SECTION - III
MATERIALS AND METHODS
MATERIALS AND METHODS

After giving a brief account of the study area, it is equally important to throw light on the materials taken and methods used in the present investigation. For a better understanding these are discussed below for each observation separately in detail.

FENCING OF STUDY SITE

The fencing of the study site in a region like bundelkhand was very painstaking because of high grazing pressure.

Although the Bohadpura Sheep Farm was already fenced but the research site was further fenced by barbed wire alongwith barriers made up of dried Lantana bush to avoid all types of biotic interferences.
CLIMATIC PARAMETERS

To describe the climatic conditions of study site during study period the meteorological data i.e. temperature, humidity, wind velocity and rainfall of Orai was collected from the meteorological observatory, civil aerodrome, Lucknow.

The solar radiation data have been obtained from meteorological observatory, Pune.

SOIL ANALYSIS

Before analysis, each soil sample taken from the study site was air dried, powdered and passed through 2 mm sieve and then taken into the soil testing laboratory, Department of Agriculture, Government of Uttar Pradesh and research laboratory of D.V. Postgraduate College, Orai.
As per methods given by Piper (1944) and Jackson (1958) are used to analyse the edaphic parameters which are given below separately.

Physical Properties of Soil:

- Soil Texture

About 5 g soil was slowly moistened to make a paste which form the basis to determine the texture type by field touch method. It is confirmed by mechanical analysis based on proportion of different sized particles.

- Soil Colour

Soil colour was obtained by taking the dry powdered soil on a white paper and comparison with Munsell's colour chart.
-Soil Moisture

To estimate the soil moisture, the fresh soil of different depths were collected on each harvesting period from the study site and brought to the laboratory in the polythene tied up properly by thread. About 100g fresh soil was put in the petridish. There after the petridish along with fresh soil was weighed and placed into the oven at the temperature 105°C for 24 hours. Next day the petridish was taken out from the oven, cooled and reweighed. The difference between two weights was the moisture percentage which was calculated by the following formula:

\[
\text{Moisture (\%)} = \frac{\text{Loss of weight on drying}}{\text{Dry weight of Soil}} \times 100
\]

-Soil Porosity

Scrapped the soil surface to a flat plain. A straight walled pit (10 X 10 X 10 cm) was dug out and so the soil removed
was collected in a polythene. The soil sample was oven dried at 105°C and weighed. To find out the volume of pit, the measuring cylinder was filled by sand with the help of a funnel. The pit in turn filled completely by dropping sand from the measuring cylinder through the same funnel. The volume of the sand needed to fill the pit was noted. Porosity of the soil was calculated as under:

\[
\text{Percent pore space} = 2.6 - \frac{D}{2.6} \times 100
\]

where,

\[
D = \frac{\text{Volume of soil}}{\text{weight of soil}} = \frac{\text{Oven dry weight of soil}}{\text{Volume of the pit}}
\]

\[
2.6 = \text{approximate specific gravity of soil particle.}
\]
-Water Holding Capacity

To obtain water holding capacity of the soil, the Keen-Raczkowki's boxes (Piper, 1944) were used. The boxes were circular and made up of brass with numerous perforations in the bottom. An average amount of the water retained by one circular Whatman's filter paper No.1 has been estimated by weighing 5 moistened filter papers and the sum weight of those filter papers divided by 5. The circular filter paper was placed in each box. The empty boxes with moistened filter paper were weighed. The soil was levelled to boxes with the help of glass slide. Then the boxes were placed on the stand and water was filled in the space, made for the purpose on the stand, till the saturation of the soil. The boxes with saturated soil were kept for over-night. Next day weights of boxes with moisture saturated soil was taken and placed into the oven under 105°C for 24 hours. Oven dried soil was reweighed. To
calculate the water holding capacity, the values were substituted in the formula given below:

\[
\text{W.H.C.} = \frac{b - c - d}{c - a} \times 100
\]

Where,

\( a = \) weight of unfilled brass box + filter paper

\( b = \) weight of box + water saturated soil (over-night)

\( c = \) weight of box + oven dried soil

\( d = \) Average amount of water retained by one filter paper.
Chemical Properties of Soil

- Soil pH

Elico pH meter composed of glass electrode and reference electrode was used in the estimation of pH of the soil. Before the use of the apparatus, the glass electrode was activated by putting it into N/10 HCl solution for 24 hours. The reference electrode was filled with saturated KCl solution. The pH meter was allowed to warm up by switched on, sometimes earlier of its use. The accuracy and sensitivity of pH meter was checked by using solutions of known pH made by dissolving the buffer tablets of different pH values (standardisation).

