

# Crop growth and forage quality of perennial grass and clover as sole and mixed crop

## Abstract

Field experiment was conducted at Agronomy Farm University of Agricultural Peshawar during summer 2013 in Randomized Complete Block Design. Crop growth rate of clover and grass planted as sole and mixed was studied. Grass showed significant increase in growth 2.22 (g m<sup>2</sup> d<sup>1</sup>). Crude protein was recorded maximum in grass when planted as sole crop (28.47 %). Mixture of alfalfa and grass (40:60) was the highest in crude fiber (31.48 %). *In Vitro* Dry Matter Digestibility (IVDMD) of alfalfa and grass as mixed crop (20:80) showed highest digestibility (33.61 %). It was concluded in light of results obtained that combination of clover and grass with a ratio of (40:60) have better results among the other mixture combination.

**Keywords:** Alfalfa (*Medicago sativa*. L), IVDMD, Crude Protein, Crude Fibre, Dry Matter

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## Introduction

Alfalfa, *Medicago sativa*, also called Lucerne, is a perennial flowering plant in the pea family Fabaceae cultivated as an important forage crop in many countries around the world. Alfalfa is a small-seeded crop, and has a slowly growing seedling, but after several months of establishment, forms a tough 'crown' at the top of the root system. This crown contains many shoot buds that enables alfalfa to regrow many times after being grazed or harvested. This plant exhibits auto toxicity, which means it is difficult for alfalfa seed to grow in existing stands of alfalfa. Therefore, alfalfa fields are recommended to be rotated with other species (for example, corn or wheat) before reseeded. Its primary use is as feed for high-producing dairy cows, because of its high protein content and highly digestible fiber, and secondarily for beef cattle, horses, sheep, and goats other legumes, its root nodules contain bacteria, *Sinorhizobium meliloti*, with the ability to fix nitrogen. Alfalfa can be sown in spring or fall, and does best on well-drained soils with a neutral pH of 6.8 – 7.5. Alfalfa requires sustained levels of potassium producing a high-protein feed phosphorus to grow well. It is moderately sensitive to salt levels in both the soil and irrigation water. Alfalfa is the most cultivated forage legume in the world. A fair assessment regarding the quality of forage grass originating from meadows require an overall analysis on the data regarding the botanical composition of pasture the nutrients and mineral content and digestibility of fodder produced.<sup>1</sup> Worldwide production was around 436 million tons in 2006. In 2009, alfalfa was grown on approximately 30 million hectares (74,000,000 acres) worldwide; of this North America produced 41% (11.9 million hectares; 29,000,000 acres), Europe produced 25% (7.12 million hectares; 17,600,000 acres), South America produced 23% (7 million hectares; 17,000,000 acres), Asia produced 8% (2.23 million hectares; 5,500,000 acres), and Africa and Oceania produced the remainder. *Setaria (splinda setaria)* is a summer-growing perennial grass suited to the moist subtropics of the Northern Rivers, Mid Coast and Manning districts of New South Wales. It performs best on coastal lowlands receiving more than 1000 mm average annual rainfall. *Setaria* is widely grown for grazing by dairy and beef cattle, but high oxalate levels make it undesirable for horses and donkeys. *Setaria*, (*splinda setaria*) are very palatable and widely used for beef and dairy. Perennial with vigorous growth habit – very palatable to livestock but may contain oxalate at certain stage of growth, thus affecting grazing. It combines well with legumes such as Glycine and alfalfa has been

described as the most cold tolerant tropical grass can withstand 3°C below freezing point. It is best to choose a suitable sowing time for any associated legume.

## Materials and methods

The present study was conducted on perennial clover and grass at Agronomy Farm, University of Agriculture Peshawar during summer 2013. Aim of this study was to investigate growth and re-growth potential and qualitative of the perennial clover (*Medicago sativa* L.) and grass (*splinda setaria* L.) species as sole and mix in different proportions.<sup>2</sup> The qualitative study was carried out at the Animal Nutrition laboratory, the University of Agriculture Peshawar. The experiment was conducted in already established field, planted sole and mixtures of different proportion under randomized complete block design (RCBD). Both clover and grass samples were harvested at two different location at about 10 days interval for growth and re-growth in the month of July, September and October, 2013. Crop growth rate (CGR) was determined by simple regression analysis between the dry matter and days after the cut and the slope of the regression line was assumed as CGR for grass and clovers in growth and re-growth, respectively. The herbage sample was dried in oven at 60°C for not less than 48 h and/or until the constant weight obtained. The dried samples were ground separately in bags and immediately ground in fine powdered at 1.00 mm (Thomas Scientific Grinder Mill USA). After drying and grinding, the ground samples of both species (clover and grass) were mixed in proportion (w/w) as per following details as treatments:

From the mixtures combinations, the following qualitative parameters were studied in the animal nutrition lab at University of Agriculture.

