

EXTRACTION OF PROTEINS FROM GRASSES**

by

F.H. Shah, M.H. Sedi and B.A. Nadeem¹

Summary. Ten grasses were evaluated of their nutritive value. Percentage of total and extractable proteins was maximum in the first regrowth harvested at one month interval. It then decreased with an increase in age of the crop. Maceration of the grasses with water after pulping increased the extractability of protein upto 183%. The yield of the extractable protein/per hectare/per year was upto 502 kg.

Introduction. Tropical grasses are photosynthetically very efficient and are a great potential for protein extraction (Hutton, 1970; Loomis, et al 1971; Ludlow and Wilson, 1968). However, extractability of protein from grasses was reported to be low due to their high fibre contents and lesser capacity to take up moisture on maceration (Arkcoll, 1971). Arkcoll and Festenstein (1971) obtained 1670 kg/ha of protein from cocksfoot. Protein preparation from young and mature leaves was reported by Arkcoll (1971) and Davies et al (1952). Extractability of protein from leaves was studied by Byers (1961) and from leaves abundantly available in Pakistan by Nazir and Shah (1966, 1968). Gore et al (1974) extracted protein from hybrid Napier Grass.

The present study deals with changes in the proximate composition and extractability of protein from various grasses with seasonal changes and maturity.

Material and Methods. The grasses were propagated in lines of plots measuring 10 x 3 metres. All the grasses, except Napier Millet hybrid, were grown from seeds. The distance between each line being 45 cm. Root stocks or Napier Millet hybrid were planted at a distance of 20 cm.

Seed rates for millet, sudan grass, maize and sorghum were 12, 20, 50 and 50 kg/hectare respectively. The plots were watered soon after sowing and then after every 2 weeks or when needed. Weeding was done at suitable intervals and the use of insecticides, pesticides, fertilizer or manure was totally avoided. Harvesting was done in the morning using steel sickle and the herbage yield was calculated at the spot.

Grasses were analysed for their ash, fibre, lipid and protein contents by A.O.A.C. (1970). For dry matter determination 20-30 gm. of the pulped leaves were dried at 100-110° in an oven for 8-10 hours.

*This research has been financed by the United States Department of Agriculture under PL-480.

+Presented at the Punjab Forest Conference held at Lahore, March, 1975.

¹Pakistan Council of Scientific and Industrial Research Laboratories, Ferozepur Road, Lahore.

Sr.	Species	Source of Seeds or Root Stocks	Date of Growing
1.	Maize (Neelum)	Agriculture Research Farm, Yusufwala, Sahiwal.	26-5-1972
2.	Maize (Syn. 200)	—do—	—do—
3.	Maize (Sy. 545)	—do—	—do—
4.	Millet (B-18)	Agriculture Research Farm, Lyallpur.	7-6-1972
5.	Millet (Ex-Borneu)	—do—	—do—
6.	Millet (Giant)	—do—	—do—
7.	Napier Millet Hybrid	Forest Range Management Nursery, Lahore.	8-3-1972
8.	Napier Millet Hybrid	—do—	8-2-1974
9.	Sorghum (D.G. Pearl).	Agriculture Research Farm, Lyallpur.	29-8-1972
10.	Sorghum (J.S. 263)	—do—	—do—
11.	Sudan Grass (Sorghum)	—do—	12-5-1972

Protein Extraction. Three successive extractions of proteins from grasses were made using IBP Pulper and the belt press by the method of Nazir and Shah (1966). Loss of the juice was minimized by processing 2 kg. of the grass before actually extracting the juice from 5 kg of the crop. Proteinous and non-proteinous N was calculated as Trichloroacetic acid (TCA)-insoluble and TCA-soluble N respectively.

