

# EVALUATION CRUDE PROTEIN BY MICRO-KJELDHAL METHOD OF SOME PALATABLE GRASSES OF MELGHAT TIGER RESERVE, AMRAVATI, MAHARASHTRA STATE.

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## Abstract

*The study aim to determine the crude protein by Micro-Kjeldhal method of some palatable grass from Melghat Tiger Reserve, Amravati, Maharashtra State. Early blooming stage and Mature stage leaf and stem of 14 species of grasses are selected from Melghat Tiger Reserve Dist. Amravati Maharashtra State was studied.*

*Study carried out by the selection of early blooming stage and mature stage of grasses leaf and stem it's shows variation in the Crude protein content in the early blooming stage and mature stage of grasses leaf and stem. During this study in more of the grasses leaf and stem contained higher amount of crude protein content in the mature stage of grasses leaf and stem than the early blooming stage of grasses leaf stem respectively*

**Key words :** *Wild some palatable grasses, Crude protein value by Micro-Kjeldhal method Melghat Tiger Reserve Maharashtra State..*

## Introduction:

Nitrogen is one of the major element found in living organism. It is an essential constituent of a number of compound such as amino acid, amides, proteins, nucleotides, vitamins, hormones etc. Proteins are the major source of nitrogen. In most protein, nitrogen constitutes nearly 16% of the total composition and hence, the total nitrogen content of the sample is multiplied by 6.25 to calculate protein content. The micro-Kjeldahl method is the suitable method for estimating the total nitrogen of food material, agriculture and clinical samples. Grass is not only difficult to digest, but time-consuming to eat. Many herbivores depend on grass for a majority of their diet, but grass doesn't contain as much protein as meat does. A predator can get enough protein by eating perhaps only a handful of meaty meals a day. However, animals who eat grass must spend most of their day munching away on grass to get enough protein and other nutrients in their diet. Protein is an important component of every cell in the body. Body uses protein to build and repair tissues. Protein also to make enzymes, hormones, and other body chemicals. Protein is an important building block of bones, muscles, cartilage, skin, and blood.

Grasses are very important group of plants not only to human beings but also to animals. The grass family, scientifically known as Poaceae is a 5<sup>th</sup> largest family of flowering plants in the world, coming after Asteraceae, Fabaceae, Orchidaceae and Rubiaceae. Grasses range from tiny inconspicuous herbs less than an inch to the giant bamboos that grow up to 130 feet tall. It is difficult to calculate the exact number of species of family Poaceae; however, according to latest estimate by Tzvelev (1989), Poaceae consists of 10,300 species belonging to 898 genera. In India, it is represented by about 1200 species belonging to 268 genera (Karthikeyan *et al.* 1989; Moulik, 1997). Grasses are widely distributed than any other family of flowering plants and grow from sea level to the highest elevations. Grasses bind the soil and prevent loss of top-soil. About 25% of earth's vegetation is covered by grasslands.

Grasses play important role in man's economic activity and in the composition of natural plant communities. Grasses grow in various habitats and they are terrestrial, aquatic, lithophytic and epiphytic. The grasses show high adaptability with respect to changing environments, ability to coexist with grazing animals and with man.

The grass vegetation broadly divided into two types depending upon their life-span, Ephemeral vegetation consisting mainly of the grasses that complete the life cycle during rainy season or after rainy season. The species like

*Arthraxon lancifolius* Trin., *Arundenella pumila* Hochst. ex A.Rich., *Sporobolus diander* (L.) R.Br., *Digitaria ternata* (A. Rich.) Stapf., are the chief components of farmers category. On the contrary the species like *Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult., *Andropogon pumulus* Roxb., *Chrysopogon fulvus* (Spreng.) Chiov., *Dichanthium caricosum* (L.) A. Camus., *Setaria intermedia* Roem. & Schult., *Pennisetum pedicellatum* Trin. which form the autumn vegetation are either perennial vegetation forming large tufts.

