al.*, 1995).

Seed yield (t/ha) determination: Seed yield was determined by harvesting both alfalfa and oats from each plot when the seeds were mature and after cautiously separating the seed from the

straw. Then, the seed yield (t/ha) was determined by weighing the seed and expressed at 10% moisture content for vetch and 12.5% for oats using the following formula (Salem *et al.*, 2015).

$$\text{Seed Yield (t/ha)} = \frac{\text{Seed yield per 0.3m} \times 4\text{m area (Kg)} \times 10 \times \text{sample dry weight(g)}}{\text{Sub sample pre drying weight (g)} \times \text{harvest area 0.3m} \times 4\text{m}}$$

3.6. *In Vitro* Gas and Methane Production Measurement

3.6.1. Rumen fluid collection and *in vitro* fermentation

Rumen fluid was obtained from the rumen of three fistulated sheep from the Dilla University farm by following the procedure (Wang *et al.*, 2017). A sample of rumen content (about 1 liter) was collected using a rumen tube before the morning feeding, squeezed into pre-warmed thermos flasks, taken immediately to the laboratory, strained through two 100µm cheese cloths, and kept at 39°C under a CO₂ atmosphere. About 200 mg of dry sample (milled through a 1.0 mm sieve) was incubated *in vitro* with rumen fluid in a calibrated glass syringe of 100 ml in triplicate. Vaseline was applied to the pistons to ease movement and prevent the escape of gas. The syringes were pre-warmed at 39°C before the addition of 30 ml of buffer mixture and rumen liquor to each syringe. The syringes were shaken for 30 minutes after incubation started and every hour for the first 10 hours of incubation.

Blanks with buffered rumen fluid without feed samples were also included in triplicate. All syringes were incubated in a water bath maintained at 39°C under CO₂. Gas production was recorded after 3, 6, 12, 24, 48, and 72 hours of incubation. The gas production characteristics were estimated by PROC NLIN of SAS (2008) fitting to the equation $G = a + b(1 - e^{-c(t - L)})$ (Dhanao, 1988), where G is the gas production (ml/200mg DM) at time t, a is gas production from the immediately soluble fraction (ml), b is gas production from the insoluble but degradable fraction (ml), a + b = potential gas production (ml); c = the rate constant of gas production (fraction/h); L - lag time.

During the incubation, gas was released from the bottles every hour to prevent an increase in head space pressure that could be high enough to force carbon dioxide into solution and so lower the pH of the incubation medium. After the incubation period, 4 ml of (10M) sodium hydroxide (NaOH) was dispensed into each incubated sample. Sodium hydroxide was added to absorb

carbon dioxide produced during fermentation, and the remaining volume of gas was recorded as methane, according to Fievez *et al.*, 2005. The average volume of gas produced from the blanks was deducted from the volume of gas produced from the samples. The methane concentration was calculated as (Jayanegara *et al.*, 2009):

3.6.2. Determination of total gas, methane, and short-chain fatty acid production

Total gas production: was determined by reading the position of the piston at each time, following the procedure of Menke and Steingass, 1988. Net gas production (ml/200 mg) at t hours was calculated as:

$$G_t = [(V_t - V_0 - G_0) \times 200] / WS$$

Where: G_t = gas production value (ml/200 mg) at t hours; V_t = volume in ml at t hours; V_0 = initial volume in ml; G_0 = gas production of blank syringes (ml); WS = weight of the dried sample in mg.

Methane Production: Methane production at 72 h of incubation was measured using the procedure described by Fievez *et al.*, 2005. For measuring methane production at the end of incubation and after recording the final gas volume, the lower end of the syringe was connected to the lower end of another syringe containing 4.0 ml of 10 M sodium hydroxide (NaOH). Sodium hydroxide was then introduced from the latter into the incubated contents, thereby avoiding gas escape. The mixing of the contents with NaOH allowed the absorption of CO_2 , and the gas volume remaining in the syringe was considered to be CH_4 . Net methane production was calculated by dividing the difference between the methane in the syringe and the equivalent blank. The methane concentration was calculated according to Jayanegara *et al.*, 2009.

$$\text{Methane concentration (\%)} = (\text{net methane production} / \text{net gas production} \times 100)$$

Short-chain fatty acids (SCFA): To 720 μ l of the incubation supernatant, formic acid (80 μ l) containing the internal standard (1% methylbutyric acid) was added. SCFA were determined according to Hoelersshinken *et al.*, 1997 using gas chromatography (GC-14A, Shimadzu Corp.,

Japan) in a GC-14A (Shimazu Corp., Kyoto, Japan) equipped with an auto sampler using a stainless steel column packed with GP 10% SP 1000 1% H₃PO₄ Chromosorb WAW (Suppelco Inc., Bellafonte, PA).

$$\text{SCFA (mmol/g DM)} = (0.0239 \text{ GP}) - 0.0601$$

Where: GP = gas production at 72 hours of incubation (Getachew *et al.*, 1999). The samples were analyzed at the Animal Nutrition Laboratory of Dilla University.

3.7. Soil Sample Collection And Analysis

The soil sample collection procedure from a plot followed a “transverse” method to get a reliable soil test (Estefan, 2013). The collected soil samples were dried, thoroughly mixed (composed), and prepared to determine the soil properties: pH, organic matter (OM), organic carbon (OC), electron conductivity (EC), available phosphorus (P), exchangeable potassium (K), total nitrogen (N), and texture. Accordingly, the soil textural analysis was performed using the hydrometer method, and the total nitrogen found in the soil samples was analyzed using the Kjeldahl method. Soil organic matter (SOM) was determined by multiplying the percentage of OC by the factor of 1.724 (Brady, 1990). Available phosphorus (P) using the Olsen method (Olsen and Dean, 1965). EC in water suspension with a soil-to-water ratio of 1:2.5. Cation exchange capacity (CEC) was analyzed by ammonium acetate. Exchangeable cations (Na) and (K) were extracted by a flame photometer. Mg and Ca were extracted by EDTA titration. The soil analysis was taken at the Batu soil laboratory.

3.8. Silage Preparation

Harvested plants were wilted in shade for three hours. The plants were chopped into approximately 2–3 cm pieces. Plant materials prepared for silage were weighed, mixed, and placed in a plastic bag. All mixtures were kept in a dark and cool environment for 45 days. Packs were checked every week to observe the condition of the materials. When the fermentation process was completed, the bags of mature silage were opened, and some physical and chemical analyses were made. Physical examinations of the odor, structure, and color of the samples in the opened silage bags were conducted subjectively by five experts. The scoring method developed by Anonymous, 1987 was used in the evaluation of physical analyses. The silage quality class of the silage was determined according to the DLG (Deutsche Landwirtschafts-Gesellschaft) score.

The DLG score is the total physical score (0–20 points) obtained by the sum of the scores of odor, structure, and color (Anonymous, 1987; Ergun *et al.*, 2013).

The total dry matter loss (TDML) was calculated by DM weight loss in the silage,

$(\text{DM of forage} - \text{DM of silage}) / \text{DM forage} \times 100$ (Pedroso *et al.*, 2011).

Effluent losses = E = $[(\text{empty bag weight} + \text{sand weight at opening (kg)} - \text{empty bag weight (kg)}) - (\text{empty bag weight} + \text{sand weight at sealing (kg)} - \text{weight empty bag (kg)})] / [\text{forage mass at sealing (kg)}] \times 100$ (Zanine *et al.*, 2010).

Gas losses (% DM) = G = $(\text{weight of full silo at sealing} - \text{the weight of full bucket at opening}) / (\text{forage mass at sealing (kg)} - \text{forage dry matter concentration at closing (\%)}) \times 100$ (Zanine *et al.*, 2010).

DMRR = dry matter recovery = dry matter recovery rate (%) = $[\text{forage mass at opening (kg)} \times \text{forage dry matter concentration at opening (\%)}] / [\text{forage mass at sealing (kg)} \times \text{forage dry matter concentration at sealing (\%)}] \times 100$ (Zanine *et al.*, 2010).

For the determination of silage DM content (%), 300 g of wet samples were taken from the mature silage and dried in an oven at 65°C for 48 hours. The DM ratio was determined by weighing the dried silage samples on a precision scale and proportioning to the wet weight (Bulgurlu, 1978). For pH value, a part of the silage materials representing the bags was taken from each silage bag and then mixed homogeneously, and a 25-gram wet silage sample from this mixture was weighed on a precision balance before being put into a mixer. 250 ml of distilled water was placed on the sample and mixed for 10 minutes, then filtered through filter paper and taken into glass beakers. The pH in the filter of approximately 200 ml of material was determined by a pH meter (Anonymous, 1993).

In order to determine the crude protein (CP) of silages, the samples were dried at 65 °C until they reached a constant weight, then ground in a mill with a sieve diameter of 1 mm in the laboratory and prepared for analysis. The total N values of these samples were determined by the Kjeldahl method, and CP was determined by multiplying the N values by the coefficient of 6.25 (Bulgurlu, 1978). The analysis of acid detergent fiber (ADF) and neutral detergent fiber (NDF)

in silage applications was determined by using a fiber analyzer according to the principles reported by Van Soest, 1963 and Van Soest & Wine, 1967.

3.9. Chemical Analysis

The feed sample was taken from each treatment and dried in an oven at 60°C for 72 hours to a constant weight and Willey mill to pass through a 1mm sieve. The ground samples were kept in airtight plastic bags prior to analysis for chemical composition. The dry matter (DM) was determined by an oven drying at 105°C overnight and ash content was determined by ground in a igniting the dry samples in a muffle furnace at 550°C for 6 hours to burn off all the organic material. The inorganic material which does not volatilized at that temperature is ash. The difference between sample DM and ash gives the organic matter (OM). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) was analyzed (Van Soest and Robertson, 1985). For nitrogen (N) analysis, the Kjeldhal method was used and crude protein (CP) content was estimated from the N content by use of a multiplier of 6.25.

3. 10. Statistical Analysis

Collected data was organized, summarized and analyzed by using SAS. LSD test at 0.05 probability levels was used to compare the treatment means (Steel and Torrie, 1984). The treatment means were separated by least significant difference (LSD).

The model used for data analysis was: $Y_{ij} = \mu + T_i + B_j + E_{ij}$ Where; Y_{ij} = Response variable, μ = Overall mean, T_i = Treatment effect, B_j = Block effect, E_{ij} = Random error

4. RESULTS AND DISCUSSION

Table 2. The agronomic performance of oats inter cropped with alfalfa at the first cutting

Treatm ent	PH		LL		Tillers		LSR		TDM(ton/ha)			SY(q/ha)			LER
	oats	alfalfa	oats	oats	alfalfa		oats	alfalfa	total	Oats	alfalfa	total			
T1	105.65		44.9	11 ^b		1.37	11.91 ^{ab}		11.91 ^b	24.33 ^a		24.33 ^a			
T2		92.9			12.68 ^a	1.29		5.05 ^a	5.05 ^d		9.88 ^a	9.88 ^e			
T3	105.25	93.83	45.4	10.75 ^b	9.83 ^b	1.33	12.13 ^a	2.02 ^c	14.15 ^a	19.79 ^b	1.41 ^d	21.2 ^b			0.95
T4	105	97.23	45.58	13.18 ^a	8.5 ^c	1.4	11.38 ^b	3.04 ^b	14.41 ^a	17.06 ^c	3.31 ^c	20.37 ^c			1.14
T5	103.58	93.9	46.75	10.83 ^b	9.65 ^b	1.48	8.82 ^c	2.88 ^{bc}	11.69 ^b	9.74 ^d	6.64 ^b	16.38 ^d			1.07
Mean	104.87	94.46	45.66	11.44	10.16	1.37	11.06	3.25	11.24	17.73	5.31	18.43			1.06
CV	1.75	7.07	5.19	8.84	6.74	16.87	3.46	18.33	6.36	2.29	5.41	2.17			
LSD	2.83	10.28	3.65	1.56	1.05	0.35	0.59	0.92	1.08	0.63	0.44	0.6			
P-value	0.44	0.8	0.73	*	***	0.2	***	***	***	***	***	***			

T1-Sole oats , T2- sole alfalfa , T3- 75% oats + 25%alfalfa, T4- 50% oats + 50% alfalfa, T5- 25% oats + 75% alfalfa, PH- plant height, LL- leaf length, TDM- total dry matter, SY- seed yield, CV – Coefficient variation, LSD – Least significant deference, PH – plant height, LL – leaf length, LSR – leaf stem ratio, TDM – total dry matter, SY – seed yield, Land equivalent ratio

Table 3. The agronomic performance of oats inter cropped with alfalfa at the second cutting

Treat ment	PH		LL		Tillers		LSR		TDM(ton/ha)			sy(q/ha)			LER
	oats	Alfalfa	oats	oats	alfalfa		oats	alfalfa	total	Oats	alfalfa	total			
T1	109.5		46.75	13 ^b		1.07	10.92 ^{ab}		10.92 ^c	22.98 ^a		22.98 ^a			
T2		94			12.75 ^c	1.19		5.99 ^a	5.99 ^c		10.83 ^a	10.83 ^e			
T3	109.25	93.75	48.75	12.75 ^b	13.83 ^b	1.18	11.55 ^a	2.96 ^c	14.51 ^a	18.44 ^b	2.38 ^d	20.8 ^b			1.02
T4	107.5	97.75	48.75	14.93 ^a	16.43 ^a	1.06	10.66 ^b	3.98 ^b	14.64 ^a	15.71 ^c	4.26 ^c	19.97 ^c			1.15
T5	107.75	94.75	49	12.75 ^b	13.65 ^b	1.15	8.36 ^c	3.82 ^{bc}	12.18 ^b	8.39 ^d	7.59 ^b	15.98 ^d			1.07
mean	108.5	95.06	48.31	13.36	14.17	1.13	10.37	4.19	11.65	16.38	6.27	18.11			1.08
CV	1.91	4.77	6.77	7.89	5.15	16.86	4.99	14.21	6.17	2.48	4.59	2.2			
LSD	3.19	6.98	5.04	1.63	1.12	0.29	0.78	0.92	1.08	0.63	0.44	0.6			
P- value	0.44	0.5	0.75	*	***	0.79	***	***	***	***	***	***			

T1-Sole oats , T2- sole alfalfa , T3- 75% oats + 25%alfalfa, T4- 50% oats + 50% alfalfa, T5- 25% oats + 75% alfalfa, PH- plant height, LL- leaf length, TDM- total dry matter, SY- seed yield, CV – Coefficient variation, LSD – Least significant deference, PH – plant height, LL – leaf length, LSR – leaf stem ratio, TDM – total dry matter, SY – seed yield, Land equivalent ratio

4.1. The Effect of Oat-Alfalfa Inter Cropping on Agronomic Performance

The result of all agronomic performances in both the first and second cuttings of this experiment is shown in Table 1&2. Except for plant height, leaf length, and leaf stem ratio, all other evaluated agronomic parameters have statistically significant differences ($P < 0.05$). The analysis of variance revealed a significant difference for all the parameters studied (Table 1&2). The findings of Ninama, 2020 indicated that this variance difference was due to the effect of different ratios of oats and alfalfa under an intercropping. In this trial, the number of tillers of alfalfa and oats plants grown in the seed proportion T4 (50% oats and 50% alfalfa) was statistically significantly higher ($P < 0.05$) than all other treatments both at the first and second cuttings, which reveals that there was no competition for space.

