PROTEIN ESTIMATION OF SELECTED FODDER GRASSES FROM PUNJAB, PAKISTAN

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Abstract

The study was carried out to assess the protein analysis of eight fodder grasses of Punjab, Pakistan to find out which fodder grass possessed high protein value and is more viable as fodder. Among the eight grass species *Phragmites australis* showed highest protein value (22.5 %) followed by *Cynadon dactylon* (20.63 %), *Dactyloctenium aegyptium* (18.75), *Polypogon monspeliensis* (18.12), *Phallaris minor* (16.88), *Cenchrus ciliaris* (13.13), *Setaria viridis* (11.88) and *Bothriochloa multiflora* (11.25 %). Conclusive results of fodder grass protein estimation had given criteria for suitable fodder selection for the variety of cattle. This nutritive information provided by this project can be used on commercial scale for the healthy maintenance of cattle farms. Not only the milk production can be enhanced but also wool and meat industry can be benefited by this knowledge.

Keywords: Fodder grasses, protein estimation, nutrient analysis

Introduction

Fodder crops are significant and economical source of food for animals so in this way it plays a remarkable role in agricultural economy of many developing countries. In Punjab (Pakistan) the total land which has been used for fodder cultivation are about 2.7 million hectares. This produced almost 57 million tons annually, providing average of 21.1 tons per hectare. This cultivated area is approximately about 14-16 % of the total cropped area (Bilal *et al*., 2001). But fodder cultivated areas are extensively reduces by 2 % every 10 years, which cause extensive shortage. Reduction in cultivated areas and minimum yield reduced the supply of fodder about 50-60 % than total requirement (Sarwar *et al*., 2002).

Fodder herb, shrub and trees have always played a significant role in feeding domestic animals. For proper and good growth of their animals dairy farmers spend a lot of money. As about 60 % expenses are only expend on feeding. Different verities of fodder are present which are beneficial for animal health and make dairy farming a profitable business. Fodders are significant and economical source of food for animals and valuable source of nutrients, protein, carbohydrates and metabolic energy. If fodder has good quality, it increases the milk production up to 100 % (Maurice *et al*., 1985). Usually, forage which has high content of protein and other digestible nutrients and low content of lignin and fiber considered as good quality forage. Also, forage quality is also determined by animal performance. Therefore, the profit in dairy farming is mostly depends upon the quality of fodder especially in developing countries (Sarwar *et al*., 2002).

Feed and fodder price comprising about 60-70 % of price of milk production. So cultivation and quality of fodder plays a remarkable role in cost effective production of milk. Feeds provide to livestock not only fulfill their nutrient needs but also fill up the rumen to satisfy the animal. A feed must

meets the needs of animal as well as microbes which lives in rumen, as these microbes promote digestion. Fodder crops give all the essential elements like protein, carbohydrates, fats and minerals. Green fodders excellent source of B-carotene (precursor of vitamin A) (Shah *et al.*, 2011).

Ruminant animals during grazing have to increase forage digestion as this is directly linked to performance, weight and milk production. Fodder's energy and protein content are major factors that control the animal's capability to attain production goals (Koster*et al*., 1996). Different body parts like muscles and blood protein are synthesis with the help of amino acid (protein) present in animal food. In most of countries cattle's diet is primarily based on forage. Nutrients especially protein quality is affected by forage species, soil nutrients and fodder maturity. Forages that grow in winters has more protein content as compare to warm-season forages. But forage maturity and nitrogen fertilizers also affect the concentration of crude protein. During summer protein quantity in fodder become low due to extensive grazing during winter, insufficient nitrogen fertilization especially when forage is in its early stages of growth. Extensive rainfall also decreases the nitrogen level in soil as nitrogen leach out from soil, which ultimately affect the protein production (Parish and Fike, 2005; Patra *et al*., 2011).