Grasses belongs to family Poaceae. Poaceae is the largest family of the Monocotyledones in Angiosperms. Grasses are classified into two main parts annual and perennials, palatable and non-palatable. Grasses with more moisture content and less silica content in the upper aerial parts like stem, leaves are considered as the palatable grasses. Grasses with low moisture content and high percentage of silica are considered as non-palatable grasses. On the basis of morphological characters grasses are also classified palatable and non-palatable grasses.

The Melghat Tiger Reserve is one of the most important Tiger reserve of Vidarbha region of Maharashtra in, India with 2747 Square Km. area. The Melghat Tiger reserve is divided into five division i) Googamal wildlife division ii) Melghat wildlife iii) Sipna wildlife division and iv) Akot wildlife division and v) Akola wildlife division

The Melghat Tiger Reserve comprises herbivorous animals like Barking deer's, Spotted deers, Sāmbar, Bison, Nilgai and omnivorous like sloth Bear. The dominant grasses are *Diachanthium annulatum* (Forssk.) Stapf., *Diachanthium caricosum* (L.) A. Camus., *Diachanthium pertusum* (L.) Clayton., *Diachanthium tuberculatum* (Hack.) Cope., *Themeda quadrivalvis* (L.) Kuntze., *Themeda triandra* Forssk., *Heteropogon contortus* (L.) Beauv. ex Roem. & Schult., *Chloris virgata* Swartz., *Chloris gyana* Kunth, *Cynodon dactylon* Roem. & Schult., *Eragrostis unioides* (Retz.) Nees ex Steud., *Eragrostis. Viscose* (Retz.) Trin. These grasses shows the association with the wild leguminous plant. The grasslands in Melghat Tiger Reserve are of three types, Taller grasslands, Intermediate grasslands and Smaller grasslands. On the basis of grasses distribution and composition grasslands are of two types Homogenous grasslands and Heterogeneous grasslands. The soil moisture content of the forest determines the palatability of the grasses

## Review of literature

Grass family was recognized by Adanson as early as in 1763 by the name Gramineae which was later on named as Poaceae by Barnhart (1895).

Family Poaceae are represented by about 10,300 species belonging to 898 genera (Tzvelev, 1989).

Alasa M.C., Falola O.O. and Babayemi O. J., (2014). Evaluate nutritive value of *Panicum maximum* Jacq. ensiled with two cultivators of *Lablab purpureus* (L.) Sweet. He observed that *Panicum maximum* Jacq. content Dry matter, Crude protein, Crude fibre, Ether extract and Ash were 46.39%, 9.01%, 33.08%, 8.15%, and 10.01% respectively.

Cooke (1901-1908) provided an account of grasses in 'Flora of the presidency of Bombay'. Gamble (1896) compiled 'the Bombusaceae of British India' and 'Flora of presidency of Madras' in Fischer (1934) provided account of Madras presidency. An illustrated account of grasses of Bombay was published by Blatter and Mac Cann (1935). Achariyar and Madaliyar (1921) published an account of South Indian grasses.

Gawali A., Mayekar A.J., Kumar S., Desai B.G., Dhekale J.S. and Burte R.G., (2017). Nutritional evaluation of Themeda (*Themeda mooneyi*) grass in Konkan Kanyal Goats. Themeda (*Themeda mooneyi*) grass, harvested at mature stage, was fed to 4 Konkan Kanyal male goats for 28 day to assess the nutrient utilization and nutritive value. Themeda grass contained 23.73% dry matter (DM), 91.60% organic matter (OM), 6.83% crude protein (CP), 2.20% ether extract (EE), 8.40% total ash (TA), 4.20% acid insoluble acid (AIA), 48.57% nitrogen free extract (NFE), 72.30% neutral detergent fibre (NDF), 53.47% acid detergent fibre (ADF), 16.35% acid detergent lignin (ADL), 38.40% cellulose, 18.83% hemicellulose, 3.30% lignin, 1.63% tannin, 0.49% Ca and 0.32% P in DM basis.