4.1.1. Dry matter yield (t/ha)

The total forage dry matter yield of harvested from T1(11.91 t/ha), T2(5.05 t/ha), and T5(11.69 t/ha) was lower ($P < 0.05$) than T3(14.15 t/ha) and T4(14.41 t/ha) at the first cutting, and T1(10.92 t/ha), T2(5.99 t/ha), and T5(12.18 t/ha) were lower ($P < 0.05$) than T3(14.51 t/ha) and T4(14.64 t/ha) at the second cutting. The TDMY t/ha of T3 (14.15 t/ha, 14.51 t/ha) and T4 (14.41 t/ha, 14.64 t/ha) were not significantly different, respectively, at the first and second cuttings. The highest total dry matter yields of 14.64 t/ha and 14.41 t/ha were recorded from a seeding ratio of 50:50 T4 at the second and first cuttings, respectively. The lowest dry matter yield was recorded from the sole alfalfa treatment at the two cuttings. The combined analysis result also showed that significantly ($p < 0.05$) the highest dry matter yield was obtained from a seeding ratio of 50% *oat* : 50% *alfalfa* (T4), followed by a seeding ratio of 75% *oat* : 25% *alfalfa* (T3), both at the first and second cutting.

The higher biomass yield obtained from these seeding ratios could be due to the fact that the proportion of oats produced much more forage yield through increased number of tiller productions than the other mixtures and sole oats. As the result shows, the biomass of the inter crop was superior and advantageous compared to their pure stands. This may be due to higher fertility levels and suitable seed ratios under intercropping system, where both crops behave as component crops. The addition of the legume crop to cereals leads to increased availability and absorption of nutrients by the plants, which results in more vegetative growth and helps increase

plant height and tillers on account of enlargement of cells and enhanced photosynthesis, which results in a higher total dry matter yield. The results are in conformity with the findings of (Verma and Jeengar, 2015; Bhagat *et al.*, 2017; Singh *et al.*, 2017; Ganvit *et al.*, 2018, and Iqbal *et al.*, 2018) number of tiller productions than the other mixtures and sole oats.

As the result shows, the biomass of the intercrop was superior and advantageous compared to their pure stands. This may be due to higher fertility levels and suitable seed ratios under the intercropping system, where both crops behave as component crops. The addition of the legume crop to cereals leads to increased availability and absorption of nutrients by the plants, which results in more vegetative growth and helps increase plant height and tillers on account of enlargement of cells and enhanced photosynthesis, which results in a higher dry matter yield. The results are in line with the findings of (Verma and Jeengar, 2015; Bhagat *et al.*, 2017; Singh *et al.*, 2017; Ganvit *et al.*, 2018; Iqbal *et al.*, 2018). In line with this study, scholars emphasized the advantages of a grass-legume mixture (Albayrak *et al.*, 2011; Dawit *et al.*, 2022). It was also reported that the total dry matter yield of mixed grass and legume pastures was greater than that of grass-based pastures, and the overall feed value was better maintained throughout the grazing season when pastures included legumes (Lusture *et al.*, 2014).

This revealed that the overall yield is not affected by the competition effects of oats as the companion crop (Table 1&2). The analysis of this trial has suggested that the total dry matter yield of alfalfa grown without oats was greater ($P<0.05$) than that grown with oats. However, this difference was not associated with the effect of companion plants on the performance of alfalfa; rather, it could largely be attributed to the proportion of land used and seed proportions for growing this plant. The land area used for growing alfalfa with companion plants was about half of the land used for growing alfalfa without companion plants, indicating that the dry matter yield per area of land was greater for alfalfa grown without oats than alfalfa grown with oats. This result was supported by Befekadu and Yunus, 2015, who reported that the total dry matter yield of alfalfa grown without companion was greater ($P<0.05$) than that of those grown with companion plants.

On the other hand, the lowest performance of the seeding ratio (25% oats and 75% alfalfa) as compared to the other mixtures could be due to the fact that alfalfa dominates and suppresses the growth of oats in terms of light and nutrient utilization. Other findings also indicated that mixtures gave higher green forage yields than pure stands (Karadau, 2003). In addition, other scholars who reported that a higher mean biomass yield was obtained from the mixture as compared to its components grown in monoculture (Arturi *et al.*, 2012). Generally, the result shows that a seeding ratio of 50% oats: 50% alfalfa and 75% oats: 25% alfalfa showed the highest total biomass yields.

The basic reason for higher forage biomass productivity might be due to the utilization of symbiotically fixed nitrogen (Whitehead, 1995), more enhanced interception of light (Hay & Walker, 1989), allelopathic and synergistic effect (Pudnam & Duke, 1978), and some other effects. These factors created a micro environment that favored higher yields than those obtained from sole legume or grass stands (Sengul, 2003). Besides, legumes can cover the N₂ demand of grasses from atmospheric N₂, and therefore legumes inter cropped with grasses compete for less soil mineral N₂. Eskandari *et al.*, 2009a indicated that there was an increase in forage quality compared to cereal monoculture and an increase in dry matter in comparison to legume monoculture. Several studies showed that the dry matter yield increased with the increasing rate of oats in mixtures with legumes (Walton, 1975; Osman & Nersoyan, 1985).

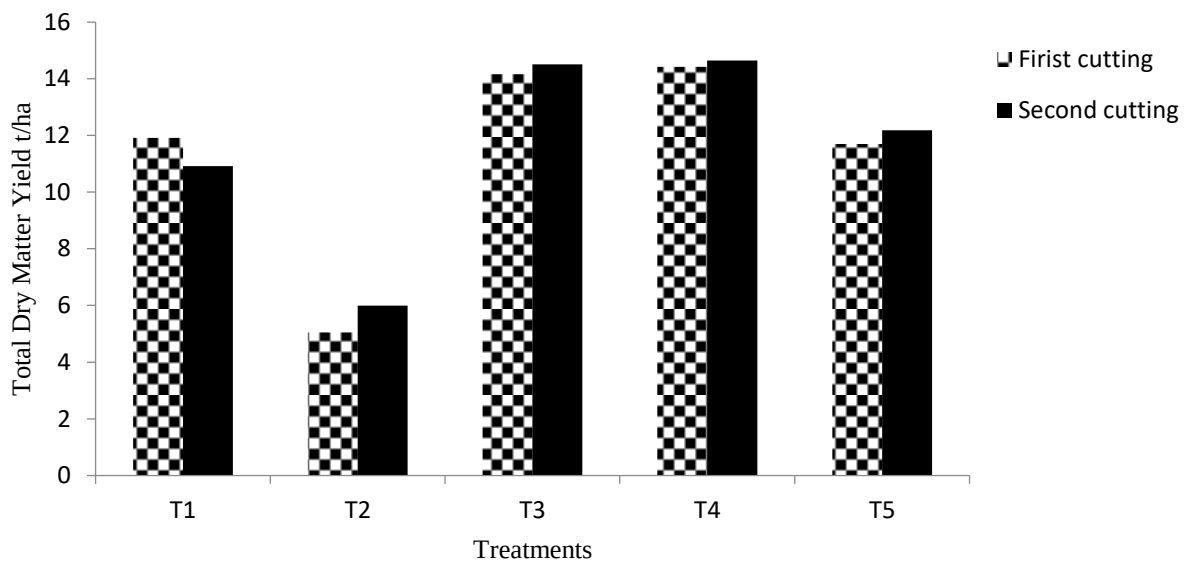


Figure 3 Total Dry Matter Yield of Oats with Alfalfa Both at 1st and 2nd Cutting

4.1.2. Plant height

The average plant height of alfalfa and oats grown in a sole was not different ($P > 0.05$) from those grown in an inter crop on the same plot both at the first and second cuttings under this experiment (Table 1). The non-significant ($p > 0.05$) differences observed for alfalfa and oat plant height illustrate the mutual benefits of the forage crops from inter crop establishment. This indicates that alfalfa grown with oats on the same plot was not affected by the height and root of oats used as companion crops to obtain sun light and nutrients, respectively, if oats were seeded at 30cm intervals in a row with alfalfa. Dawit *et al.*, 2022 reported that a non-significant ($p > 0.05$) effect was observed on plant heights for the mixture of alfalfa among the tested seeding ratios. However, Meyer and Nudell, 2008 indicated that companion crops compete with under-seed alfalfa, which can affect the stand that can be established. In contrast to this study plant height recorded at different forage growth stages showed significant differences between inter cropping treatments of alfalfa and oats (Mosebi *et al.*, 2018). The data also indicated that all seed ratios of oats and alfalfa showed a yield advantage over sole oats and sole alfalfa. The increase in total dry matter yields in the inter cropping systems might be owing to better utilization of space and light interception, coupled with the nutrient contribution of leguminous fodder to cereal. The results are in agreement with those of Kumar, 2005, Sharma *et al.*, 2009, and Deore *et al.*, 2013.

4.1.3. Total seed yield (q/ha)

The total seed yield (table 1) has a statistically significant difference ($P < 0.05$), which indicates an increment order of T1 (24.33). T3(21.2)>T4(20.37)>T5(16.38)>T2(9.88) at first, and T1(22.98)>T3(20.8)>T4(19.97)>T5(15.98)>T2(10.83) at second cutting. In this experiment, the seed yield of sole oats was significantly higher than that of oats inter cropped with alfalfa. The highest seed yields were obtained from sole oats (T2) with 24.33 t/ha, followed by T3 (75% oats + 25% alfalfa) with an average yield of 21.2 t/ha. The lowest mean seed yields (10.83 t/ha and 15.98 t/ha) were recorded from T2 (sole alfalfa) and T5 (25% oats + 75% alfalfa) seed ratios, respectively. The sole oats were significantly different from the sole alfalfa and their inter crop groups and produced the highest seed yield in the present study. The yield differences between the treatments might be due to the effective tiller in oats attributed to the differences.

4.1.4. Land equivalent ratio (LER)

LER's values were calculated for inter cropping treatments in two consecutive cuttings (Table 2). All the inter crops showed LER greater than one except T3 of the first cutting. The maximum LER values were obtained from T4 (50% oats + 50% alfalfa) seed proportion of oats + alfalfa inter cropping system, followed by T5 (25% oats + 75% alfalfa) and T3 (75% oats + 25% alfalfa) both at first and second cutting. The reason for the higher LER values for the seeding ratio of 50% oats and 50% alfalfa could be due to the better benefit of the oats from the fixed nitrogen through the alfalfa. The values of LER greater than one from the results show that inter cropping is advantageous. Inter cropping systems that constantly give LERs greater than one are considered to be more efficient systems from a land use point of view than mono crops. LER ranged from 0.95 to 1.14 in the first cutting and from 1.02 to 1.15 in the second cutting.

Therefore, 0.05% to 14% of the first cutting and 2% to 15% more land should be used in mono cropping in order to obtain the same yield as inter cropping, which indicated the advantage of inter cropping over mono cropping in terms of the use of water, nutrients, carbon dioxide, and light for plant growth. It was found that LER greater than one was primarily due to nutrient cycling and an increase in nitrogen content (Singh *et al.*, 2012). The present study result is in line with the report of Ganvit *et al.*, 2018, which ranged between 1.24 and 1.44). In addition, legume-grass mixtures generally provide more consistent and greater forage yields across a range of environments than grass or legume monoculture (Papadopoulos *et al.*, 2012). Hence, the value of LER for seeding ratios shows that inter cropping of alfalfa and oats is more advantageous than sole cropping. Where it was more than 1, indicating the suitability of the practice in the quantitative term. The present findings are in accordance with those of Patel and Rajgopal, 2003, Kumar *et al.*, 2005, Surve *et al.*, 2012, and Ganvit *et al.*, 2018.