## Dry matter

For the estimation of dry matter (DM), about 2g samples were taken in clean and pre-weighed crucibles in duplicate. The crucibles were then placed in laboratory oven for 18h at 100°C. After drying in an oven the samples were cooled in a desiccator for 30 minutes and reweighed. The DM% was determined by using the following formula.

$$DM (\%) = \frac{C - A \times 100}{B - A}$$

A = weight of empty crucible

B = weight of crucible + sample (pre drying)

C = weight of crucible + sample (post drying).

### Crude protein (CP)

Crude Protein in the representative sample of feed and was determined with Kjeldhal Method (AOAC, 1990). In this method samples were digested with concentrated Sulphuric acid ( $H_2SO_4$ ) and were followed by distillation and titration. Samples (about 0.3gm) were taken in the Tecator digestion tubes and were added with 5 gm of catalyst (Potassium Sulphate 93%, Copper Sulphate 7%) and 10 ml concentrated sulfuric acid. Acetanilide (0.1 gm) was processed as standard for recovery of nitrogen. The digestion tubes were heated in Tecator digestion block. The tubes were then allowed to cool at room temperature. About 15 ml distill water was added with the tubes containing digested samples. After dispensing required amount of sodium hydroxide (NaOH) solution (40% W/V) in the tubes to alkaline the sample and the contents were distilled for about seven minutes. The resulting ammonia was collected in conical flask containing 10 ml boric acid (2%) and 3-4 drops of methylene red indicator.

The titration of distillate was carried out with 0.5N sulfuric acid solutions. To determine the blank values tubes were taken containing 15ml distilled water and 5ml NaOH were also processed for distillation and titration. The percentage of crude protein was calculated as under:

$$(CP \%) = \frac{(V1 - V2) * 20 * 0.0104 * 0.014 * 100}{(Sample \text{ in } g)}$$

V1= Titration reading of sample

V2= Titration reading of blank sample

### Crude fiber (CF)

It is the organic residues that remain when a moisture free sample is digested first with weak acid solution ( $H_2SO_4$ ) and then with a weak alkaline solution (NaOH). The residues collected after digestion is ignited and the loss in weight on burning is registered as crude fiber. Moisture free sample (1-2gm) was taken in a tall from beaker. Two hundred ml boiling dilute  $H_2SO_4$  was added and was digested for 30 minutes on crude fiber extraction apparatus. Then was filtered through glass buchner funnel with an aid of suction air pump. Then was washed with hot water until it became acid free (15ml filtrate is collected and 1 drop N/10 NaOH and 1 drop Phenolphthalein indicator is added. Pink color is an indicator of being acid free).<sup>3-5</sup> Transferred again to tall beaker and 200ml boiling dilute NaOH was added. Then was filtered through glass buchner funnel with an aid of suction air pump. Then was washed with 10ml hot dilute  $H_2SO_4$  and then with hot water until it became acid free. It was transferred to a prepared gooch crucible, and then with 10ml ethanol crucible was washed. Then sample was dried it in an oven at 135°C for 2 hours. Then were cooled in dessicator for 30 minutes and weighed. Samples were further ignited in muffle furnace at 600°C for 30 minutes. Ignition residues were cooled in dessicator for 1 hour and reweighed. The percentage of CF was calculated as under:

$$\%CF (sample) = \frac{(crucible \text{ wt.} + \text{dried residue}) - (crucible \text{ wt.} + \text{ash residue}) \times 100}{(Crucible \text{ wt.} + \text{sample}) - \text{empty crucible weight}}$$

### In Vitro dry matter digestibility

**Principle:** This method has two stages. In the first stage a small quantity 0.5 mg (0 mm size). Simple of dried forage is digested an aerobically with rumen microorganisms at 37.9 c. then in the second stage the residues are again digested with acid (HCL) and pepsin to convert the protein into water soluble products. The dried residues weighed and then calculate the digestibility.