Results and Discussion. *Yield of Crop.* Table I shows that the herbage yield depended on species, harvesting interval, climatic conditions and age of the grasses. Neelum maize gave maximum yield (6.46 tons/hectare) when harvested after 45 days of sowing. The crude protein, lipid and fibre contents were higher in "Neelum" than the other two maize varieties but ash and total extractable N was lesser than the other two. The maximum extractability (47.58%) was observed in case of Maize (Syn. 200). This

appears to be due to higher moisture content (89.41%) of the crop. The yield of millet (B-18) in the first cut, was 10.84 tons/hectare, which decreased to 7.37, 3.40 and 1.57 in the 1st, 2nd and 3rd regrowths harvested at 30 days intervals. Dry matter and lipid contents showed increase in the regrowths, whereas ash, crude fibre and protein contents increased till 2nd regrowth, after which these decreased. A similar pattern was observed in case of millet (Ex-Borneu) and Giant millet.

Out of the two Sorghum varieties J.S. 263 gave higher herbage yield, and higher protein, lipid, ash and fibre contents than D.G. Pearl. Percentage extractability in case of J.S. 263 was also higher because of its higher moisture content (83.75%) than that of the other variety (77.63%). The herbage yield of Sudan Grass was maximum 22.55 tons/ha) in the 1st regrowth of 60 days but % extractability of protein was the minimum (3.00%) as compared with the crops harvested at 30 days interval. The difference in extractability of Napier Millet hybrid from 31.57 to 88.55% appeared to be due to variation in the moisture contents. The herbage yield and lipid contents increased while protein contents decreased with an increase in the age of the crop. Appearance of new leaves in case of Sudan Grass and Napier Millet hybrid resulted in an increase in the protein contents (Table-I) which was observed in 64 days and 81 days old crops. Although an improvement in herbage yield was noticed during monsoon it was species variant as is evident from the Table-I. The yield of Millet (Ex-Borneu) was more in August than that in July, 1972 while the herbage yield of other two varieties decreased in August. The herbage yield of Sudan Grass was maximum in the month of November. Our findings are in agreement with the observations of Vartha et al (1973), who reported changes in herbage yield and extractability by irrigation and with change in season.

Extractability. Table-I shows that the extractability of proteins from Sudan grass and Maize (Syn. 200) was 3.00 and 72.27% respectively. The difference seems to be due to variation in the moisture contents of the plants. The extractability improved with an increase in moisture contents of the plants. Our results agree with the findings of Dev. et al (1974) and Deshmukh et al (1974) that the extractability was more in monsoon (i.e. due to high moisture content) than that of summer. Dev. et.al. (1974) and Gore et al (1974) on their trial on Hybrid Napier grass had noted an improvement in the extractability during monsoon season. Vartha et al (1973) had recorded an increase in extractable protein from lucern by irrigation. It is also evident from Table-I that the extractability is not totally dependent upon the amount of total N. These findings are in agreement with the results of Byers (1961) and Byers and Sturrock (1965).

Extractability Vs Age. The effect of age on the extractability of protein is shown in Table-I. It is evident that with an increase in the age of Napier Millet hybrid, the extractability of both PN and NPN decreased from 36.91 to 16.70% and 23.91 to 14.87% respectively in 30 and 50 days regrowths during 1973 and from 57.56 to 32.73% and 30.99 to 30.29% in 30 and 60 days regrowths during 1974. In case of Sudan grass the decrease in extractability of both PN and NPN was from 5.31 to 0.78% and 5.99 to 2.22% respectively. Byers and Sturrock (1965) made a similar observation while extracting protein from different crops. The decrease in extractability is also due to an increase in D.M. % with

an increase in the age of the crop. These findings agree with the observations of many workers (Deshmukh et al 1974; Dev et al 1974; Nazir and Shah, 1966; Vartha, et al 1973).

Extractability Vs Water Contents. Table II depicts the effect of maceration of the crop with water on the extractability of protein. It is evident that 12.5 to 182.9% more protein could be extracted when moisture content of the grasses increased by the addition of water. In case of Giant Millet the % protein extracted was increased from 59.13 to 94.92%, which was maximum among the grasses reported. However, Oelshlegel et al (1969) had reported that Sur (1967) was successful to extract upto 96% of the protein from the water-hyacinth by lowering the pH of the juice to 3.5 before heating.