Kauthale V., Kulkarni S. and Nalawade A., (2017). Nutritional evaluation of selected fodder species from Wardha District of Maharashtra, India. The study was carried out to evaluate the nutritional analysis of some fodder plant species in Wardha district. Fourteen fodder species viz., *Apluda mutica* (L.) Hack., *Sehima sulcatum* (Hack.) A. Camus., *Dichanthium* sp., *Themeda quadrivalvis* (L.) Kuntze., *Spodiopogon rhizophorus* (Steud.) Pilg., *Chrysopogon fulvus* (Spreng) Chiov., *Cleistachne stocksii* Hook. f., *Sehima nervosum* (Rottler) Stapf., *Pennisetum pedicellatum* Trin., *Eulalia fimbriata* (Hack.) Kuntze., *Heteropogon ritchiei* (Hook.f.) Blatt. & McCann., *Cymbopogon martini* (Roxb.) Wats., *Thelepogon elegans* Roem. & Schult. and *Stylosanthes hamata* (L.) Taub.

were analyzed for crude protein, crude fiber, oil/ether extract, ash and silica content. The crude protein content of the investigated fodder species ranged from 2.81% to 10.17%, the crude fiber content from 24.56% to 35.73%, the ether extract from 0.59% to 1.01%, ash content from 8.17% to 11.55% and silica content from 3.87% to 7.47%. Findings of the present analysis indicated that fodder species showed variations in nutrients status before seed maturity stage and local fodder species provided partly required nutrients for indigenous livestock.

Kumar K. and Soni A., (2013). Study the nutrient content of commonly available species of forage in the region of Rajasthan. Common forage species such as *Pennisetum Typholdenum* Pers., *Cenchrus ciliaris* L., *Cenchrus setigerus* Vahl. and *Lasiurus Indicus* Henrard. from Jodhpur district of Rajasthan were analysed for their nutritional constituent. The Crude protein content ranged from 6.5 to 9.0%, Cellulose from 28.6 to 30.8%, Hemicellulose from 28 to 32.5%, Lignin from 6.9 to 7.9%, Crude Fiber from 30.43 to 31.9%, Neutral detergent fiber from 68.8 to 71.3% and Acid detergent fiber from 38.1 to 40.8% on dry matter basis.

Muratkar G. D. and Kokate U. R. (2012), studied the Taxonomy of Palatable and non palatable grasses of Melghat Tiger Reserve, in this field work the exploration of grasses from Melghat Tiger Reserve with reference to the fodder value of the grasses for wild herbivorous animals of the protected areas of the Melghat Tiger Reserve.

### Material and Method:-

**Plant collection:-** Melghat Tiger Reserve possesses a unique position, the forest is of mixed dry deciduous with dominance of teak (*Tectona grandis* L). The annual rainfall varies from 1200 – 1400mm, humidity 67% – 89% and the temperature range varies from 8°C – 39°C and there is various diversity of flora and fauna. Collection of the grasses plant species from selected areas of Melghat Tiger Reserve especially from Gugamal Wildlife division, Melghat wildlife division and Akot wildlife division. In the month of September, October and November by arranging the regular field visits in the protected area.

#### Grasses selected for study:

Sr. No.	Botanical Name	Common Name	Location
1	<i>Apluda mutica</i> L.	Motitura	Gullargaht
2	<i>Chloris barbata</i> Sw.	Gonde	Vairat
3	<i>Chloris virgata</i> Sw.	Gonde	Vairat
4	<i>Cynodon dactylon</i> (L). Pers.	Harali	Gullarghat
5	<i>Diahanthium annulatum</i> (Forssk.) Stapf.	Mothi Marvel	Gullarghat
6	<i>Diachanthium caricosum</i> (L.) A. Camus.	Lahan Marvel	Gullarghat
7	<i>Digitaria bicornis</i> (Lam.) Roem. & Schult.	Rai Gavati	Pili
8	<i>Heteropogon contortus</i> (L.) P.Beauv. ex Roem. & Schult.	Kusal Kali	Pastalai
9	<i>Iseilema laxum</i> Hack.	Moshan	Bori
10	<i>Paspalidium flavedium</i> (Retz.) A. Camus.	Bodilya	Dhargad
11	<i>Setaria pumila</i> (Poir.)Roem. & Schult.	Ran Bajara	Dhargad