The 20 g of soil was dissolved in 40ml of flat bottom flask. It is stirred well with glass rod and thereafter, electrode assembly dipped in it. The needle of pH meter indicated the pH value. The electrode assembly was carefully cleaned with soft paper or towel before measuring the next sample.
- Soil Organic Matter

Organic matter was determined by Walklay's and black's rapid titration method (Piper, 1944). One gram soil powder was taken into 500 ml Erlenmeyer flask and 10 ml normal potassium dichromate solution was added in flask. 20 ml conc. $\text{H}_2\text{SO}_4$ was poured rapidly directing the stream into solution. The solution in the flask was immediately swirled vigorously for 1 minute and the flask was put on asbestos sheet for 30 minutes. Afterwards 200 ml of water, 10 ml of phosphoric acid and 0.5 % solution of diphenyle amine indicator (about 2 ml) was added in the flask. The solution became dark blue. The flask solution was titrated by adding ferrous ammonium sulphate (F.A.S.) drop by drop until the colour flashes to light green. This process was also repeated for blank. The percentage organic carbon was calculated as follows:

$$\% \text{ Organic Carbon} = B - S \times 0.003 \times 100$$
where,

\[ B = \text{F.A.S. for blank} \]

\[ S = \text{F.A.S. for sample of soil} \]

\[ 0.003 = \text{amount of carbon used for 1ml of pot. dichromate} \]

\[
\text{Organic Matter Percentage} = B - S \times 0.003 \times 100 \times 1.724
\]

(Because organic matter consist with 58% carbon)

Or \[ 1.724 = 100 / 58 \]

- **Soil Nitrogen**

Soil nitrogen was determined by micro-Kjeldahl method following Jackson (1958). The digestion of the material
was done prior to the titration in sulphuric acid using CuSO₄, K₂SO₄ and Selenium powder as catalysts. After digestion, the solution was extracted with distilled water and the volume was made up. The extract was distilled with strong NaOH in the 'Markham' distillation unit and the distilled NH₃ was collected in dilute H₂SO₄ mixed with bromcresol green - methyl red indicators. The boric acid was blank titrated with 0.05N HCl and the percentage of nitrogen (N) was calculated, following the formula given by Jackson (1958).

\[
\% N = (T - B) \times N \times \frac{1.4}{5}
\]

where,

\( T = \) Sample titration, ml standard acid

\( B = \) Blank titration, ml standard acid

\( N = \) Normality of standard acid

\( S = \) Sample weight, g
The total nitrogen was determined by the modified Kjeldahl method (A.O.A.C., 1765).

- **Soil Phosphorus (Available)**

An aliquot of the extract solution of the soil was taken in a 50ml volumetric flask and pH was adjusted at 3 using Na₂CO₃ and dinitrophenol as an indicator. In this solution sulphonolybdic and molybdophosphoric acids were added. Blue colour was developed after adding a few drops of chlorostanous acid in the test solution, and within the specific time, transmission was read photometrically at 660nm wave length (Jackson, 1958).

- **C/N Ratio**

The percentage of soil organic carbon and nitrogen already estimated have been used to calculate the C/N ration i.e. the total amount of carbon by nitrogen.
PLANT MATERIALS

Experimental Approach

Field experiment was conducted during 1996-97 on the one year old protected and established grassland dominated by *Iseilema laxum* at Bohadpura Sheep Farm, Orai. The treatments consisting of 5 levels of nitrogen (0, 30, 60, 90 and 120 kg N/ha) and 3 levels of phosphorus (0, 30 and 60 kg P₂O₅/ha) were laid out in randomised block design with three replications. The plot size was 5 X 4 m. Full dose of nitrogen through urea were broadcasted and mixed in the plots soon after the first soaking rains and the remaining half dose of urea was applied after one month of the first application. To obtain dry matter forage production, 4 cuttings i.e. on August 9 and 29, September 20 and October 16 were taken as per growth.

The seeds of *Stylosanthes hamata*, *S. scabra*, *S. guianensis* and *S. humilis* were inter-cropped in the grassland
community at the rate of 5 kg/ha seeds in the first week of July, 1986. In each treatment there was one control i.e. without application of nitrogen, phosphorus and legume indicated as 0 (Zero) treatment.

Harvesting and Drying of Plant Materials

To obtain forage production, above ground clip method i.e. harvest method (Odum, 1960) was adopted laying down 25 X 25 cm quadrat in experimental plots. The forage thus obtained were oven dried at the temperature of 70ºc for over night and calculated in t/ha.

Grinding and Storage

The oven dried plant materials were grinded by electric grinder and sieved through 1 mm size of sieve.