Yield of Protein. Yield of crude and extractable protein of 7 grasses is depicted in Table III. The amount of extractable protein varied from 42.7 to 502.9 kg./ha/yr. in Maize (Syn. 545) and Millet (Giant) respectively. The low extractability could be due to the fact that the grasses were propagated without the use of any fertilizer or manure. Gore et al (1974) with liberal application of fertilizers, have reported 2000 kg/ha/yr. of extractable protein from Hybrid Napier grass in India.

Acknowledgement. The authors acknowledge with thanks the Director, P.C.S.I.R. Laboratories, Lahore for providing facilities.

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TABLE I
Proximate Analysis and Extractability of Protein from Grasses

Sr. No.	Name of Species.	Date of Cut.	Age in days.	Yield Tons/ha.	Dry Wt. %	Ash %	Crude Protein % (Nx6.0)	Lipid %	Fibre %	% Extractability. (Total of three extractions)		
										PN	NPN	PN+NPN
1.	Maize (Neelum)	July, 1972	45 old.	6.46	14.55	4.14	10.56	2.59	31.91	25.80	14.42	40.22
2.	Maize (Syn. 200)	July, 1972	45 old.	2.18	10.59	5.54	9.92	2.51	28.74	47.58	25.69	73.27
3.	Maize (Syn. 545)	July, 1972	45 old.	4.29	15.62	6.71	8.39	2.80	28.67	33.60	14.04	47.64
4.	(i) Millet (B-18)	July, 1972	45 old.	10.84	12.74	9.58	9.20	2.21	28.08	38.55	20.26	58.81
	(ii) —do—	Aug, 1972	30 reg.	7.37	14.88	10.04	10.37	2.38	28.64	31.17	13.03	44.20
	(iii) —do—	Sept, 1972	30 reg.	3.40	24.10	10.25	11.58	3.32	26.75	14.67	5.49	20.16
	(iv) —do—	Oct, 1972	30 reg.	1.57	25.09	8.36	10.39	4.87	26.96	35.22	11.47	46.69
5.	(i) Millet (Ex-Borneu)	July, 1972	45 old.	7.83	16.34	9.24	8.75	2.54	27.74	25.04	16.02	41.06
	(ii) —do—	Aug, 1972	30 reg.	8.67	15.33	7.31	9.21	2.18	26.12	26.71	9.64	36.35
	(iii) —do—	Sept, 1972	30 reg.	3.83	14.15	9.47	10.02	3.95	27.00	37.76	10.21	47.97
	(iv) —do—	Oct, 1972	30 reg.	1.17	21.56	9.28	7.72	2.21	20.58	45.65	19.66	65.31
6.	(i) Millet (Giant)	July, 1972	40 old.	21.07	12.25	10.81	11.84	3.14	23.91	32.38	29.09	61.47
	(ii) —do—	Aug, 1972	30 reg.	12.20	16.32	9.48	12.88	1.89	21.19	23.65	10.90	34.55
	(iii) —do—	Sept, 1972	30 reg.	6.30	12.47	9.19	9.23	2.60	27.25	45.58	13.58	59.16
	(iv) —do—	Oct, 1972	30 reg.	3.50	18.50	8.30	10.80	1.57	26.72	41.50	16.11	57.61
7.	Sorghum (D.G. Pearl)	Oct, 1972	55 old.	3.64	22.37	7.16	8.16	3.80	27.66	9.53	3.52	13.05
8.	Sorghum (J.S. 263)	Oct, 1972	50 old.	4.76	16.25	8.55	9.60	4.71	29.36	34.00	19.75	53.75
9.	(i) Sudan Grass (Sorghum)	July, 1972	65 old.	2.94	15.63	8.23	9.08	2.44	28.59	19.69	13.22	32.91
	(ii) —do—	Aug, 1972	30 reg.	6.33	21.78	8.06	7.62	2.25	22.65	5.31	5.99	11.30
	(iii) —do—	Aug, 1972	60 reg.	21.25	26.01	9.46	8.82	2.18	26.47	0.78	2.22	3.00
	(iv) —do—	Sept, 1972	30 reg.	11.06	16.69	8.58	8.34	1.99	24.53	15.40	9.42	24.82
	(v) —do—	Oct, 1972	30 reg.	11.09	12.67	9.91	11.48	2.35	22.36	66.01	20.87	86.88
	(vi) —do—	Nov, 1972	35 reg.	13.55	14.64	9.30	9.36	2.13	23.92	64.44	15.37	79.81
10.	(i) Napier Millet hybrid.	May 1973	80 old.	28.82	14.52	12.76	14.54	2.60	29.92	27.23	14.68	41.91
	(ii) —do—	June 1973	30 reg.	31.03	10.33	16.36	13.20	1.92	29.55	36.91	23.91	60.82
	(iii) —do—	June 1973	50 reg.	44.31	13.98	12.45	11.76	2.41	31.42	16.70	14.87	31.57
	(iv) —do—	Aug, 1973	45 reg.	21.53	9.63	14.52	10.38	2.68	29.55	45.60	19.95	65.55
	(v) —do—	April 1974	60 old.	11.46	17.01	13.04	11.76	3.52	27.94	54.09	30.60	84.69
	(vi) —do—	May 1974	30 reg.	24.73	12.26	14.77	16.26	1.65	25.63	57.65	30.99	88.55
	(vii) —do—	June 1974	45 reg.	28.37	12.36	12.52	11.16	3.42	28.15	45.16	41.34	86.50
	(viii) —do—	June 1974	60 reg.	103.64	20.84	15.10	9.90	3.47	28.28	32.73	30.29	63.02