Table 4 The Chemical composition of oats and alfalfa inter cropped forage with different seed ratios at first cutting

Treatment	DM	OM	Ash	CP	NDF	ADF	ADL	IVDMD
T1	90.3	92.03 ^b	7.97 ^a	11.53 ^e	46.59 ^a	30.94 ^a	4.79 ^a	62.28 ^d
T2	90.23	93.53 ^a	6.47 ^b	21.2 ^a	39.29 ^d	22.65 ^e	2.14 ^c	73.3 ^a
T3	89.98	92.28 ^b	7.72 ^a	13.65 ^d	44.84 ^b	28.52 ^b	3.85 ^b	62.73 ^d
T4	90.48	92.08 ^b	7.92 ^a	17.33 ^c	42.27 ^c	26.11 ^c	2.39 ^c	65.81 ^c
T5	89.9	92.85 ^{ab}	7.15 ^{ab}	18.55 ^b	39.98 ^d	23.64 ^d	2.19 ^c	68.47 ^b
mean	90.18	92.55	7.45	16.45	42.59	26.37	3.07	66.52
CV	0.38	0.65	8.02	3.63	1.67	1.58	5.54	0.56
LSD	0.51	0.9	0.9	0.9	1.07	0.63	0.26	0.57
P-value	0.15	0.01(*)	0.01(*)	***	***	***	***	***

Means with the same letter are not significantly different at $P < 0.05$. T1-Sole oats, T2- sole alfalfa, T3- 75% oats + 25%alfalfa,T4- 50% oats + 50% alfalfa, T5- 25% oats + 75% alfalfa, DM- dry matter, OM- organic matter, CP- crude protein, NDF- neutral detergent fiber, ADF- acid detergent fiber, ADL- acid detergent lignin, IVDMD- *in vitro* dry matter digestibility, ME- methabolizable energy, CV – Coefficient of variation, LSD – Least significant deference

Table 5 The Chemical composition of oats and alfalfa inter cropped forage with different seed ratios at second cutting

Treatment	DM%	OM	Ash	CP	NDF	ADF	ADL	IVDMD
T1	86.78	94.1 ^b	5.91 ^a	13.02 ^e	39.91 ^a	29.44 ^a	3.62 ^a	63.96 ^d
T2	84.18	95.65 ^a	4.35 ^b	22.8 ^a	30.67 ^e	20.01 ^e	2.02 ^c	74.99 ^a
T3	87.1	94.27 ^b	5.73 ^a	14.27 ^d	39.15 ^b	27.29 ^b	3.47 ^a	64.28 ^d
T4	87.98	94.06 ^b	5.95 ^a	18.27 ^c	35.8 ^c	25.35 ^c	2.89 ^b	67.42 ^c
T5	85.55	94.91 ^{ab}	5.09 ^{ab}	19.19 ^b	32.62 ^d	21.82 ^d	2.63 ^b	70.03 ^b
mean	86.28	94.59	5.4	17.51	35.63	24.78	2.93	68.14
CV	2.64	0.62	10.82	1.92	1.01	1.82	7.36	0.62
LSD	3.43	0.88	0.88	0.5	0.54	0.68	0.32	0.77
P-value	0.21	0.04(*)	**	***	***	***	***	***

Means with the same letter are not significantly different at $P < 0.05$. T1-Sole oats, T2- sole alfalfa, T3- 75% oats + 25%alfalfa,T4- 50% oats + 50% alfalfa, T5- 25% oats + 75% alfalfa, DM- dry matter, OM- organic matter, CP- crude protein, NDF- neutral detergent fiber, ADF- acid detergent fiber, ADL- acid detergent lignin, IVDMD- *in vitro* dry matter digestibility, ME- methabolizable energy, CV – Coefficient of variation, LSD – Least significant deference,

4.2. The Chemical Composition and Variability of the Forage

The results of the analysis of the nutrient contents of oats and alfalfa with different seeding ratio combinations are indicated in Tables 3 and 4. The result indicated that the combinations of different seeding ratios did not significantly ($p > 0.05$) influence the percent of dry matter under both mixtures and pure stands in DM content. However, numerically, the DM percent ranged

from 89.9% to 90.48% and 84.18% to 87.98%, with an overall mean of 90.18% and 86.28% for both the first and second cuttings, respectively, under pure stand and mixture. The result of the current study is lower than the result of Tesfaye *et al.*, 2020, who reported that DM% was between 91.8% and 93.78% in both mixtures and pure stands, respectively. The difference may be due to soil and ecology difference.

The DM percentage recorded in the first cutting being higher than that of the second cutting may be due to the maturation effect. On the other hand, the different seeding ratios of oats and alfalfa inter cropped showed a significant ($p < 0.05$) variation among the values of OM%, Ash%, CP%, NDF%, ADF%, ADL%, and IVDMD both at first and second cutting. The highest OM% (93.53%, 95.65%) was observed from the pure stand of alfalfa both at the first and second cuttings, respectively. The percentage content of OM% is directly related to the protein content, which means that when OM% increases, the CP% content will also increase.

Total ash content of oats and alfalfa: Total ash content was significantly affected ($P > 0.05$) both at the first and second cuttings (Tables 3 and 4). A higher ash content was recorded at the first cutting. The ash content of the first cutting mean data is higher than that of the second cutting, which may be due to the maturation stage difference between the first and second cutting periods. The ash content obtained in this study was in agreement with the results of Yehalem (2004), who reported an increase in total ash contents with advancing plant maturity. However, the results of the current study disagree with those of Diriba and Vaars, 2000, who reported that the mineral contents of plants declined during the maturing process due to the natural dilution and translocation of nutrients from the vegetative part to the root system. McDonald *et al.*, 2002 also reported that mineral concentration declines with age and is influenced by soil type, soil nutrient levels, and seasonal conditions. In general, variation in the concentration of minerals in forages can be induced by factors like varieties, plant developmental stage, morphological fractions, climatic conditions, soil characteristics, and fertilization regime (Jukenvicius *et al.*, 2007; Gezahegn *et al.*, 2013).

Crude protein yield of oats-alfalfa inter cropping: The highest CP% content (21.2%, 22.8%) was recorded from the pure stand of alfalfa (T2), followed by the seeding ratios T5 (25% oats: 75% alfalfa) (18.55%, 19.19%), T4 (50% oats: 50% alfalfa) (17.33%, 18.27%), and T3 (75%

oats: 25% alfalfa) (13.35%, 14.27%), while the pure stand of oats produced the least T1 (11.53%, 13.02%) CP value both at the first and second cutting, respectively. The CP content of the intercropped groups in the present study is similar to the report of NinamaSD *et al.*, 2020, 16.72% from oats and alfalfa. The average values of CP content were directly related to the Alfalfa seeding ratios in the intercrop. The CP content of oats-alfalfa produced in a mixture is much improved due to the alfalfa component in the intercrop. Similarly, alfalfa is an excellent forage because of its high yield, rich content and high quality of protein, abundant vitamins and minerals, good palatability, and high digestibility (Wang *et al.*, 2003), and because it produces more protein per unit area than other forage legumes (Capstaff and Miller, 2018).

The current result agrees with Diriba and Vaars (2000), who suggested a higher CP% as indicative of the importance of the forages. The CP% result of the present study Similar to the report of Shoaib *et al.*, 2014, crude protein yield was significantly influenced by the seed proportions of inter crops. Studies also indicated that the production of grass-legumes inter crop increased fresh fodder yield and protein contents, as well as enhanced fodder palatability (Karadag, 2003; Bakhashwain, 2010). Even though the value of CP% content in 25% oats: 75% alfalfa seeding ratio was higher than other seeding ratios in the intercrop, the high proportion of alfalfa in this ratio is undesirable since these normally have a low dry matter yield.

Fiber and lignin contents: Of the tested seeding ratios, the pure stand of oats had the highest NDF (46.59%, 39.91%), ADF (30.94%, 29.44%), and ADL (4.79%, 3.62%) content, followed by the seeding ratios of 75% oats and 25% alfalfa, which produced NDF (44.84%, 39.15%), ADF (28.52%, 27.29%), and ADL (3.85%, 3.47%) both at the first and second cutting, respectively (tables 3 and 4). The lowest values of NDF (39.29%, 30.67%), ADF (22.65%, 20.01%), and ADL (2.14%, 2.02%) were recorded from the pure stands of alfalfa, both at the first and second cuttings, respectively. The fiber result of the present study is lower than the previous finding reported (NinamaSD, 2020). NDF, ADF, and ADL concentrations in forage were directly proportional to the oats percentage in the intercrop. As the seeding ratios of the oats increased in the intercrop, the content of NDF, ADF, and ADL increased. Findings from Paulson *et al.*, 2008 also suggested that the grass contains higher fiber content than the legumes.

The present study shows that the DM, NDF, and ADF contents increased with advancing maturity for first-cutting forage, which is consistent with Sikora *et al.*, 2019. This is due to the moisture and leaf-to-stem ratio decreasing and the cell wall content in the stem increasing with advancing maturity (Yari *et al.*, 2012). Grasses tend to increase the fiber fractions, such as NDF and ADF, of mixtures owing to the abundant cell wall materials (Buxton, 1997), and legumes are usually richer in CP than grasses due to their substantial biological fixation of N from the atmosphere (Jahanzad *et al.*, 2014). Similar to the current study, increasing the alfalfa proportion in mixtures increased CP and decreased NDF, ADF, hemicelluloses, and cellulose concentrations, suggesting that forage mixtures improved nutritive values and reduced the need for purchased protein supplements in ruminant rations (Adesogan *et al.*, 2002). Other studies also showed significant positive correlations between legume inclusion in pastures and forage quality traits like crude protein and negative correlations with NDF, ADF, and ADL (Bosworth, 2007). In other ways, the fiber content of the first cutting is higher than that of the second cutting, as supported by NinamaSD, 2020), who stated that there was an increase in fiber content with the advancement of the growth stage.

The *in vitro* dry matter digestibility of oats was significantly affected by the treatment. The highest IVDMD (73.3, 74.99) was observed from the pure stand of alfalfa (T2) both at the first and second cuttings. The IVDMD result observed from the second cutting was higher than that of the first cutting due to the regrowth at the second cutting, which implies the best result of IVDMD. On the other hand, the lowest IVDMD (62.28, 63.96) was observed from the pure stand of oats (T1) sole oats both at first and second cutting. The IVDMD results of the three intercropped groups (T3, T4, and T5) were higher than those of the pure stand of oats but lower than those of the pure stand of alfalfa, which revealed an improvement in digestibility. Similarly, intercropping improved the forage yield of dry matter, crude protein, and dry matter digestibility (Metekia *et al.*, 2023).

In the present study, the increase in the alfalfa proportion in the mixtures increased the IVDMD and OMD compared with that from sole oats for both harvests. In agreement with a previous study (Dal Pizzol *et al.*, 2017), IVDMD increased when the proportion of alfalfa increased in axonopus-alfalfa and tall fescue-alfalfa mixtures. The present study indicates that digestibility could be increased when Alfalfa is mixed with other crops (Chen *et al.*, 2017; Bai *et al.*, 2018;

Chen *et al.*, 2019). This may be because balanced digestible nutrients from oats and alfalfa silage mixtures set off a ruminal synergistic effect on the fractional rate of degradation and the extent of fermentation, followed by better nutrient availability and utilization efficiency for rumen microorganisms (Kiran, 2007). The association between fermented nutrients from grass and legume mixtures may also lead to synergistic effects on the dominant microbial populations and shifts in the microbial community composition (Pandit *et al.*, 2018), and different metabolic pathways might be simultaneously driven through niche compartmentalization and functional dominance between abundant bacteria (Hart *et al.*, 2018). Higher fiber content means a lower degradation rate and longer fermentation time (Diaz *et al.*, 2014), and indigestible fiber, e.g., ADL, in particular, is the main physical barrier interfering with microbial attachment and degradation, and this is negatively correlated with fiber digestibility (Buxton, 1997).

Table 6 Effect of oats inter cropped with alfalfa silage on the chemical composition variability.

Treat ment	DMi	DM%	ASH	OM	CP	NDF	ADF	ADL	EE	ME	IVDMD
T1	29.41 ^a	27.28 ^a	8.97	76.07 ^d	9.43 ^d	49.78 ^a	25.67 ^a	4.25 ^a	1.98	11.42 ^e	60.74 ^d
T2	24.91 ^c	21.45 ^b	8.2	79.97 ^a	19.07 ^a	42.68 ^d	17.44 ^e	1.67 ^c	2.01	13.65 ^a	71.76 ^a
T3	28.47 ^{ab}	26.69 ^a	8.7	77.53 ^c	11.47 ^c	47.78 ^b	23.49 ^b	3.31 ^b	1.88	11.88 ^d	61.19 ^d
T4	27.51 ^b	25.54 ^a	9.07	77.00 ^c	15.1 ^b	45.39 ^c	20.98 ^c	1.84 ^c	1.99	12.58 ^c	64.27 ^c
T5	27.19 ^b	24.67 ^a	8.03	78.5 ^b	16.07 ^b	43.17 ^d	18.52 ^d	1.69 ^c	2.05	12.92 ^b	66.93 ^b
mean	27.5	25.13	8.6	77.81	14.23	45.76	21.22	2.55	1.98	12.49	64.98
CV	3.16	6.93	6.67	0.58	4.54	1.72	2.1	7.6	2.4	1.15	0.58
LSD	1.58	3.17	1.04	0.82	1.17	1.43	0.81	0.35	0.21	0.26	0.57
P- value	**	*	0.19	***	***	***	***	***	0.23	***	***

Means with the same letter are not significantly different at $P < 0.05$. T1- Sole oats, T2- sole alfalfa, T3- 75% oats + 25%alfalfa, T4- 50% oats + 50% alfalfa, T5- 25% oats + 75% alfalfa, DMi- initial dry matter, DM- actual dry matter, OM- organic matter, CP- crude protein, NDF- neutral detergent fiber, ADF- acid detergent fiber, ADL- acid detergent lignin, IVDMD- *in vitro* dry matter digestibility, ME- methabolizable energy, CV – Coefficient of variation, LSD – Least significant deference.