Usage of grasses as fodder is another remarkable view and a splendid diversity of herbivores feed on them. They are appraised to be most worthy fodder as they are effortlessly attainable, high nutrient content and various groups of animals are adjustable to consume them. In Pakistan Central Punjab is habitat of many fodder grasses that are rich in nutrients but they are not economically up to the mark (Arshadullah *et al*., 2011).On the other hand for proper maintenance of animals approximately 110.3 million tons of TDN (total digestible nutrients) and 13.5 million tons CP (crude protein) are required (Anon, 2006). While in Pakistan there is only 75 % TDN and 40 % CP provision to the livestock from feed (Younas and Yaqoob, 2005). As this diet is nutrient deficit so the livestock are not healthy and vulnerable to different diseases, pathogens and cause different breeding problems. In Pakistan local farmers are not thoroughly known about dietetic content of fodder. But it is high time that farmers should have knowledge about different variety of fodders especially related to their nutrient composition

Aims and objectives

- To estimate protein content of different grasses from different areas of Punjab (Pakistan).
- To analyze the nutritive proposition of fodder grasses through protein analysis. This makes fodder selection more suitable according to the animal diet needs. Good fodder selection will give healthy livestock nurturing and this will straight lead progress and profitability of our country milk, wool and meat industry.

Materials and Methods

The research work was confined to protein analysis of selected fodder grasses found commonly in Pakistan (Punjab).

Sample collection: Samples of selected fodder grasses were collected from Jallo Park, Changa Manga, KilaRutas and Pabbi Hills.

Chemicals required: Digestion mixture (Copper sulphate 1 g, Potassium Sulphate 9 g, Selenium dioxide 0.02 g), sulphuric acid, distilled water,

Phenolphthalein, 2 % boric acid, 40 % NaOH, $KMnO_4$, N/70 HCl

Protein estimation

a. **Sample digestion:** Kjeldhel method was utilized for protein analysis. In this methoda mixture of 20 mL of sulphuric acid and 0.2 g of digestion mixture were used for digestion of 0.2 g sample. All the process of digestion was done under fume hood at high temperature so that no residue was left in flask and a clear solution was obtained. After digestion the solution was diluted with distilled water up to 100 ml in a cooled bulb of flask, the bulb of heated flask was cooled down after process. This diluted solution was then stored in plastic bottle for further use for protein determination.

b. **Sample titration:** Kjeldhel apparatus was utilized for this purpose. In this method phenolphthalein acts as an indicator and 2-3 drops of it was added into 5 mL of 2 % boric acid and put under the condenser tube. Digested sample (10 mL) was taken in distillation flask. Then conversion of ammonia into ammonium complex took place by addition of 15 mL of 40 % NaOH solution from the top of the distillation flask. Cup was fixed with stopper after washing with distilled water. Then the distillation process was started by heating $KMnO_4$containing round bottom flask. Flask containing boric acid captured nitrogen in the form of ammonium. On the formation of ammonium borate complex pink color of boric acid was disappeared which indicated the completion of reaction. Then solution was titrated against N/70 HCl when solution volume became 25 mL. pink color appeared which was and point. Three concordant readings were taken. Below mentioned formula was used for the estimation of nitrogen and crude protein present in sample.

Calculations for protein determination:

Weight of sample (mg) = Weight of sample (g) × 1000

Titration values = used volume of acid

Titration values/ factor 5 / factor 10 × 100 × 100

Percentage nitrogen = Titration reading after dividing and multiplying / weight of sample (mg)

Percentage protein = Percentage nitrogen × factor 6.25

Factor 10 is the sample taken for distillation

100 ml represents the total volume of sample

The factor is multiplied with N to convert nitrogen into protein and this factor varies from product to product (5.7 – 6.38).

Results and Discussions

The current study was done for protein estimation of fodder grasses. This analysis provide protein basis for fodder selection. By knowing the protein estimation of different grasses good quality of fodder can be produced for livestock with the help of which we can increase the quality as well as yield of different products. Protein estimation can be helpful in production of balanced diet for livestock. With fodder animal can get extra nutrition they need. Hence current research was preliminary focused to estimate either fodder grass has more potential than other fodder trees and shrubs and moreover among these grasses which grass has good protein quality.