*Water Added — 1000 ml.

**Water Added — 2000 ml.

TABLE II
The Effect of Moisture Contents on the Extractability and Quality of Proteins.

Sr. No.	Species.	Water added.	1st Extraction		2nd Extraction		3rd Extraction		Total Extraction		+ Increase in Total Extractability	
			PN	NPN	PN	NPN	PN	NPN	PN	NPN	PN	NPN
1.	Millet (B-18)	—	4.40	1.20	5.76	2.12	4.51	1.45	14.67	4.77	19.44	—
		1000 ml.	10.03	4.56	8.50	2.28	5.60	1.45	24.13	8.29	32.42	66.77
2.	Millet (Ex-Borneu)	—	13.83	6.35	13.83	2.63	10.09	3.23	37.75	12.21	49.86	—
		1000 ml.	19.52	7.00	13.11	3.89	9.76	2.75	42.39	13.64	56.03	12.15
3.	Millet (Giant).	—	18.26	6.37	16.57	4.48	10.72	2.73	45.55	13.58	59.13	—
		1000 ml.	33.40	12.86	22.61	12.86	10.00	3.18	66.01	28.90	94.91	60.51
4.	Sorghum (D.G. Pearl).	—	3.59	1.47	3.08	1.25	2.86	0.81	9.53	3.53	13.06	—
		1500 ml.	8.36	7.77	8.21	3.81	6.82	1.98	23.39	13.56	36.95	182.92
5.	Sorghum (J.S. 263).	—	13.88	7.50	12.31	7.31	7.81	4.93	34.00	19.74	53.74	—
		1500 ml.	17.94	12.81	13.88	8.00	8.44	4.25	40.26	25.06	65.32	21.55

TABLE III
Crude and Extractable Protein in Grasses

Sr. No.	Species	Yield Tons/ha.	Total Cuts/year.	Protein % N+6.00	PN % (Extractable)	Crude Protein	Yield of Ext. Protein Kg/ha/yr
1.	Maize (Neelum)	13.0	1	10.6	25.8	181.4	46.8
2.	Maize (Syn. 200)	8.8	1	9.9	47.6	94.8	45.2
3.	Maize (Syn. 545)	10.7	1	8.4	33.6	127.0	42.7
4.	Millet (B-18)	30.0	5	10.4	30.0	622.6	186.8
5.	Millet (Ex-Borneu)	26.0	5	8.9	42.0	335.8	141.0
6.	Millet (Giant)	50.0	5	11.2	55.0	914.3	502.9
7.	Sudan Grass (Sorghum)	55.0	7	8.5	50.0	702.4	351.2