12	<i>Spodiopogon rhizophorus</i> Trin.	Pochali	Pastalai
13	<i>Themeda triandra</i> Forssk.	Lahan Gondhal	Bori
14	<i>Themeda quadrivalvis</i> (L.) Kuntze.	Mothi Gondhal	Gullarghat

### Determination of Crude proteins by Micro-Kjeldahl method:

Nitrogen is one of the major element found in living organism. It is an essential constituent of a number of compound such as amino acid, amides, proteins, nucleotides, vitamins, hormones etc. Proteins are the major source of nitrogen. In most protein, nitrogen constitutes nearly 16% of the total composition and hence, the total nitrogen content of the sample is multiplied by 6.25 to calculate protein content. The micro-Kjeldahl method is the suitable method for estimating the total nitrogen of food material, agriculture and clinical samples.

### Principle:

The sample is digested with concentrate sulphuric acid in the presence of a catalyst to convert the nitrogen in protein or any other organic material to ammonium sulphate. By steam distillation of this salt in presence of a strong alkali, ammonia is liberated and collected in boric acid solution as ammonium borate which is estimated against a standard acid by titration. On an average most protein have 16% nitrogen in their composition. In other words, 1mg nitrogen equals 6.25 protein. Thus, by finding out the amount of ammonia formed, from a known amount of sample, one can calculate the amount of protein present.

**Requirements:** Micro-Kjeldahl apparatus, electronic balance, burette, pipette, conical flask, measuring cylinder and distilled water.

**Reagents:** Conc. Sulphuric acid, mercuric oxide, potassium sulphate, 40% sodium hydroxide, 4% boric acid, mixed indicator and 0.02N sulphuric acid or hydrochloric acid.

### Procedure:

1. Weight about 0.5-1g of finely powdered homogenous into a digestion flask.
2. Add 10 to 15ml sulphuric acid 5gm of catalyst mixture to the sample.
3. Add boiling chips/glass beads and digest the sample over digestion (takes approximately 40 min. at 370°C. The time of digestion will vary with regard to the size of the sample, temperature and the mode of digestion).
4. The sample turns light green colour or colorless at end of the digestion process.
5. Cool and add minimum quantity of water along the sides of the flask to dissolve solids and transfer quantitatively to the distillation apparatus with successive rinsing with water.
6. Place a 100ml conical flask containing 20ml 4% boric acid with mixed indicator in such a way that the tip of the condenser dipping inside the solution.
7. Add 40 ml of 40% sodium hydroxide solution distillation apparatus through funnel and rinse with water.
8. Distill and collect the ammonia in boric acid. The colour change from violet to green is an indication of ammonia absorbed (collect at least 15-20ml of distillate).
9. Rinse the tip of the condenser with water and titrate the distilled sample against the standard hydrochloric acid or sulphuric acid (0.02N) until the appearance of original violet colour as the end point.
10. Run a blank digested similarly with an equal volume of water after washing the distillation apparatus by back suction with excess of water.

Calculation

$$N\% = \frac{(\text{ml HCl in sample}) - (\text{ml of HCl in blank}) \times \text{normality of acid} \times 14 \times 100}{\text{Weight of sample (mg)}}$$

$$\text{Crude protein content}\% = N\% \times 6.25$$



**Table 1. Showing Evaluation of Crude protein content by Micro-Kjeldahl method early blooming stage of grasses**

Sr. No.	Botanical Name	Crude Protein	Crude Protein
		in % (Leaf)	in % (Stem)
1	<i>Apluda mutica</i> L.	10.62	5.62
2	<i>Chloris barbata</i> Sw.	6.87	6.25
3	<i>Chloris virgata</i> Sw.	7	6
4	<i>Cynodon dactylon</i> (L). Pers.	10.25	6.25
5	<i>Diahanthium annulatum</i> (Forssk.) Stapf.	11.75	9.62
6	<i>Diachanthium caricosum</i> (L.) A. Camus.	11.12	8.5
7	<i>Digitaria bicornis</i> (Lam.) Roem. & Schult.	8.12	6.12
8	<i>Heteropogon contortus</i> (L.) P.Beauv. ex Roem. & Schult.	13.87	12.62
9	<i>Iseilema laxum</i> Hack.	9.5	8
10	<i>Paspalidium flavedium</i> (Retz.) A. Camus.	8.87	8.12
11	<i>Setaria pumila</i> (Poir.)Roem. & Schutt.	9.12	8.62
12	<i>Spodiopogon rhizophorus</i> Trin.	11.37	9.8
13	<i>Themeda triandra</i> Forssk.	8.75	7.75
14	<i>Themeda quadrivalvis</i> (L.) Kuntze.	7.75	9.25