4.3. Silage Dry Matter Ratio and Some Chemical Properties

The DM ratio and some chemical properties of silage obtained with different ratios of mixtures of oats and alfalfa are given in Table 5. The chemical composition of the fermented silage for the seven treatments after 45days of ensiling is shown in Table 5. There were significant differences ($P < 0.05$) between the treatments. T1 and T4 had the lowest values of OM compared to the other treatments. T2 (sole alfalfa) had the higher OM, CP, ME, and IVDMD content. T5 had the

second higher OM, CP, ME, and IVDMD content. T4 and T5 had similar content in DM and CP. The DM content of the plants before ensiling ranges from 24.91% to 29.41% (Table 3). After ensiling, the DM content ranges from 21.45% (sole alfalfa) to 27.28% (sole oats). There was up to 5.5% variability in the treatment values at baseline and 5.83% variability at day 45. These variations are likely due to the differences in seed ratios in the oats (*A. sativa*) used and the properties they exhibited when inter cropped with alfalfa. It was determined that the silage DM ratio increased in parallel with the increase in the ratio of oats and alfalfa in the intercrop groups (Table 5).

The DM ratio is of great importance in the complete realization of chemical events during silage formation, and this ratio is accepted as an important criteria in determining silage quality (Geren, 2001; Cakmak *et al.*, 2013). One of the factors that affects the ensiling process is the DM content of the plant. It predicts the insolubility of forage crops (Knicky, 2005). This situation led to an increase in the DM ratio in silage obtained by adding oats to alfalfa, which has a lower DM content. It was also reported in many research findings that the silage DM ratio increases due to the increase in the cereal ratio in the mixture and that quality silages were obtained in this sense (Demirel *et al.*, 2010; Aykan *et al.*, 2018; Seydosoglu, 2019; Goru, 2020; Karadeniz *et al.*, 2020; Gulumser *et al.*, 2021). According to these results, sole silage of alfalfa will reduce silage quality. Therefore, there was a positive relationship between silage quality and the DM ratio. Ensiling plants with excessively high moisture content affects the lactic acid fermentation in the silo negatively and increases the formation of butter acid (Filya, 2002). For this reason, the silage material must contain highly soluble carbohydrates per unit dry matter.

The crude protein ratio is considered an important indicator in determining the quality of forages. In the study, the highest CP rate was determined in sole alfalfa silage with 19.07%. The lowest CP ratio was found in sole oat silage (9.43%). It was observed that the CP values of the silage belonging to the oats-alfalfa inter crop, where the ratio of alfalfa was 25%, were in the low group statistically. In other words, silage CP values increased in parallel with the increase in the amount of alfalfa in the mixture (Table 5). This is due to the fact that alfalfa is a legume. In some other studies, CP ratios of silage increased with the increase in legume ratios in the mixtures (Geren *et al.*, 2008; Geren, 2014; Turan, 2019; Can *et al.*, 2020; Goru, 2020; Mut *et al.*, 2020).

The NDF, ADF, and ADL ratios of the silage belonging to the sole and different mixtures of oats and alfalfa were analyzed. The highest NDF, ADF, and ADL ratio is sole oats (49.78%, 25.67%, and 4.25%, respectively), and the lowest is sole alfalfa silage (42.68%, 17.44%, and 1.67%, respectively) (Table 5). The fiber composition followed the order of T1 > T3 > T4 > T5 > T2, with some variations between the fiber fractions. This result implies that the fiber density of leguminous plants is lower than that of grass (Ozyazici *et al.*, 2022)

In the present study, it was also observed that the NDF, ADF, and ADL contents of the silage obtained decreased as the amount of alfalfa added to the mixture increased (Table 5). This can be explained by the proportional decrease in cell wall substances in mixtures where alfalfa, which is rich in cellular substances such as CP, is a legume. In other words, the low NDF, ADF, and ADL content of alfalfa was effective in reducing the low NDF, ADF, and ADL content of the silage it was mixed with. The ratio of NDF, ADF, and ADL, which are plant cell wall components, is a good indicator of total digestible nutrients, and it is desirable to have a low ratio of ADF and NDF in roughage (Gursoy, 2014; Van Soest, 2018). As another result of our study, considering the ratio of NDF, ADF, and ADL, it can be said that silage obtained from the oat-alfalfa inter crop produced higher quality silage than both sole oats and alfalfa (Table 5).

In silage made with different grass-legume mixtures, Can *et al.*, 2019 found the ADF ratio to be 29.61–35.88% and the NDF ratio to be 41.66–57.49% in teder (Bituminaria bituminosa L.) and oats mixed silage; Turan, 2019 found the ADF ratio of 30.19%–33.11% and the NDF ratio to 43.00–47.84% in Hungarian vetch and barley mixtures; Karadeniz *et al.*, 2020 found the ADF ratio to be 32.76–34.63% and the NDF ratio to be 46.87–49.70% in the mixtures of grass pea and triticale; Goru and Seydosoglu, 2021 reported the ADF ratio between 31.85–42.99% and the NDF ratio between 47.47–68.12% in some cool climate cereals (oats, barley, rye, and triticale) and common vetch mixtures. In the same studies, the researchers reported that ADF and NDF ratios of silage decreased with the increase in legume ratios in the mixtures, consistent with the findings of our study.

The fiber fractions (NDF, ADF, and ADL content) of T2, T4, and T5 were comparable and lower ($p < 0.001$) than the others. Low fiber content improves nutrient utilization by animals (Stanley, 2004). ADF and ADL levels critically affect feed quality. Research has shown a

negative correlation between their high levels and the potential digestibility of a feed. As ADF increases, the feed becomes less digestible. This has the potential to increase animal intake and digestibility (Ball *et al.*, 2004). According to Stanley, 2004, heterofermentative bacteria convert simple glucose into organic acids (acetic, lactic, propionic, and butyric), which leads to a drop in fiber concentration.

In this study, the overall nutritive value results of the original forage (tables 3 and 4) were higher than those of silage (table 5), except for Ash% and NDF%. This result is supported by different scholars who argue that during the forage conservation process, there are losses of dry matter (DM) (Wilkinson, 2015) and a reduction in the concentration of nutrients as a result of physical and chemical processes that decrease the nutritional value of hay and silage (Borreani *et al.*, 2018; Van Soest, 2018). Proteins and water-soluble carbohydrates (WSC) are among the nutrients that decrease during the conservation process of fresh forages (Charch, 1991; Duniere *et al.*, 2013). The decrease in the content and availability of these nutrients is the reason why the nutritional value of the preserved forage is commonly lower than that of the original forage (Grabber, 2009).

Table 7 Average scores and quality class of physical properties of silage of oats with alfalfa.

Treatment	smell	color	moldiness	structure	DLG	Quality class
T1	3	2.77	2.67	3.27	17.87	Very good
T2	2.2	2.23	2.97	2.77	12.54	medium
T3	3.5	3.5	3.63	3.6	18.32	Very good
T4	3.83	3.83	3.87	3.87	19.16	Very good
T5	3.37	3.07	3.17	3.1	16.69	good
means	3.3	3.08	3.26	3.32	16.92	good

T1-Sole oats, T2- sole alfalfa, T3- 75% oats + 25%alfalfa, T4- 50% oats + 50% alfalfa, T5- 25% oats + 75% alfalfa, DLG =Deutsche Landwirtschafts-Gesellschaft score. CV – Coefficient of variation, LSD – Least significant deference. Means with the same letter are not significantly different at P<0.05.

4.4. Silage Physical Properties

In determining the quality of silage feed, physical methods such as color, smell, and structure, which are simple and can be applied under all conditions, are also used, and with this method, preliminary information about the quality of silage can be obtained (Aykan *et al.*, 2018). The scores and quality class results of the physical observation values (smell, moldiness, structure, and color) of the silage obtained by mixing oats and alfalfa in different proportions are given in

Table 6. Silage smell is one of the most important sensory characteristics used to determine silage quality by physical methods. The average smell value of all applications was determined to be 3.3 points, and the smell values of oats and alfalfa silage and their mixtures varied between 2.2 and 3.83 points. In terms of smell, it was observed that sole alfalfa silage, with a score of 2.2, was close to the "irritative or offensive; alcohol, acidic" smell. Other applications have a good and excellent silage smell in terms of smell in general. In particular, silage consisting of 50% oats and 50% alfalfa gave the best result with 3.83 points (Table 6).

In good silage, leaves and stems are required to preserve their physical structure. In the study, it was determined that the structure of the leaves in sole alfalfa silage was slightly deteriorated and showed low structure characteristics with an average score of 2.77. It was observed that the structure of the leaves and stems of the other silage was not deteriorated, and the silage structures were at the desired levels. In this sense, it has been determined that alfalfa has the highest structure feature of silage at 50:50 mixing ratios with oats (Table 6). The main factor in the preservation of the structure is the fermentation stage, and since the amount of lactic acid increases in a short time in the successfully fermented silo feed, deterioration, wear, or mold formation does not occur on the leaves and stems. Similarly, the average scores of the moldiness variability ranged from 2.67 to 3.87. In terms of moldiness, sole *oat* silage, with a score of 2.67, revealed moderate moldiness. The other treatments are slightly moldy and without mold silage. Particularly, T4 and T3 gave the best results with 3.87 and 3.63, respectively.

Another physical feature used to determine the quality of silage is color. As can be seen from Table 6, the average color value of the applications was determined to be 3.08 points, and the color score values of the silage varied between 2.23 and 3.83. These values show that all silage applications preserve their color when prepared, which physically indicates good silage. Especially among the mixed silage, 50% oats and 50% alfalfa, followed by 25% oats and 75% alfalfa, gave the best results in terms of color (Table 6). Total physical scores (DLG) of silage materials ranged between 12.54 and 19.16. In terms of DLG, sole alfalfa silage was in the "medium" silage quality class, while the other silages were in between "good" and "very good" class. According to the average value of all applications, the physical quality of the silage was found to be "good." In general, when the physical properties and DLG scores are evaluated together, it can be said that better quality silage was obtained from T4 (50% oats and 50%

alfalfa) and T3 (75% oats and 25% alfalfa) mixed silage compared to sole silage materials (Table 6).

It was also revealed in some other studies that the physical properties of silage obtained by mixing legume forage species and cereals in different proportions differ depending on the mixing ratios. In general, higher scores in terms of DLG were obtained from the mixture of legumes with cereals at low rates, as in the findings of our study. For example, the highest DLG scores were reported from silage obtained by mixing white clover with barley at 20%+80% (Demirel *et al.*, 2010), forage peas with barley at 25%+75% (Aykan *et al.*, 2018; Seydosoglu, 2021); grass pea with triticale at 20%+80% (Karadeniz *et al.*, 2020); and common vetch with oats at 25%+75% (Goru and Seydosoglu, 2021).

Table 8 PH, temperature, gas loss, dry matter recovery, and effluent loss of oats with alfalfa.

Treatment	PHi	45 days PH	T°C	TDML	GL	DMRR	EL
T1	6.17	4 ^b	29.7 ^a	3.03	3.52 ^a	68.08 ^c	6.73 ^b
T2	6.1	5.1 ^a	28.2 ^b	2.99	3.35 ^b	73.1 ^{bc}	9.02 ^a
T3	6.03	4.08 ^b	26.9 ^c	2.91	3.04 ^c	79.23 ^{ab}	6.25 ^b
T4	6	3.74 ^c	26.23 ^c	2.93	2.94 ^c	87.2 ^a	5 ^c
T5	6.23	4.97 ^a	28.2 ^b	3.03	3.06 ^c	71.59 ^{bc}	5.33 ^c
means	6.12	4.37	27.69	2.98	3.18	75.84	6.47
CV	2.12	3.16	1.86	3.2	2.8	5.98	4.12
LSD	2.54	0.25	0.94	0.17	0.16	8.25	0.48
P-value	0.39	***	***	0.47	***	**	***

Phi=initial pH, TDML= total dry matter loss, GL=gas loss, EL= effluent loss, DMRR= dry matter recovery rate. Means in same with different superscript letters differ significantly at $p < 0.05$. T1-Sole oats, T2- sole alfalfa, T3- 75% oats + 25%alfalfa, T4- 50% oats + 50% alfalfa, T5- 25% oats + 75% alfalfa, CV – Coefficient of variation, LSD – Least significant deference. Means with the same letter are not significantly different at $P < 0.05$.

4.5. Silage Ph, Temperature, and Dry Matter Loss of the Ensiled Oats with Alfalfa

The pH, temperature, and dry matter loss are shown in Table 7. The pH of the pre-ensiled materials from the treatments with the lowest pH was T4, and the highest was T1. However, the final pH did not follow a trend similar to that seen during pre-ensiling. There is a difference between the pH of the treatments on day 45 ($p < 0.05$). The result from the beginning shows that T4 initially had a low buffering capacity, which meant that T4 needed less acid than the rest of the treatments to lower its pH value. T2 (5.1) and T5 (4.97) had relatively higher final pH than

the others. Silage pH is one of the main determinants that influence the extent of fermentation and quality of ensiled crops, and well-preserved silage usually has a low pH but high lactic acid concentration.