To avoid from moisture the dried and sieved plant materials were stored in polythene tied up properly with thread.
The dried powdered plant materials were used in different analysis i.e. quality (Nutritive value) like protein, fat, fibre and carbohydrate.

**Chemical Analysis of Plants**

The dried and powdered plant materials were used for plant analysis. Under the plant analysis contents of plant viz. Carbohydrate, protein, fibre, fat and ash were estimated.

- **Crude Protein**

0.2g of dry powder of plant material was mixed with 10ml of conc. sulphuric acid and a very small quantity of selenium powder, copper sulphate and potassium sulphate were also added. This mixture was transferred into long neck digesting flask and carefully digested on sand bath till it became colourless. Then 50ml or distilled water was added to dilute it. This colourless product with 40% sodium hydroxide solution was poured in kjeldahl flask.
The flask was heated gently up to boiling by which ammonia was released and absorbed in sulphuric acid (10 ml of 1 N). Sulphuric acid with ammonia was titrated (least count of burette was 0.01) with 1N sodium hydroxide using two drops of Tisharo's indicator (0.08 % of Methyl red w/v and 0.02 of Methylene blue w/v in 100 cc of Ethyl Alcohol). Pink colour of the solution became colourless. Reading of the burette was noted and the difference of this reading and blank titrated solution was calculated by the following formula:

\[
\% \text{ Nitrogen} = \frac{\text{T-B} \times N \times 1.40}{W}
\]

where,

**T** = Titrated reading

**B** = Blank reading

**N** = Normality of sulphuric acid

**W** = Weight of powder of plant material
Thus, nitrogen content was estimated by micro-
kjeldahl method as described earlier (Jackson, 1958). Crude protein
was obtained by multiplying the nitrogen content of the forage by
6.25 as described by William (1970).

- Fat

10g of oven dry powder of plant material was taken in
extraction thimble which was then plugged with cotton. The thimble
was placed in extractor. A clear dry soxhlet flask was carefully
weighed and attached to the bottom of extractor. Sufficient quantity
of petroleum ether (B.P. 40-60°c) was poured in the extractor to
start the siphon. Heating mantle was switched on so that the
petroleum just boiled. Heating was continued for atleast 8 hours
when the extraction was completed.

Fat content of the material was collected in the flask. After the
distillation of ether a soft and fatty material remained, it is
the fat. Flask was removed and dried to a constant weight in oven at
100°C for half an hour. Increase in the weight of flask is the weight of fat.

To obtain percentage of fat the following formula was used.

\[
\% \text{ fat} = \frac{\text{Weight of fat}}{\text{Weight of plant material}} \times 100
\]

-Crude Fibre

The residue obtained from the fat determination was taken out and 2 g of this residue was thoroughly washed. This residue was transferred to 1000ml beaker for determination of crude fibre and 175 ml of distilled water and 25 ml of 10% sulphuric acid (prepared by diluting 54 ml of conc. sulphuric acid to 1 litre) were added to it. A mark was made on the beaker to indicate 200ml level. A round bottom flask of 500 ml placed on the top of beaker containing material was brought to boil and gentle boiling was continued for 30 minutes. After 30 minutes the content of beaker
minutes. After 30 minutes the content of beaker was filtered and residue was washed free from acid (tested with litmus). 25 ml of 10% sodium hydroxide was added, followed by sufficient amount of distilled water to bring the liquids up to 200 ml mark, already made on beaker. The flat bottom flask with water was placed on the top of beaker and again boiled for 30 minutes. The residue was filtered and made alkali free (tested with litmus).

Finally the residue was washed with small quantity of methanol. Methanol treated residue was transferred to a small porcelain crucible and it was dried to a constant weight in an oven at 100°C for 24 hours. Crucible was cooled in a desiccator and weighed. The crucible was placed in muffle furnace at 550°C for 6 hours and then put in desiccator and reweighed. Percentage crude fibre was estimated by following formula:

\[
\% \text{ crude fibre} = \frac{\text{Wt. of digested plant material} - \text{wt. of ash}}{\text{wt. of plant material}}
\]
-Ash

Percentage of ash was determined by the method given by William (1970). The ashing was done by taking 1-2 g of plant material in a porcelain basin and to ignite in the muffle furnace for about 3-4 hours at 450 - 500 °C. The amount of ash was determined by weighing the ignited material. The difference of two weights was the amount of ash. Percentage of ash calculated with the help of following formula;

\[
% \text{ Ash} = \frac{\text{weight of Ash}}{\text{wt. of plant material}} \times 100
\]

-Carbohydrate

It was obtained by getting difference between 100 and the sum of crude protein, fat, fibre and ash as described by William (1970).