**Table 2. Showing Evaluation of Crude protein content by Micro-Kjeldahl method matured stage of grasses**

Sr. No.	Botanical Name	Crude Protein	Crude Protein
		in % (Leaf)	in % (Stem)
1	<i>Apluda mutica</i> L.	7.3	8.62
2	<i>Chloris barbata</i> Sw.	8.12	5.26
3	<i>Chloris virgata</i> Sw.	10.37	8.12

4	<i>Cynodon dactylon</i> (L). Pers.	7.62	9.5
5	<i>Diahanthium annulatum</i> (Forssk.) Stapf.	10.25	9.12
6	<i>Diachanthium caricosum</i> (L.) A. Camus.	13.5	11.62
7	<i>Digitaria bicornis</i> (Lam.) Roem. & Schult.	10.62	9.87
8	<i>Heteropogon contortus</i> (L.) P.Beauv. ex Roem. & Schult.	9.87	11.25
9	<i>Iseilema laxum</i> Hack.	8.87	8.62
10	<i>Paspalidium flavedium</i> (Retz.) A. Camus.	8.37	8.87
11	<i>Setaria pumila</i> (Poir.)Roem. & Schutt.	13.2	12.25
12	<i>Spodiopogon rhizophorus</i> Trin.	7.87	8.5
13	<i>Themeda triandra</i> Forssk.	10.75	9.5
14	<i>Themeda quadrivalvis</i> (L.) Kuntze.	9.37	7.25

### Result & Discussion:-

Crude protein (CP) content in early blooming stage of grasses leaf are presented in Table 1. *Apluda mutica* L. 7.3%, *Chloris barbata* Sw. 6.87%, *Chloris virgata* Sw. 7%, *Cynodon dactylon* (L). Pers. 10.25%, *Diahanthium annulatum* (Forssk.) Stapf. 11.75%, *Diachanthium caricosum* (L.) A. Camus.11.12%, *Digitaria bicornis* (Lam.) Roem. & Schult. 8.12%, *Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult. 13.87%, *Iseilema laxum* Hack. 9.5%, *Paspalidium flavedium* (Retz.) A. Camus. 8.87%, *Setaria pumila* (Poir.)Roem. & Schutt. 9.12%, *Spodiopogon rhizophorus* Trin. 11.37%, *Themeda triandra* Forssk. 8.75% and *Themeda quadrivalvis* (L.) Kuntze. 7.75%

From the above observation of early blooming stage of grasses leaf it is concluded that more value of crude protein content found in *Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult. 13.87%, *Diahanthium annulatum* (Forssk.) Stapf. 11.75%, *Spodiopogon rhizophorus* Trin. 11.37% and *Diachanthium caricosum* (L.) A. Camus.11.12%.

Crude protein (CP) content in early blooming stage of grasses Stem(Culm) are presented in Table 1. *Apluda mutica* L. 5.62%, *Chloris barbata* Sw. 6.25%, *Chloris virgata* Sw. 6%, *Cynodon dactylon* (L).Pers. 6.25%, *Diahanthium annulatum* (Forssk.) Stapf. 9.62%, *Diachanthium caricosum* (L.) A. Camus. 8.5%, *Digitaria bicornis* (Lam.) Roem. & Schult. 6.12%, *Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult. 12.62%, *Iseilema laxum* Hack. 8%, *Paspalidium flavedium* (Retz.) A. Camus. 8.12%, *Setaria pumila* (Poir.)Roem. & Schutt. 8.62%, *Spodiopogon rhizophorus* Trin. 9.8%, *Themeda triandra* Forssk. 7.75% and *Themeda quadrivalvis* (L.) Kuntze. 9.25%.