In our current study, the increase in the alfalfa proportion in mixtures increased the silage pH, in agreement with the previous study from Contreras-Govea *et al.*, 2011 showing that legumes such as alfalfa, pea, and red clover do not easily make good silage because of their high buffering capacity and low WSC content, as well as their high level of proteolysis under the combined effects of both plant and silage microbial enzymes, which compromises the ensiling quality compared with that associated with grasses. Higher production of acetic acid facilitates enterobacteria activity, leading to poor fermentation during the early stages, lowering DM and energy, and the process of acetic acid production directly competes with lactic acid bacteria for nutrient use McDonald *et al.*, 1991, resulting in a tendency and shift toward acetate production, especially when sugar concentrations are low.

In our current study, silage from inter crop mixtures of oats and alfalfa tended to have higher lactic acid compared with those from an alfalfa monoculture, probably because the higher content of WSC in oats provided a more readily available fermentation substrate, e.g., soluble sugars for lactic acid-producing bacteria, associated with a more rapid decline in pH for successful fermentation compared with that in sole alfalfa silage (Nguyen *et al.*, 2004). The lower silage pH from mixtures than from sole alfalfa crops might also be attributed to the activity of in oats and the mediated and reduced alfalfa proteolysis (Lee *et al.*, 2006). In the course of this study, other scholars reported that well-preserved alfalfa silage is characterized by a lower pH, which may be due to the effect of treatment with different additives (Muck and Kung, 1997; Zhang *et al.*, 2009).

However, the DMRR followed an opposite trend, with T4 showing the highest value, followed by T3. The temperature of the treatments on day 45 shows a trend of T1 > T2 > T3 > T5 > T4 that varies between treatments ($p < 0.05$). The total DM loss of the treatments ranged from 3.03 (T1 and T5) to 2.91 (T3). The highest gas loss was observed in T1 (sole oats), followed by T2 (sole alfalfa). The lowest effluent treatment was T4 (2.94), and the highest was T1 (3.52). The highest total DM recovery rate for which the highest score was obtained was from T4, followed

by T3. Silage pH value is one of the main factors affecting fermentation quality (McDonald *et al.*, 1991; Geren, 2014; Elis and Ozyazici, 2019). In this study, the highest pH was obtained from sole oats (5.1) and sole alfalfa (4.97), and the lowest was obtained from the inter crop of oats 50% and alfalfa 50% (3.74) silage (Table 5).

It is reported that the silage pH level should be kept at low levels in order to obtain quality silage (Liu *et al.*, 2013), while Filya, 2001 stated that the pH value of a good silage should be below 5. Other researchers (Danley *et al.*, 1973; Comberg *et al.*, 1974; Roth, 2001; Kilic, 2006) reported that the pH value of good silage was between 3.5 and 4.5. It was observed that the pH value was above the desired value in all silage applications examined according to these critical values in the literature. It is thought that this situation is probably due to a lack of good compaction in the silo. However, when Table 7 is examined, reductions in the oat ratio in mixtures resulted in reductions in the pH values of silage. In other words, as the proportion of cereals included in the mixture increased, the pH value of the silage increased (Table 7). It has been reported in some research results that the silage pH values of the silage consisting of a mixture of leguminous forage plants and some cereals differ according to the mixing ratios.

Silage pH value according to the mixing ratio was 5.05–5.34 according to Demirel *et al.*, 2010 in barley and white clover mixtures; 3.90–4.00 according to Aykan and Saruhan, 2018 and Seydoşoglu *et al.*, 2019 in barley and forage pea mixtures; 3.99–4.99 according to Karadeniz *et al.*, 2020 in mixtures of triticale and grass pea; 5.03–6.04 according to Goru and Seydoşoglu, 2021 in mixtures of some cool season cereals (oats, barley, rye, and triticale) and common vetch; 4.51–4.83 according to Gulumser *et al.*, 2021 in oats and forage pea mixtures. The silage treated with sole oats (T1) had higher temperature values ($p < 0.05$) compared to the others, indicating that it was more susceptible to deterioration. This is probably due to the soluble carbohydrate content of the oats and the creation of a less favorable environment for microbial fermentation compared to the others.

A temperature of 26.9 °C and 26.23 °C was recorded for T3 and T4, respectively. This is an indicator of good silage where adequate fermentation has occurred (McDonald *et al.*, 1991). According to Kong, 2011, the temperature of the silage in small silos should be similar to the ambient temperature or a few degrees warmer. In this study, the amount of heat generated was

low, indicating that little aerobic degradation occurred. The browning (Millard) reaction occurs when aerobic oxidation generates too much heat and creates a protein and carbohydrate combination that prevents protein and fiber digestion (Bolsen *et al.*, 1996). These high temperatures are the result of aerobic bacteria oxidizing the extra air trapped in the forage mass. Importantly, these temperatures should drop quickly as more packaging forces air out of the mass and fermentation takes place.

Heat-damaged protein can result from sustained high temperatures in excess of 45–50 °C (Kung *et al.*, 2018). Temperatures in the core of the silo typically drop gradually by 25–30 °C once the active phase of fermentation is over. Big bale and bag silos are examples of small silos that are designed to cool faster than larger silos. Rarely, especially after several months of storage, is the temperature above 35 °C. Huge masses of fodder act as insulation in large silos, resulting in very slow heat dissipation, and in core silage, temperatures often remain high for long periods of time (Kung *et al.*, 2018). The inter cropped groups (T3, T4, and T5) resulted in a significant reduction in gas losses (table 7). Secondary fermentation by enterobacteria, clostridium bacteria, and aerobic microorganisms causes gas losses in silage.

These microbes typically thrive in poorly fermented silage, while well-fermented silage with high levels of lactic acid fermentation produces minimal nutrient content (Kung *et al.*, 2018). This happens when the sugar content is particularly high. Yeast development leads to alcohol fermentation, which leads to gas losses in the form of ethanol. According to McDonald *et al.*, 1991, moisture loss through the stomata peaks soon after cutting and stops within 2 hours as a result of complete stomata closure. However, moisture loss through the cuticle can still occur after that. Effluent production was lower for T4 than for the others. Abundant effluent production in silage results in high losses of organic substances such as sugars, organic acids, and proteins (Knicky, 2005).

The absence of organic substrates reduces the nutritional value of silage (Li *et al.*, 2022). Increasing the DM content in the silage by mixing the two forages (T4 and T3) led to a reduction in effluent production. Silage effluent is thought to transport nitrogen molecules, sugars, organic acids, and mineral salts (McDonald *et al.*, 1991). Effluent (or leachate) may be produced, typically when the ensiled crop has a high moisture content (Gebrehanna *et al.*, 2014). The

moisture of pre-ensiled crops can be influenced by plant factors (McDonald *et al.*, 1991). There is high variability in reported effluent production rates for different crops with different moisture contents (Gebrehanna *et al.*, 2014). Fermenting a crop is said to lower the pH to inhibit putrefactive bacteria, thus preserving the protein content of the fodder (McDonald *et al.*, 1991).

Table 9 Effect of alfalfa-oat inter cropping on soil physical and chemical properties before forage sowing and after harvest

Treat ment	PH		OC		OM		EC		CEC		TN		Exch K		Av.P(ppm)	
	BP	AH	BP	AH	BP	AH	BP	AH	BP	AH	BP	AH	BP	AH	BP	AH
T1	4.87	6.15 ^a	3.89	4.08 ^c	6.63	7.02 ^b	0.08	0.31	30.72	31.68 ^d	0.9	1.6 ^d	3.98 ^{ab}	4.4 ^d	8.89	13.74 ^e
T2	4.47	6.3 ^a	3.85	4.16 ^c	6.7	7.16 ^b	0.08	0.17	30.46	40.86 ^a	0.89	4.37 ^a	3.99 ^{ab}	6.19 ^a	8.93	16.82 ^a
T3	4.43	5.9 ^b	3.99	5.02 ^b	6.86	11.8 ^a	0.09	0.13	30.71	33.56 ^c	0.98	2.28 ^c	3.92 ^b	4.41 ^d	8.9	14.41 ^d
T4	4.17	5.67 ^{bc}	3.89	5.45 ^a	6.71	11.53 ^a	0.08	0.14	30.75	34.98 ^c	0.92	3.37 ^b	4.14 ^a	5.08 ^c	8.96	15.1 ^c
T5	4.19	5.47 ^c	3.88	5.65 ^a	6.67	11.47 ^a	0.07	0.16	30.06	37.44 ^b	0.93	4.03 ^a	3.95 ^{ab}	5.75 ^b	8.92	15.95 ^b
Mean	4.43	5.9	3.9	4.87	6.7	9.79	0.08	0.18	30.54	35.7	0.92	3.13	3.4	5.17	8.92	15.2
CV	5.73	2.6	3.27	3.91	3.26	4.39	30.09	9.7	4.25	3.03	7.95	10.59	3.5	4.08	6.59	7.41
LSD	0.61	0.23	0.19	0.29	0.33	0.65	0.04	0.27	1.96	1.63	0.11	0.5	0.21	0.32	0.1	0.04
Pvalue	0.02	***	0.64	***	0.63	***	0.95	0.61	0.93	***	0.54	***	0.26	***	0.35	***

T1-Sole oats, T2- sole alfalfa, T3- 75% oats + 25%alfalfa, T4- 50% oats + 50% alfalfa, T5- 25% oats + 75% alfalfa, OC = Organic carbon, OM = Organic matter, TN = total nitrogen, Av.P (ppm) = Available phosphorous(parts per million), CEC (meq/100g) = Cation Exchange Capacity (mills equivalent per 100 gram), Exch.K (meq/100 g) = Exchangeable potassium (mills equivalent per 100 g), BP= before planting, AH= after harvest, CV – Coefficient of variation, LSD – Least significant deference. Means with the same letter are not significantly different at P<0.05.

4.6. Physico-Chemical Properties of the Soil

The soil physico-chemical analysis results before planting forage are shown in Table 8. The pre-soil analysis showed that the experimental soil had a pH of 4.43, indicating that the soil was strongly acidic, which was also reported by Hazelton and Murphy, 2007 and Mesfin, 2007. The preferable pH ranges for most crops are in the range of 6.0 and 7.5 (Hazelton and Murphy, 2007; Hall, 2008). However, the pH of the experimental soil was below the recommended level, which is strongly acidic and more likely to be deficient in some of the available nutrients for optimal plant growth. Therefore, selecting and growing species and varieties adaptable to acidic soils is

one solution (Scott *et al.*, 1997). Similarly, Heluf and Wakene, 2006, reported that agronomic and management options to correct acid soils, improve nutrient use efficiency, and increase crop production on acidic soils include the application of organic materials, appropriate crop rotations, and crop mixtures.

The soil analysis result showed that the available phosphorous rated at 8.92 ppm in the experiment site before forage planting was low according to the classification of a relative range of ex-tractable phosphorous of <5 ppm, 5-10 ppm, 11-15 ppm, 16-20 ppm, and 21-25 ppm as very low, low, medium, high, and very high, respectively (Marx *et al.*, 1999). The total average nitrogen, organic carbon, and organic matter content of soil in the study area before planting was 0.92%, 3.9%, and 6.7%, respectively. According to Hazelton and Murphy's, 2007 rating, nitrogen is very high and organic carbon contents are medium in the present finding. The analysis of soil samples after forage harvest showed an increased level of some of the soil nutrients based on treatment combinations (Table 8).

All treatment groups indicated a slight change in pH values above the initial recorded average value of 4.43 in the experiment. The pH of the soil after forage harvest was statistically higher in sole oats (6.15) and sole alfalfa (6.3) than that of their mixtures in T3 (5.9) and T4 (5.67). This slightly higher change after forage harvest in soil pH might be attributed to an increase in organic matter and exchangeable bases, or the basic cation content of the soil, that influenced the change in soil pH value. The present result is in line with that of Belayneh *et al.*, 2020, who reported that the soil treated with inter cropping had a slight change in pH value after harvesting. Wong and Swift, 2003 reported that the most effective way of managing and correcting soil acidity is through appropriate crop rotations, crop mixtures, and the use of plant species and varieties, which can ameliorate the effect of soil acidity on crop growth.

The available phosphorus (av.P) for soil samples after forage harvesting alfalfa-based treatments was comparatively higher (14.41 ppm to 15.95 ppm) than pure stand oats (13.74 ppm) treatments (Table 8). The highest av.P value was obtained in sole alfalfa (16.82). The highest av.P pure stand alfalfa might be due to the fact that alfalfa increased phosphorus availability by mobilization of soil mineral due to its nodulation that enhance the soil fertility. This result is in line with the result of Belayneh *et al.*, 2020, who reported that including legumes in inter

cropping increases phosphorus availability due to their deep roots. The lowest value of soil available phosphorus in pure stand oats-based treatment showed that there was more utilization of phosphorus by oats plants. Organic carbon percentage (OC%) of the soil was slightly higher, ranging from 4.16% to 5.65% after forage harvest in all alfalfa and oats-alfalfa mixture treatments, whereas it was lower in sole oats treatments, ranging from 4.08% compared to pre-planting.