Basic human structures such as bone, muscles, hair, skin, milk, organ and other tissues all are made up of proteins. Protein is used for repairing of body tissues, proper growth and milk production. Amino acids are basic units of proteins. These amino acids are used to replace and repair body tissues and

animals get them from digested proteins. If animal diet has enough protein then they can make all the necessary amino acids with the help of rumen microbes. Feed protein content is usually considered a good analytic of quality. Livestock usually get protein by plants, plant protein is the primarily sources of protein. Crude protein is the sum of Protein present in feed and total requirement of cattle. Crude protein content is varies across feeds, but within a feed, quality of feed is directly linked with protein content. This certainly is true in forages.

1. *Cynodondactylon*(L) Pers

Common Name(s)	Arampandrotra, Bahama grass, Australian couch, Balama grass, Bamyudaa, devil grass, devil's grass and couch grass.
Family	Poaceae
Origin	Native to the Mediterranean regions of Europe and now found throughout the world.
Habitat	Rroad sides, gardens, overgrazed, uncultivated lands.
Distribution in Pakistan	Punjab and NWFP.
Distribution in World	Europe, Middle East, North Africa, NorthAustralia.
Protein analysis	20.63 % protein.

2. *Polypogonmonspeliensis*(L.) Desf.

Common Name(s)	Annual beard grass
Family	Gramineae.
Origin	Southern Europe.
Habitat	Annual, herbaceous, found in pools and marshes.
Distribution in Pakistan	Punjab, Sind, N.W.F.P., Baluchistan and Gilgit
Distribution in World	South Africa, India and China.
Protein analysis	18.12 % protein.

3. *Cenchrusciliaris* L.

Common Name(s)	Buffel grass, foxtail buffalo grass and blue buffalo grass
Family	Poaceae.
Origin	Africa, Asia and Europe.
Habitat	Well-drained soils and roadsides.
Distribution in Pakistan	Punjab, Baluchistan, Sind & N.W.F.P.
Distribution in World	Africa, Arabia &Middle East
Protein analysis	13.13 % proteins.

4. *Phalarisminor* Retz.

Common Name(s)	Little seed canary grass and small canary grass,
Family	Poaceae.
Origin	North Africa, South Asia and Europe
Habitat	Waste places.
Distribution in Pakistan	Punjab and Baluchistan,
Distribution in World	All the world
Protein analysis	16.88 % protein.

5. Phragmitesaustralis (Cav.) Trin. exSteud.

Common Name(s)	Common reed and Danube grass
Family	Poaceae.
Origin	North America and Europe
Habitat	Wet areas
Distribution in Pakistan	Punjab & temperate regions.
Distribution in World	All parts of world
Protein analysis	22.5 % protein.

6. *Dactylocteniumaegyptium*(L.)

Common Name(s)	Crowfoot grass
Family	Poaceae.
Origin	America.
Habitat	Marshy lands
Distribution in Pakistan	Sind and Punjab
Distribution in World	Africa and warm regions of old world.
Protein analysis	18.75 % protein.

7. *Bothriochloamultiflora* (L.) A. Camus

Common Name(s)	Indian bluegrass & pitted beard grass,
Family	Poaceae.
Origin	Asia.

Habitat	Alonroadsides.
Distribution in Pakistan	Punjab & Sind
Distribution in World	Kenya, Uganda, Southeast Asia.
Protein analysis	11.25 % protein.

8. *Setariaviridis* (L.) Beauv.

Common Name(s)	Green foxtail
Family	Poaceae.
Origin	Europe
Habitat	Waste places, gardens and fields.
Distribution in Pakistan	Punjab, Baluchistan, Gilgit.
Distribution in World	North America, temperate countries.
Protein analysis	11.88 % protein.