From the above observation of early blooming stage of grasses stem it is concluded that more value of crude protein content found in *Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult. 12.62%, *Spodiopogon rhizophorus* Trin. 9.8%, *Diahanthium annulatum* (Forssk.) Stapf. 9.62%, and *Themeda quadrivalvis* (L.) Kuntze. 9.25% respectively.

Crude protein (CP) content in matured stage of grasses leaf are presented in Table 2. *Apluda mutica* L. 7.3%, *Chloris barbata* Sw. 8.12%, *Chloris virgata* Sw. 10.37%, *Cynodon dactylon* (L).Pers. 7.62%, *Diahanthium*

*annulatum* (Forssk.) Stapf. 10.25% *Diachanthium caricosum* (L.) A. Camus. 13.5%, *Digitaria bicornis* (Lam.) Roem. & Schult. 10.62%, *Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult. 9.87%, *Iseilema laxum* Hack. 8.87%, *Paspalidium flavedium* (Retz.) A. Camus. 8.37%, *Setaria pumila* (Poir.)Roem. & Schutt. 13.2%, *Spodiopogon rhizophorus* Trin. 7.87%, *Themeda triandra* Forssk. 10.75%, and *Themeda quadrivalvis* (L.) Kuntze. 9.37%.

From the above observation of matured stage of grasses leaf it is concluded that more value of crude protein content found in *Diachanthium caricosum* (L.) A. Camus. 13.5%, *Setaria pumila* (Poir.)Roem. & Schutt. 13.2%, *Themeda triandra* Forssk. 10.75%, *Digitaria bicornis* (Lam.) Roem. & Schult. 10.62%, *Chloris virgata* Sw. 10.37%, and *Diahcanthium annulatum* (Forssk.) Stapf. 10.25% respectively.

Crude protein (CP) content in matured stage of grasses stem(Culm) are presented in Table 2. *Apluda mutica* L. 8.62%, *Chloris barbata* Sw. 5.26%, *Chloris virgata* Sw. 8.12%, *Cynodon dactylon*(L).Pers.9.5%, *Diahcanthium annulatum* (Forssk.)Stapf. 9.12%, *Diachanthium caricosum* (L.) A. Camus. 11.62%, *Digitaria bicornis* (Lam.) Roem. & Schult. 9.87%, *Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult. 11.25%, *Iseilema laxum* Hack. 8.62%, *Paspalidium flavedium* (Retz.) A. Camus. 8.87%, *Setaria pumila* (Poir.)Roem. & Schutt. 12.25%, *Spodiopogon rhizophorus* Trin. 8.5%, *Themeda triandra* Forssk. 9.5%, and *Themeda quadrivalvis* (L.) Kuntze. 7.25%.

From the above observation of matured stage of grasses stem(Culm) it is concluded that more value of crude protein content found in *Setaria pumila* (Poir.)Roem. & Schutt. 12.25%, *Diachanthium caricosum* (L.) A. Camus. 11.62% and *Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult. 11.25%.

From the observation of Table 1. and Table 2. It concluded that there more % of crude protein content present in Mature stage of grasses leaf and stem than the early blooming stage of grasses leaf and stem.

### Conclusion :-

The study of the Evaluation of Crude protein content by Micro-Kjeldahl method of Palatable grasses from Melghat Tiger Reserve Dist. Amravati, State Maharashtra.. Study carried out by the selection of early blooming stage and mature stage of grasses leaf and stem it's shows variation in the Crude protein content in the early blooming stage and mature stage of grasses leaf and stem. During this study in some grasses leaf and stem contained higher amount of crude protein in the mature stage of grasses leaf and stem than the early blooming stage of grasses leaf stem.

Crude protein play an important component of every cell in the body. Body uses protein to build and repair tissues. Protein also to make enzymes, hormones, and other body chemicals. Protein is an important building block of bones, muscles, cartilage, skin, and blood of herbivores animal of Melghat Tiger Reserve Dist. Amravati State Maharashtra.

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