The higher organic carbon percentage in the tested soil after forage harvest in all alfalfa-based treatments was probably due to improved soil structure and fertility. The lower OC% in pure stand oats crop treatments showed that there was more utilization of organic carbon by oats plants. In the current study, an increasing amount of organic matter percentage, which ranged from 7.16% to 11.53%, was obtained in alfalfa and alfalfa-oat inter cropped treatments compared to before forage planting with an average 6.7% OM. This indicates that alfalfa-based treatments, which build up organic matter content and arrest pH decline, are likely to create soil conditions that encourage survival, persistence, and a higher population of *Rhizobium* in soil. The current result agrees with Eshetu, 2011, who reported that soil that was cultivated on acid-tolerant legume plant fields has a higher organic matter content than cereals.

According to Barber, 1984, rates of organic matter greater than 10 percent are very high, 5–10 percent high, 2–5 percent medium, 1-2 percent low, and less than 1 percent very low. Employing this rating, the organic matter percent of this study after forage harvest was ranked high for T1 and T2 and very high for inter cropped groups. The total nitrogen content of the soil increased in sole alfalfa plots, whereas it decreased in sole oats after harvest compared to pre-planting (Table 8). The total nitrogen content after forage harvest varied from (2.28% to 4.37%) in pure stand alfalfa and alfalfa-oat inter cropped treatments, whereas the lowest (1.6%) was obtained under pure stand oats. Thus, mixed cropping of oats with alfalfa in this study offers better opportunities for complementary nitrogen use in low-input farming systems without compromising the yield of both species.

The highest total nitrogen percentage after forage harvest in alfalfa was attributed to the ability of alfalfa plants to biologically fix atmospheric nitrogen (N_2) in the soil and improve the soil N status. On the other hand, the decreased total nitrogen percentage observed in the pure stand oats

crop might be due to more utilization of soil N_2 by oats than alfalfa. In mixed treatments, the percentage of total nitrogen falls between pure oats and pure alfalfa after forage harvest. This variation might be attributed to the alfalfa component of the mixtures, which may have fixed N_2 in the soil while the amount of N absorption from the soil by the associated oat crops. This result is supported by Hauggaard *et al.*, 2001a, who reported that legumes such as alfalfa can cover their N demand from atmospheric N_2 , therefore inter cropping with cereals competes less for soil mineral N_2 . Similarly, the current result is in line with the finding of Keighobadi *et al.*, 2014, who reported that inter cropping cereal with legume crops such as cowpea, soybean, common vetch, and groundnuts helps maintain and improve soil fertility because these legumes accumulate about 80 to 350 kg of nitrogen (N_2) ha^{-1} .

The soil exchangeable potassium levels were seen to increase in all treatments after forage harvest compared to before forage planting in the experiment soils. The exchangeable potassium was corrected from a very low level to low in all pure stand alfalfa and oat-based treatments after forage harvest but remained the same in pure stand oats such a level of rating was also suggested by Marx *et al.*, 1999. According to the current result, both oats and alfalfa had a positive effect on exchangeable potassium in the soil. According to Landon, 1991, who rated the top soils having a CEC of > 40 meq/100 g, 25–40 meq/100g, 15–25 meq/100g, 5–15 meq/100 g, and < 5 meq/100 g, they were classified as very high, high, medium, low, and very low, respectively. The cation exchange capacity (CEC) values of the soils obtained in all treatments after forage harvest were statistically different ($P < 0.05$) and higher than the CEC value indicated before forage planting. The CEC value of the current experiment soil ranges from 31.68 to 40.86 meq/100g after forage harvest. The highest CEC value was observed in Sole alfalfa (40.86 meq/100 g) after harvesting. Thus, the soils in the present experiment containing medium organic matter contents have a high cation exchange capacity. The current result is supported by Marx *et al.*, 1999, who reported that colloidal OM has the ability to absorb and hold positively charged ions.

Table 10 Effect of oats with alfalfa forage on in vitro total gas (ml/200 g DM) and methane (CH₄) production

Treatment	Incubation period						CH ₄ at 72 hrs	SCFA mmol/L at 72hrs
	3	6	12	24	48	72		
T1	6.75 ^e	11.39 ^e	15.51 ^e	32.29 ^c	38.65 ^e	42.74 ^e	5.33 ^d	0.78 ^d
T2	11.83 ^a	16.78 ^a	21.92 ^a	42.61 ^a	50.81 ^a	56.05 ^a	9.5 ^a	1.59 ^a
T3	7.47 ^d	12.72 ^d	16.87 ^d	34.58 ^c	40.19 ^d	46.18 ^d	6.87 ^c	0.94 ^{cd}
T4	8.63 ^c	13.81 ^c	18.04 ^c	38.51 ^b	44.63 ^c	49.98 ^c	5.93 ^{cd}	1.08 ^{bc}
T5	9.77 ^b	14.78 ^b	19.43 ^b	38.43 ^b	48.21 ^b	52.99 ^b	8.23 ^b	1.19 ^b
mean	8.89	13.89	18.36	37.28	44.5	49.59	7.17	1.12
CV	4.1	2.32	1.64	3.41	0.7	0.52	7.35	7.59
LSD	0.67	0.56	0.55	2.23	0.57	0.47	0.96	0.15
P-value	***	***	***	***	***	***	***	***

a,b,c,d,e= The same column bearing different superscript differ significantly(p<0.05), CH₄ = Methane, SCFA= Short Chain Fatty Acids (mmol), CV= coefficient variation, LSD= Least Significant Deference,T1-Sole oats, T2- sole alfalfa, T3- 75% oats + 25%alfalfa, T4- 50% oats + 50% alfalfa, T5- 25% oats + 75% alfalfa

4.7. Effect of Oats and Alfalfa Inter Cropping Forage on *In Vitro* Total Gas and Methane Production

The inter cropping ratio of oats and alfalfa forage to *in vitro* total gas, methane, and SCFA results is indicated in Table 9. *In vitro* total gas, methane, and short-chain fatty acid production were all significantly different (p< 0.001) between the treatments. The seed ratio of the inter cropped forages indicates the leading potency of total gas production throughout the incubation period. A tangible difference among the treatments in the gas production potential was primarily recorded at T2 (sole alfalfa), where it scored the highest value throughout the incubation period (11.83 ml, 16.78 ml, 21.92 ml, 42.61 ml, 50.81 ml, 56.05 ml at 3, 6, 12, 24, 48, and 72 hrs, respectively). Similarly, T2 (sole alfalfa) recorded the highest value both in methane and short-chain fatty acid production (9.5 and 1.59 at 72 hrs, respectively). In other ways, T1 (sole oats) revealed the lowest value in total gas production at all the incubation periods and also in both methane and SCFA production.

Among the inter cropped groups, T5 (25% oats + 75% alfalfa) seed ratio had a higher value than T4 (50% oats + 50% alfalfa) and T3 (75% oats + 25% alfalfa) at all the incubation periods of total gas, methane, and SCFA production. The result of this study revealed that the total gas, methane, and SCFA production value increased in parallel with the alfalfa seed ratio as it

increased in the inter cropped groups (T3, T4, and T5). The result of this study was confirmed as alfalfa has a higher potential for gas production. So, the leading potency of sole alfalfa in methane production consistently observed in the present study could be explained by its highest total gas production throughout the periods of fermentation. On the other hand, methane production represents the efficiency of microbial fermentation in the rumen and is associated with highly degradable contents (Jayanegara *et al.*, 2009). So, the least potency of sole oats in methane production could imply a lower content of the poorly digestible carbohydrate fractions.

Thus, relatively more efficient microbial fermentation could be expected in line with sole alfalfa and the inter cropped groups (T4 and T5) compared to sole oats examined in the present study. In the current study, the increase in alfalfa proportion in oats and alfalfa mixtures increased CH₄. Consistent with the previous studies (Chaves *et al.*, 2006; Jayanegara *et al.*, 2011), the amount of CH₄ produced from grass pastures was less than that from alfalfa pastures because CH₄ production was positively correlated with the concentrations of readily fermentable NFE and IVDMD. In accordance with the present result, the end-product gases (CH₄, CO₂, and H₂) are mainly produced from the process of carbohydrate fermentation rather than protein fermentation during *in vitro* incubation, and only a small proportion of gas is indirectly produced from the buffering of short-chain VFAs (Getachew *et al.*, 1998).

Gases produced in the rumen are mainly carbon dioxide and methane. It has been found that plant fiber accounts for more than 60% of gas production emissions (Niu, 2014). These gases imply not only lower efficiency and productivity in livestock systems but also considerable threats to our environment (Rojas-Downing *et al.*, 2017). Higher levels of soluble carbohydrates provided energy resources for better fermentation environments and ruminal microbe growth and coincided with higher VFA production and effective biodegradability (Cho *et al.*, 2012). Our present study also confirmed that increasing alfalfa inclusion increased the total SCFAs' production. The concentrations of branched-chain VFAs produced were mainly dependent on the composition and extent of amino acids in feeds, and higher CP was significantly correlated with the higher concentrations of SCFA production (Getachew *et al.*, 2004), which explained the higher SCFA production with increasing alfalfa proportion in the present study.

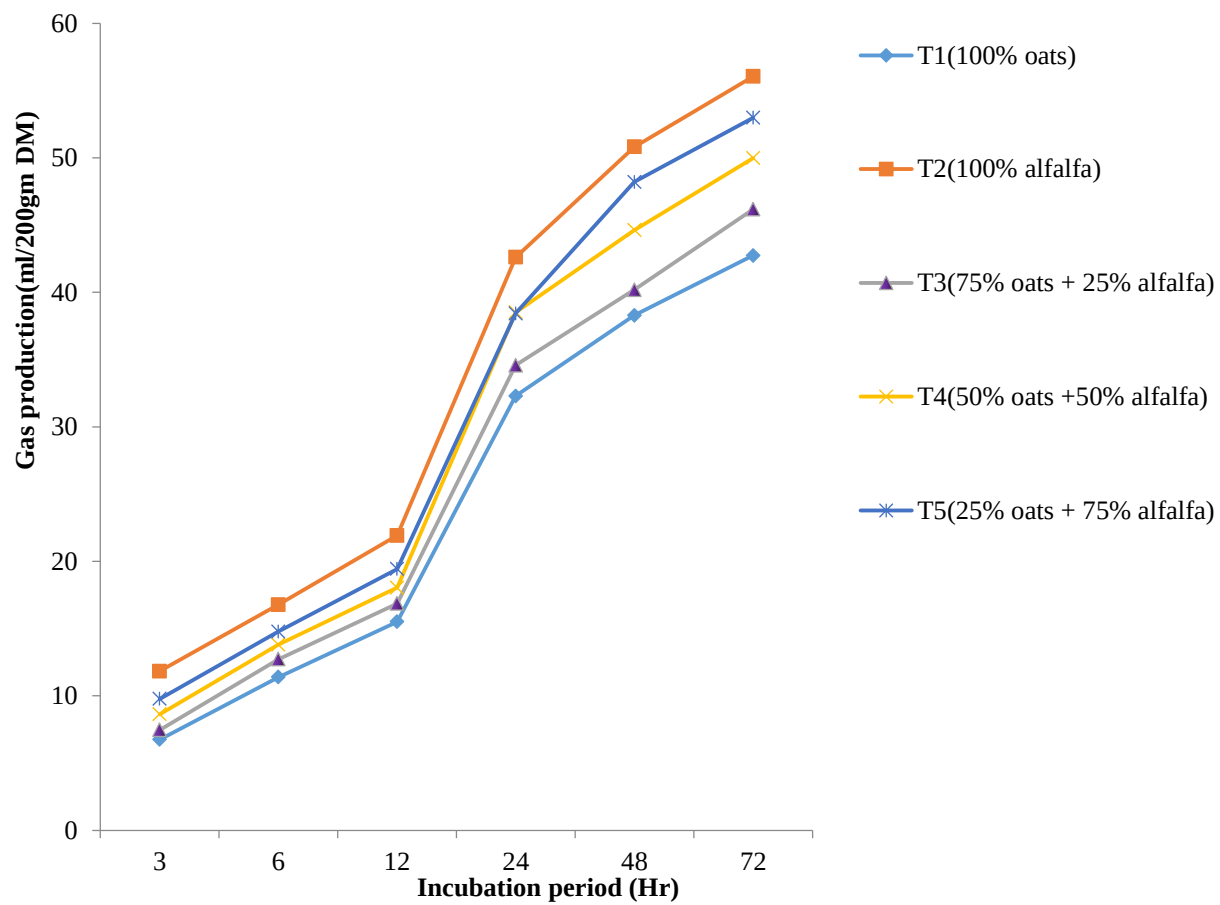


Figure 3 *In vitro* gas production of oats with alfalfa of raw forage

Table 11 Effect of oats with alfalfa forage on in vitro gas production characteristics

Treatment	Gas production characteristics				
	a	b	c	a +b	LT
T1	3.55 ^e	97.52 ^a	0.022 ^e	46.37 ^e	1.88 ^a
T2	7.18 ^a	39.19 ^e	0.052 ^a	101.07 ^a	0.62 ^d
T3	3.96 ^d	84.01 ^b	0.038 ^d	49 ^d	1.03 ^b
T4	4.46 ^c	76.23 ^c	0.041 ^c	80.69 ^c	0.96 ^b
T5	5.42 ^b	43.58 ^d	0.046 ^b	87.97 ^b	0.84 ^c
mean	4.91	68.11	0.04	73.02	1.07
CV	3.92	0.91	3.61	0.89	3.75
LSD	0.35	1.12	0.003	1.18	0.07
P-value	***	***	***	***	***

a, b, c, d, e = column means with different superscripts shows significant between-treatments difference ($P < 0.05$), a = Gas production from the immediately soluble fraction (ml), b = Gas production from the in-soluble but degradable fraction (ml), a + b = Potential gas production (ml); c = the rate constant of gas production (fraction/h); LT - lag time, CV= coefficient variation, LSD= Least Significant Difference. Means with the same letter are not significantly different at $P < 0.05$.