### Equipment

- 1) Centrifuge Tubes 60-80ml
- 2) Rubber Stoppers equipped with Bunsen Valve
- 3) Centrifuge Machine
- 4) Water Bath
- 5) Magnetic stirrer with heater
- 6) Incubator
- 7) CO<sub>2</sub> gas cylinder
- 8) Hot air lab oven
- 9) Solution dispenser
- 10) Standard simple with known digestibility
- 11) Electric Balance
- 12) cooler filled with hot water
- 13) P<sup>H</sup> meter or P<sup>H</sup> paper

### Reagents

1. CO<sub>2</sub> Gas
2. Rumen liquor
3. Buffer solution artificial saliva Composition for 1liter

Di sodium hydrogen phosphate	Na <sub>2</sub> HPO <sub>4</sub> 2H <sub>2</sub> O	5.794gm
Sodium hydrogen Carbonate	Na HCO <sub>3</sub>	9.810gm
Sodium Chloride	NaCl	0.479gm
Potassium Chloride	KCl	0.577gm
Calcium chloride	CaCl <sub>2</sub> 2H <sub>2</sub> O <sub>2</sub>	0.060gm
Magnesium Chloride	MgCl <sub>2</sub> 6 H <sub>2</sub> O	0.132gm

**Procedure:** Duplicate simples of oven dried finely grounded 1 mm particle size feed 0.5gm were weighed into pre weighted 60ml plastic centrifuge tubes. These tubes were stored at 38°C in the incubator until required. About 10 ml of the buffer solution was added to each tube 12 hours before starting the experiments. Along with the simple took two blank simple also in which we only added the rumen liquor. After 12 hours added the remaining 20ml of the buffer solution to each tube followed by 10 ml of the strained rumen liquor. Each tube contains 40ml of the solution. CO<sub>2</sub> flashed out thoroughly in each tube in order to create the anaerobic condition in the tubes and the tubes was sealed with a rubber cork with a Bunsen valve or gas released valve. 4mm slit in the plastic tubes on the valve was cut with the help of knife to release the gas fermentation from inside the tubes. After sealing the tube were incubated at 38 c for 48 hr being shaking twice a day. During the incubation the P<sup>H</sup> should be maintained within the limit 6.7-6.9. After 48 hrs centrifuge the tubes for 5-7minutes at 3000rpm. Thus discarded the supernatant and the residue remained in the tubes were dried in the oven at 60c.

## Results and discussion

Crop growth rate (CGR) was recorded in growth and re growth the same for alfalfa but different for the grass. There was significant increase in the grass re growth as compared to clover. The qualitative parameters of alfalfa and grass as sole crop and mixture was almost the same for dry matter, crude protein, and crude fibre (Table 1). Crude protein of grass sole was maximum (28.47%) than alfalfa and grass mixture (40:60) was (18.86%). Crude fiber of alfalfa and grass mixture (40:60) was maximum (31.48%) as compared to any other mixtures of sole crops among the treatments. IVDMD of mixtures of alfalfa and grass (20:80) showed the highest 33.61% as compared to any other mixtures of sole crops among the treatments. Sole grass showed the poor IVDMD than any other species i.e. clover or its combination with grass in different ratios (Table 2).

**Table 1** Crop growth rate after re-growth in summer of the perennial clovers and grasses in two consecutive cuts

Crop species	First growth CGR (g m <sup>-2</sup> d <sup>-1</sup> )	Re-growth CGR (g m <sup>-2</sup> d <sup>-1</sup> )
Clovers (Alfalfa)	1.16 a	1.24 a
Grass ( <i>Splinda sitaria</i> )	0.69 b	2.22 a

**Table 2** Qualitative parameters of the perennial grass-clovers mixtures (w/w) in different ratio as forage

Grass-clovers combinations (w/w)	DM %	CP %	CF %	IVDMD %
Alfalfa sole crop (00:100)	91	25	29.44	10.81
Grass sole crop (100:00)	91.24	28.47	28.47	18.41
Alfalfa: Grass (80:20)	90.9	20.06	29.94	14.54
Alfalfa: Grass (60:40)	91.42	21.57	28.97	14.74
Alfalfa: Grass (60:40)	91.92	18.86	31.48	19.64
Alfalfa: Grass (20:80)	91.82	22.27	28.44	33.61

## Conclusion and recommendation

Grass showed a strong variation in growth and re growth in CGR while clover showed a consistency in the cuts in September to October. Combination of mixtures of grass clover in a proportion of 20:80 (w/w) is the best combination for the highest digestibility with almost similar protein, fibre, and dry matter.

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## Conflicts of interest

The authors declared there is no conflict of interest.

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