Concluded results had indicated that *Phragmites australis* had highest protein (22.5 %) among all these eight studied fodder grasses however *Bothriochloa pertusa* has lowest protein (11.25 %). In current study estimated protein in *Cynadondactylon* were 20.63 % while in 2011 Patra *et al.* (2011) had reported lower CP value. This difference may be due to climatic conditions affecting the protein quality of fodder. Similarly Kaur *et al*. (2006) had reported 10.9 % proteins in *Phallaris minor* while in this research higher value was determined i.e., 16.88 %. However protein value of *Cenchrus ciliaris* (13.13) lied in range earlier estimated by Ashraf *et al*. (2013). Other species were also showed good protein potential such as in *Polypogonmon speliensis* (18.12), *Dactylocteniuma egyptium* (18.75) *Setaria viridis* (11.88) protein was present.

A protein comparison among fodder grass and other conventional fodder species revealed that grasses usually possess same and even higher protein potential. Such as Temel and Tan (2011) reported the 8 % and 9 % protein in *Arbutus* and *Quercus* respectively. Similarly Shenkute *et al*. (2012) had estimated proteins for different shrubs specie. They worked on *Acacia, Rubu, Veronica, Ocimum* and many more but estimated proteins were ranged from 8.95-20.9 %, however grass fodder protein ranged from 13.13-22.5 %. This signified the more protein value of grass fodder.

Table 1: Protein analysis of *Cynodon dactylon*

No. of Obs	Titration Reading	Reading 1	Reading 2	Reading 3	Mean
1	Volume of acid used	3.5	3.3	3.0	3.3

Calculations for Determination of Protein

Weight of sample (mg)= 0.2×1000 = 200 mg

Titration reading = 3.3 mL

3.3/ 5/ 10× 100 × 100 = 660

% age nitrogen = 660/ 200 = 3.3 %

% age of protein = 3.3× 6.25 = 20.63 %

Table 2: Protein analysis of *Polypogonmon speliensis*

No. of Obs	Titration Reading	Reading 1	Reading 2	Reading 3	Mean
1	Volume of acid used	2.5	2.8	3.4	2.9

Calculations for Determination of Protein

Weight of sample (mg) = 0.2×1000 = 200 mg

Titration reading = 2.9 mL

2.9/ 5/ 10× 100 × 100 = 580

% age nitrogen = 580/ 200 = 2.9 %

% age of protein = 2.9× 6.25 = 18.12 %

Table 3: Protein analysis of *Cenchrus ciliaris*

No. of Obs	Titration Reading	Reading 1	Reading 2	Reading 3	Mean
1	Volume of acid used	2.2	1.8	2.3	2.1

Calculations for Determination of Protein

Weight of sample (mg) = 0.2×1000 = 200 mg

Titration reading = 2.1 mL

2.1/ 5/ 10× 100 × 100 = 420

% age nitrogen = 420/ 200 = 2.1 %

% age of protein = 2.1× 6.25 = 13.13 %

Table 4: Protein analysis of *Phalaris minor*

No. of Obs	Titration Reading	Reading 1	Reading 2	Reading 3	Mean
1	Volume of acid used	2.4	2.6	3.0	2.7

Calculations for Determination of Protein

Weight of sample (mg) = 0.2×1000 = 200 mg

Titration reading = 2.7 mL

2.7/ 5/ 10× 100 × 100 = 540

% age nitrogen = 540/ 200 = 2.7 %

% age of protein = 7.9× 6.25 = 16.88 %

Table 5: Protein analysis of *Phragmites australis*

No. of Obs	Titration Reading	Reading 1	Reading 2	Reading 3	Mean
1	Volume of acid used	3.8	3.5	3.7	3.6