4.8. The Effect of Oats and Alfalfa Inter Cropping Forage on *In Vitro* Gas Production Characteristics

In vitro gas production characteristics of oats and alfalfa inter cropped forage are indicated in Table 10. All of the *in vitro* gas production characteristics showed a significant ($P < 0.001$) difference among the treatments. Gas production from immediately soluble fractions (a), the rate of gas production (c), and potential gas production (a+b) were higher in T2 (sole alfalfa) as compared to T1 (sole oats). Whereas, gas production from insoluble but potentially degradable fractions (b) and lag time were higher in T1 (sole oats) as compared to T2 (sole alfalfa) observations. The oats with alfalfa gas production from immediately soluble fractions (a) in the treatments were in the range of 3.55-7.18 ml with a pooled average value of 4.91 ml.

The highest and lowest 'a' values were observed in T2 (7.18 ml) and T1 (3.55 ml), respectively. The highest value of gas production from the insoluble but potentially degradable fractions (b) was recorded from T1 (sole oats) (97.52 ml) and the lowest from T2 (39.19 ml). In other words, the medium values of gas production from the insoluble but potentially degradable fractions (b) in the inter cropped groups (T3, T4, and T5) are 84.01 ml, 76.23 ml, and 43.58 ml, respectively. Correspondingly, the highest and lowest potential gas production (a+b) records in the treatments

were noted for T2 (sole alfalfa) (101.07 ml) and T5 (25% oats + 75% alfalfa) (87.97 ml), respectively. In the present study, the rate constant of gas production (c) was higher in T2 (0.052 ml) and lower in T1 (0.022). Also, medium values were observed in the inter cropped groups (T3, T4, and T5).

The higher fermentation process lag time was noted for T1 (1.88 h), followed by T3 (1.03 h), and T4 (0.96 h), respectively. While T2 (0.62 h) and T5 (0.84 h) had the shortest comparable lag time records. Similar to this study Gelgelo *et al.*, 2023, those species that have the highest immediately ferment able fraction would have the fastest rate of gas production constant as well as the shortest possible lag time. In line with this study, Tolera and Sundstol, 1999 confirmed that poorly digestible feed components require plenty of exposure time to be hydrated and colonized by rumen microorganisms before they enter the fermentation process. Getachew *et al.*, 2004 suggested that the rate of nutrient fermentation in a particular feed sample is influenced by the microbial mass and the convenience of the feed material for microbial digestion.

Correspondingly, the longest lag time of fermentation observed for sole oats could be due to the fermenting microbes requiring a reasonable amount of time to multiply and hydrate the slowly ferment able feed particles Tolera and Sundstol, 1999. As to the reflections of McDonald *et al.*, 2010, the interwoven matrix of polymers in the slowly ferment able feed components creates barriers against the microbial invasion and limits their access to the digesting enzymes of microbes, thereby decreasing their rate of fermentation and lengthening their lag time in *in vitro* gas production.

Besides, the value of gas production constant (c) was suggested to be equivalent to feed intake and therefore expected to affect the rate of ingesta passage along the gut, while potential gas production (a + b) was a correspondent of feed biodegradability (Khazaal *et al.*, 1996). To this end, depending on seed ratio oats with alfalfa observations of gas production constant, it follows that animals consuming sole alfalfa would have better feed intake, followed by those consuming (25% oats + 75% alfalfa) and (50% oats + 50% alfalfa), while comparatively lower intake could be expected for animals relaying on sole oats and (75% oats + 25% alfalfa).

Table 12 Effect of oat-alfalfa silage on *in vitro* total gas (ml/200 g DM) and methane (CH₄) production

Treatment	Incubation period						CH ₄ at 72 hrs	SCFA mmol/L at 72hrs
	3	6	12	24	48	72		
T1	5.4 ^e	10.18 ^e	14.13 ^e	31.18 ^e	37.26 ^e	42.01 ^e	4.28 ^d	0.6 ^d
T2	11.08 ^a	16.05 ^a	21.11 ^a	43.31 ^a	49.38 ^a	55.14 ^a	8.45 ^a	1.42 ^a
T3	6.12 ^d	11.52 ^d	15.62 ^d	33.26 ^d	38.59 ^d	45.36 ^d	5.16 ^c	0.76 ^{cd}
T4	7.1 ^c	12.71 ^c	16.72 ^c	37.28 ^c	41.53 ^c	49.28 ^c	4.88 ^c	0.9b ^c
T5	9.94 ^b	13.36 ^b	18.23 ^b	39.53 ^b	46.23 ^b	52.14 ^b	7.18 ^b	1.01 ^b
mean	7.91	12.76	17.16	36.91	42.6	48.78	5.99	0.94
CV	1.35	1.04	0.76	0.62	0.66	0.28	3.57	9.06
LSD	0.19	0.24	0.24	0.42	0.51	0.25	0.39	0.15
P-value	***	***	***	***	***	***	***	***

^{a,b,c,d,e}= The same column bearing different superscript differ significantly ($p < 0.05$); CH₄ = Methane; SCFA= Short Chain Fatty Acids (mmol); CV= coefficient variation; LSD= Least Significant Deference, T1-Sole oats, T2- sole alfalfa, T3- 75% oats + 25%alfalfa, T4- 50% oats + 50% alfalfa, T5- 25% oats + 75% alfalfa

4.9. Effect of Oats-Alfalfa Inter Cropping Silage on *In Vitro* Gas and Methane Production

Inter cropping ratio of oats and alfalfa silage to *in vitro* total gas, methane, and SCFA results were significantly different ($P < 0.05$) in Table 11. Similar to oats and alfalfa forage results *in vitro* total gas, methane and SCFA had the highest value in the T2 (sole alfalfa) and the lowest value in the T1 (sole oats). However, medium values were observed in the intercropping groups (T3, T4, and T5). Sole alfalfa had the highest value of total gas production through all the incubation periods (11.08 ml, 16.05 ml, 21.11 ml, 43.31 ml, 49.38 ml, and 55.14 ml at 3 h, 6 h, 12 h, 24 h, 48 h, and 72 h), respectively, and the lowest values were recorded in T1 (sole oats) in all the incubation periods. All the intercropped groups (T3, T4, and T5) have shown average values throughout the incubation periods.

Similarly, T2 (sole alfalfa) had the highest value both in methane and SCFA production (8.45 and 1.42), respectively. The methane production potential in this study increased with the proportion of alfalfa in the mixture. The highest value of methane production was recorded in T2 (sole alfalfa) and the lowest value in T1 (sole oats). The same trend also applied for the SCFA, in which T2 had the highest and T1 the lowest value. Other studies have suggested that the concentration of SCFA is positively correlated with the amount of substrate fermentation in the rumen (McDonald *et al.*, 2011; Chen *et al.*, 2019). In this study, we observed that with the

increase in the proportion of alfalfa, the digestible DM and OM also increased, which in turn resulted in an increase in the concentration of SCFA.

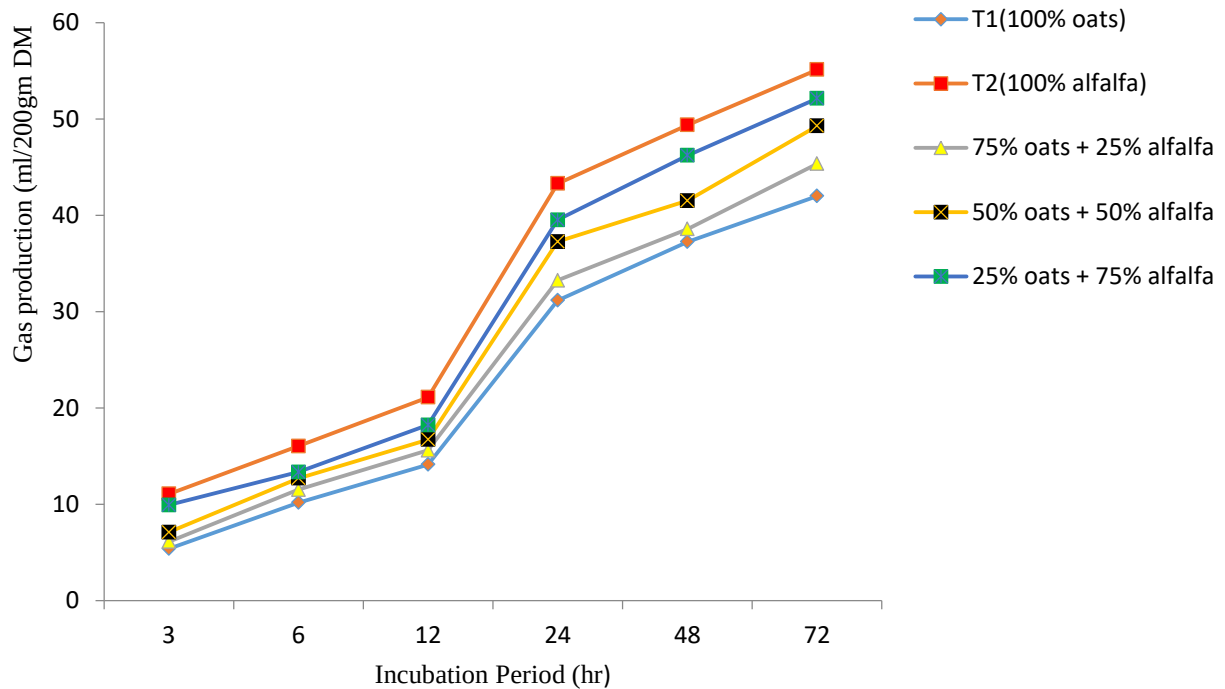


Figure 4. *In vitro* gas production of oats with alfalfa silage

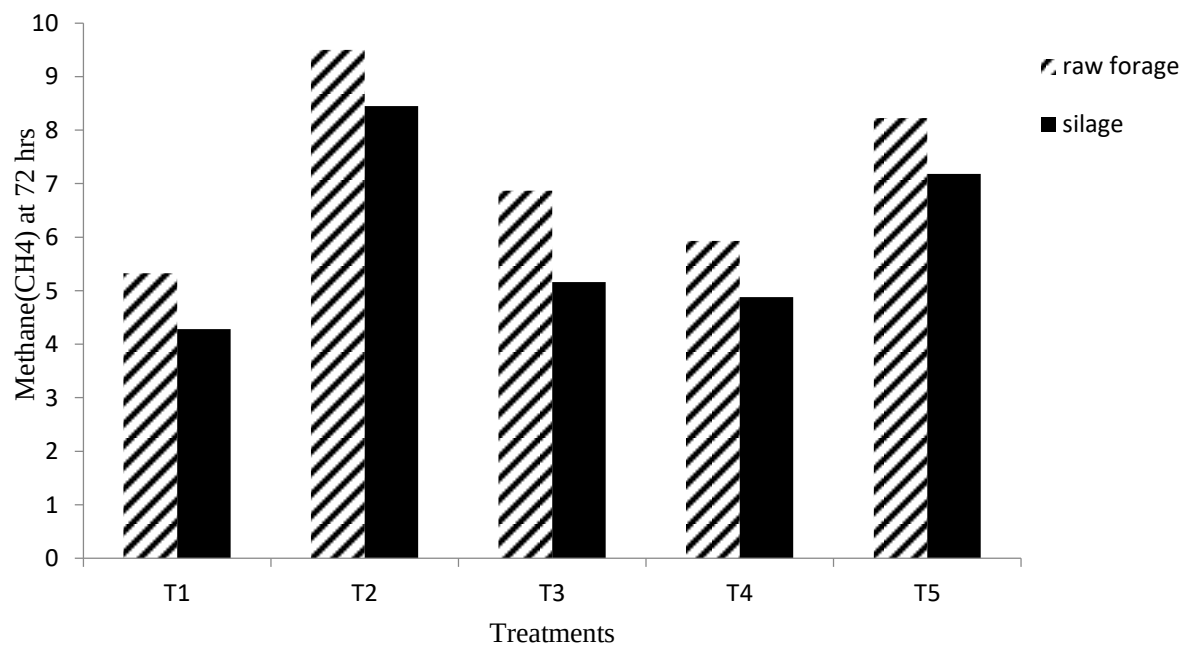


Figure 5 Raw forage vs silage methane (CH₄) production at 72hrs incubation period.

Table 13 Effect of oats with alfalfa silage on in vitro gas production characteristics

Treatment	Gas production characteristics				
	a	b	c	a + b	LT
T1	2.4 ^e	96.05 ^a	0.019 ^d	98.48 ^a	1.75 ^a
T2	6.18 ^a	37.44 ^e	0.046 ^a	43.62 ^e	0.53 ^e
T3	2.9 ^d	81.85 ^b	0.033 ^c	84.52 ^b	1.03 ^b
T4	3.27 ^c	74.1 ^c	0.039 ^b	77.37 ^c	0.9 ^c
T5	4.68 ^b	41.03 ^d	0.041 ^b	45.71 ^d	0.75 ^d
mean	3.89	66.1	0.04	69.94	0.99
CV	1.48	0.23	5.41	0.41	2.05
LSD	0.1	0.27	0.004	0.52	0.04
P-value	***	***	***	***	***

a, b, c, d, e = column means with different superscripts shows significant between-treatments difference ($P < 0.05$), a = Gas production from the immediately soluble fraction (ml), b = Gas production from the in-soluble but degradable fraction (ml), a + b = Potential gas production (ml); c = the rate constant of gas production (fraction/h); LT - lag time, T1-Sole oats, T2- sole alfalfa, T3- 75% oats + 25%alfalfa, T4- 50% oats + 50% alfalfa, T5- 25% oats + 75% alfalfa, CV= coefficient variation; LSD= Least Significant Deference.

4.10. The Effect of Oats and Alfalfa Inter Cropping Silage on *In Vitro* Gas Production Characteristics

Sole Alfalfa silage had the highest cumulative gas production (Parameter a), and the Alfalfa + oats treatments were greater than sole *oats*. *In vitro* gas production characteristics of *oats* and alfalfa inter cropped silage were significantly different (Table 12). All the *in vitro* gas production characteristics were significantly ($P < 0.001$) different among the treatments. Gas production from immediately soluble fractions (a) and the rate of gas production (c) were both higher in T2 (sole alfalfa) as compared to T1 (sole oats), similar to oats with alfalfa forage values. Whereas, gas production from insoluble but potentially degradable fractions (b), potential gas production (a + b), and lag time were all higher in T1 (sole oats) as compared to T2 (sole alfalfa) observations.

The oats with alfalfa gas production from immediately soluble fractions (a) in the treatments were in the range of (2.4–6.18 ml) with a pooled average value of 3.89 ml. The highest and lowest (a) values were observed in T2 (6.18 ml) and T1 (2.4 ml), respectively. The highest value of gas production from the insoluble but potentially degradable fractions (b) was recorded from T1 (96.05 ml) and the lowest from T2 (37.44 ml). In other words, medium values of gas

production from the insoluble but potentially degradable fractions (b) in the intercropped groups (T3, T4, and T5) are 81.85 ml, 74.1 ml, and 41.03 ml, respectively.

Correspondingly, the highest and lowest potential gas production (a + b) records in the treatments were noted for T1 (98.48 ml) and T2 (43.62 ml), respectively. In the present study, the rate constant of gas production (c) was higher in T2 (0.046 ml) and lower in T1 (0.019). Also, medium values were observed in the intercropped groups (T3, T4, and T5). The higher fermentation process lag time was noted for T1 (1.75 h), followed by T3 (1.03 h), and T4 (0.9 h), respectively. While T2 (0.53 h) and T5 (0.75 h) had the shortest comparable lag time records. However, all the values of the parameter in the oats with alfalfa silage were lower than those in the oats with alfalfa raw forage.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Leguminous forages cultivation not only provides high-quality and quantity feed for livestock, but by fixing nitrogen, they could maintain and improve soil fertility and reduce the cost of chemical fertilizers, in which prices are increasing rapidly from time to time. The compatibility of legumes forages with grasses depends on the morphology and physiological characteristics of the legume and grass, in combination with the response of the management imposition, climate, soil, and biotic conditions under which the crop was growing. Considerable variations were existed among the tested seed ratios, indicating the potential for selecting higher-performing forage biomass yield and quality. The highest forage yield in the oats-alfalfa inter crop was obtained from the seed ratio (50% oats + 50% alfalfa) and (75% oats + 25% alfalfa).

However, the highest seed yield was obtained from oats in pure stands. The analysis of variance also showed a great variation in the chemical composition (DM, Ash, OM, CP, NDF, ADF, ADL, and IVDMD) of the tested treatments, both in forage and silage. The OM% and CP% content in the pure stand of alfalfa were significantly higher than those in pure stand oats and oats-alfalfa inter cropped. The crude protein content of the intercropped groups increased as the seed ratio of alfalfa increased in the combination of both forage and silage. Even though the NDF content of sole oats was higher than that of other treatments, all treatments were observed to be below the critical level (55%) of NDF, which indicates higher digestibility and intake.

The crude protein and neutral detergent fiber were the most important nutrients that determine the quality of forages. Due to this, the highest total nutrient yields of CP% and NDF% were obtained in mixed treatments compared to pure stands. The seed ratio of oats and alfalfa inter cropping affects both the physical and chemical properties of the prepared silage. Particularly, the inter cropped groups (50% oats + 50% alfalfa) and (75% oats + 25% alfalfa) revealed acceptable silage quality in all parameters compared to pure stands of oats and alfalfa. In this study, all treatment groups indicated a slight change in the physico-chemical properties of the soil. Significant variations were observed in tested soil parameters like PH, OC, OM, EC, CEC, TN, K, and available P due to inter cropping of oats and alfalfa.

And also, the tested seed ratio of oats with alfalfa reduced, to some extent, total gas production and methane emissions compared to sole alfalfa but was higher than sole oats. The produced gas and methane increased parallel to the increased alfalfa in the mixture. So (50% oats + 50% alfalfa and 75% oats + 25% alfalfa) seed ratios were suitable combinations in the case of total gas and methane production reduction. Generally, in the present study, the oats-alfalfa mixture increased forage biomass yield, forage and silage quality, and improved soil physical and chemical components, while total gas and methane production reductions were observed.

5.2. Recommendations

Based on the results of the present study, the following recommendations are proposed: The mixture of (50% oats + 50% alfalfa and 75% oats + 25% alfalfa) seed ratio combinations performed the best in most agronomic parameters like nutritional values, soil fertility, fermentation properties of their silage, and balancing gas and methane production in both forage and silage. It is recommended for fodder production in the studied area and related agro-ecologies to enhance dry season feed shortage through conservation forages (silage) and improve livestock productivity. When growing oats for seed purposes, it is preferable to use oats in a pure stand as it gives a comparatively higher seed yield. However, this study was conducted in a single season; therefore, it is recommended that the experiment should be conducted repeatedly in order to give more concrete recommendation. Furthermore, the impact of oats and alfalfa inter cropping on animal performance should be investigated (feeding trial).

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7. APPENDICES

Appendices 1 . Rating key for nodule assessment (Corbin et al., 1977)

	Field assessment key		Mean score and indication
Score	Visual observation on field	Mean nodule score	Indication
0	No nodulation	0	No nodulation and no N ₂ fixation
1	<5 in the crown-root zone (regarded as the region up to 5cm below the first lateral roots) with no nodules on elsewhere on the root system	0-1	Very poor nodulation and probably little or no N ₂ fixation
2	5-10 in the crown-root zone with	1-2	Poor nodulation and probably little N ₂ fixation
3	>10 in the crown-root zone with	2-3	Fair nodulation; N ₂ fixation may not be sufficient to supply the N demand of the crop
4	>10 in the crown-root zone with 5-10 nodules on elsewhere on the root system	3-4	Good nodulation and good potential for N ₂ fixation
5	>10 in the crown-root zone with >10 nodules on elsewhere on the root system	4-5	Excellent nodulation; excellent potential for N ₂ fixation

Appendices 2 . Physical examination key developed by DLG and silage quality class

Odor	score
No butter acid odor, slightly sour, fruity and aromatic odor	14
A small amount of butter acid, strong sour odor, and slight escalation	8
Moderate butter acid odor, strong escalation-musty odor	4
Strong butter acid or ammonia odor, very slight sour odor	2
Strong decomposition, ammonia or musty odor	0
Structure	
Intact leaves and stems	4
A slightly deteriorated structure of leaves	2
A deteriorated structure of leaves and stems, musty and dirty	1
Rotten leaf and stalk	0
Color	

Preserved its color at the moment it was silage (brown in withered silage)	2
Slightly changed color (yellow to brown)	1
Completely changed color (reseda green)	0
Quality class according to the physical properties of silage	
Quality class	DLG score
Very good	20-18
Good	17-14
Medium	13-10
Low (low value)	9-5
Corrupted (useless)	4-0

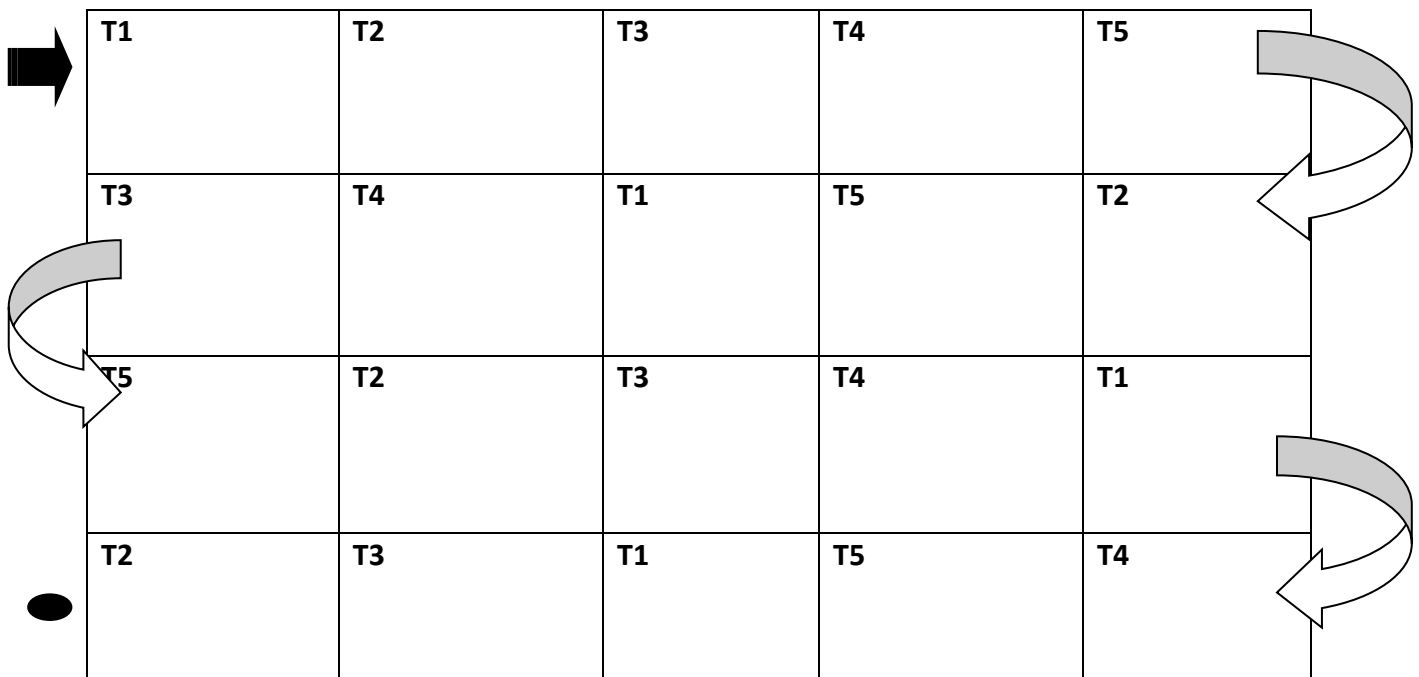
Appendices 3 Description of physical qualities and scale (1-4) for evaluating silage quality

Rating Scores	Smell	Color	Texture	Moldiness	pH
1. (Bad)	Rancid and musty smell/pungent	Dark/deep brown	Dark/deep brown	Putrefactive and agglutinative	Highly moldy
2. (Moderate)	Irritative/offensive; alcohol, acidic	Brown (Medium)	Slightly viscous/slimy	Medium	4.4–5.0
3. (Good)	Light acidic (pleasant)	Brown, yellow	Medium (loose, soft, and firm)	Slightly moldy	4.1–4.3
4. (Excellent)	Pleasant and sweet-acidic (very pleasant)	Light/greenish yellow/Olive green	Loose and soft, Firm	Without mold	≤4.0

Source: (Stanley *et al.*, 2004; Lima *et al.*, 2011)

Appendices 4 . Treatments and design

- T1** 100% sole oat
- T2** 100% sole alfalfa of (magna 788)
- T3** 75% oat + 25% Alfalfa (magna 788)
- T4** 50% Oat + 50% Alfalfa (magna 788)
- T5** 25% Oat + 75% Alfalfa (magna 788)



Plot size = 4x4 m

Space b/n plot and replication = 1m

Space b/n rows = 30 cm

No of treatment =5

No of replication = 4

Total no of plot = 20

oat variety = Bate

alfalfa variety = magna 788

Total area = 20 m width x 25 m length

Seed preparation during experiment

Oats (Bate variety)

Sole oats = 115 g / plot

75% oats = 86.25 g/plot

50% oats = 57.5 g/plot

25% oats = 28.75 g/plot

Calculated from 80 kg/ha

alfalfa (Magna 788)

sole alfalfa = 21.6 g / plot

75% alfalfa = 16.2 g/ plot

50% alfalfa = 10.8 g/ plot

25% alfalfa = 5.4 g / plot

calculated from 15 kg/ha

Appendices 5. Trial images from land preparation, planning, forage management, harvesting to laboratory analysis



Land layout, leveling, sowing, and Forage management (watering and weeding)



Forage harvesting and all important agronomic data have been collected.



Sample preparation (drying and grinding)



Silage preparation (harvesting, tilting, chopping, mixing, packing, storing, and recording)



Soil sample collection.



DM Determination (DM% were determined for 24hrs at 105 °C both for forage and silage)



Ash determination both for forage and silage at 500 °C for 3hrs.



Characterization of silage after 45 days maturation (pH, temperature, Physical Properties (smell, color, moldiness and texture) were evaluated by six experts).