Calculations for Determination of Protein

Weight of sample (mg) = 0.2×1000 = 200 mg

Titration reading = 3.6 mL

3.6/ 5/ 10× 100 × 100 = 720

% age nitrogen = 720/ 200 = 3.6 %

% age of protein = 3.6× 6.25 = 22.5 %

Table 6: Protein analysis of *Dactyloctenium aegyptium*

No. of Obs	Titration Reading	Reading 1	Reading 2	Reading 3	Mean
1	Volume of acid used	2.8	3.0	3.1	3.0

Calculations for Determination of Protein

Weight of sample (mg) = 0.2×1000 = 200 mg

Titration reading = 3.0 mL

3.0/ 5/ 10× 100 × 100 = 600

% age nitrogen = 600/ 200 = 3.0 %

% age of protein = 3.0× 6.25 = 18.75 %

Table 7: Protein analysis of *Bothriochloa pertusa*

No. of Obs	Titration Reading	Reading 1	Reading 2	Reading 3	Mean
1	Volume of acid used	1.8	1.4	2.4	1.8

Calculations for Determination of Protein

Weight of sample (mg) = 0.2×1000 = 200 mg

Titration reading = 1.8 mL

1.8/ 5/ 10× 100 × 100 = 360

% age nitrogen = 360/ 200 = 1.8 %

% age of protein = 1.8× 6.25 = 11.25 %

Table 8: Protein analysis of *Setaria viridis*

No. of Obs	Titration Reading	Reading 1	Reading 2	Reading 3	Mean
1	Volume of acid used	1.5	2.0	2.3	1.9

Calculations for Determination of Protein

Weight of sample (mg) = 0.2×1000 = 200 mg

Titration reading = 1.9 mL

1.9/ 5/ 10× 100 × 100 = 380

% age nitrogen = 380/ 200 = 1.9 %

% age of protein = 1.9× 6.25 = 11.88 %

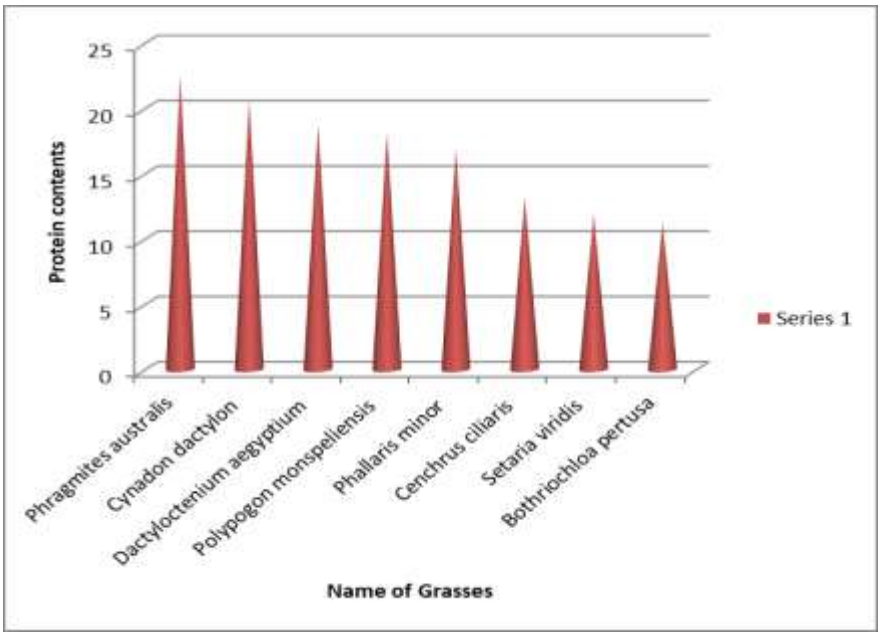

Fig. 01. Protein comparison among studied fodder grasses

Conclusion

This analysis revealed that grass fodder is valuable and in many cases more nutritionally valuable than other conventional fodders. The nutritive information based on protein analysis provided by this project can be used on commercial scale for the healthy maintenance of cattle farms. Not only the milk production can be enhanced but also wool and meat industry can be benefited by this knowledge.

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