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Nutritional profile and in situ digestion kinetics of some irrigated grasses at pre-bloom stage

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Abstract. The study was aimed to examine the nutritional value, mineral profile, gross energy and in situ digestion kinetics of 10 abundantly available grasses of irrigated areas of Pakistan. Malai grass/Blue Panic, Barru/Johnson grass, Mott grass, Khas Khas grass/Vetiver, Lemon grass, Dhaman grass/Bulled grass, Kallar grass, Rhodes grass, Khabal grass/Bermuda grass and Swank grasses with botanical names Panicum antidotale, Sorghum halepense, Pennisetum purpureum, Vetiveria zizanioides, Cymbopogon citrates, Cenchrus ciliaris, Leptochloa fusca, Chloris gayana, Cynodon dactylon and Panicum colunum were included in the study, respectively. The data collected was analyzed by using analysis of variance technique in a completely randomized design and means were compared by least significant difference (SPSS, 1999). All grasses were cut at pre-bloom stage and were subjected to drying for analysis of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), gross energy and minerals content by using their respective standard methods. For in situ digestion kinetics, data on each parameter (lag time, rate and extent of digestion of DM and NDF) was analyzed according to completely randomized design using the GLM procedure of SAS (1988). The findings revealed highest (14.28%) and lowest (7.90%) CP contents for P. antidotale and P. colunum, respectively. Gross energy content of grasses ranged from 3654 to 2799 Kcal/kg, maximum for P. antidotale and minimum for C. citrates. The C. gayana contained maximum Ca (4 g/kg DM), whereas, minimum Ca (1.6 g/kg DM) was noticed in C. ciliaris. The Mg concentration varied from 0.84 g/kg in L. fusca to 0.264 g/kg in C. ciliaris. Maximum (10.5 g/kg DM) and minimum (2.5 g/kg DM) Na contents were found in V. zizanioides and C. citrates, respectively. Likewise, K concentration varied from 27.2 g/kg to 11.6 g/kg with highest and lowest contents in P. colunum and L. fusca, respectively. Maximum (0.59 g/kg DM) and minimum (0.14 g/kg DM) P concentrations were observed in P. purpureum and C. gayana, respectively. The In Situ digestion kinetics indicated highest (74.2%) and lowest (29.5%) DM digestibility of P. antidotale and V. zizanioides grasses, respectively. The highest (67.96%) NDF digestibility was observed for P. antidotale while the lowest (27.37%) for L. fusca. Nutritional profile, shorter lag time, faster rate of digestion and high extent of digestion reflected these grasses as valuable ruminant feed.

Keywords: irrigates grasses, nutritive value, in situ digestion kinetics

1. Introduction

Grasses (C4) are the plants which contain most extensive, much needed and familiar component of range vegetation especially in extreme climates of the range areas. Grasses are more easily accessible, better in taste and quicker in digestion than shrubs and trees[1]. Ecosystems having rather less extreme climate have typical perennial grasses. These grasses have a quality of quick resprouting because they have special ecological features and moderate grazing. Natural flora of Pakistan contains some very good forage grasses. Need of time is to propagate them through artificial seeding and stump planting [1] . The efficiency of range livestock is based on properly utilization of the range forage for optimum production. Minerals are necessary both for the growth and development of plants as well as for the growth, maintenance and productivity of grazing animals in the range areas. The mineral composition of range plants depends upon various environmental factors such as geographic aspects, climate, soil minerals, grazing stress, seasonal changes and the ability of plant to get minerals from soil [2,3]. It has been concluded that minerals deficiency results in poor animal health, productivity and reproductive faults even if sufficient green fodder is present. It was observed that mineral composition of grasses changed seasonally, especially in dry climate. In productivity of grazing livestock, both the excess and deficiency of minerals are the major constraints [2,3].

Concentrations of mineral elements in forage are dependent upon the interaction of a number of factors including soil, plant species, and stage of maturity, yield, pasture management and climate [4]. Minerals protect and maintain the structural components of the body, organs and tissues, and are constituents of body fluids and tissues as electrolytes. Minerals also catalyze several enzymatic processes and hormone systems in the animal tissues. Energy feeding systems for dairy cows [5] were developed from calorimetric data. In practice, these systems are applied directly to lactating dairy cows. An activity allowance is added to the maintenance energy requirement in some systems to account for normal voluntary activity of cows that would be housed in free-stall systems [5]. However, some calorimetric studies had reported that the maintenance energy requirement recommended in these systems was lower than that for current dairy cows [6].

In situ technique has shown great potential for the evaluation and estimation of the nutrients of different types of feedstuffs in ruminants. This technique is simple, economical, rapid and reproductive. So this is widely used to examine the rate and extent of utilization of feeds and forages. The results of in vivo procedure and in situ procedure are quite comparable. So in situ technique being more advantageous is in common use for the evaluation of forages [7,8]. Digestion kinetics (lag time, rate and extent of disappearance) are helpful in understanding the mechanism of availability of nutrients from the feed taken by the animals. It helps us to understand the problems in fiber digestion. Also it gives us a slim idea to develop feeding regimes for animals to get optimum production [9]. To improve forage intake, the rate of removal from the rumen must be increased by increasing the rate of digestion, the rate of passage, or both [10]. Fermentation rate of feeds depends upon morphology and maturity of species and the sources of feedstuff [11].

As there is little information on the nutritive value of irrigated grasses (C4), so the objectives of the present study were

1. To explore the nutritional potential of 10 experimental grasses
2. To measure their digestibilities by in situ technique

2. Materials and Methods

2.1. Sample procurement and processing

Ten different types of cultivated grasses (C_4 type) of family Poacea were harvested from the ground level at the pre-bloom stage. The grass samples were dried at 55^{o}C and then each was ground at 2 mm size. The local and botanical names of experimental grasses are presented in table 1.

Table 1: Botanical names of different experimental grasses

No.	Local Names	Botanical Names
1	Malai grass/Blue Panic	*Panicum antidotale*
2	Barru/Johnson grass	*Sorghum halepense*
3	Mott grass	*Pennisetum purpureum*
4	Khas Khas grass/Vetiver	*Vetiveria zizanioides*
5	Lemon grass	*Cymbopogon citrates*
6	Dhaman grass/Bulled grass	*Cenchrus ciliaris*
7	Kallar grass	*Leptochloa fusca*
8	Rhodes grass	*Chloris gayana*
9	Khabal grass/Bermuda grass	*Cynodon dactylon*
10	Swank grass	*Panicum colunum*

***In situ* digestion trail**

Feeding regimen of bull

A mature ruminally cannulated buffalo bull was used to study *in situ* digestion kinetics of grasses. The bull was fed a blend of sorghum fodder supplemented with concentrate to meet the nutritional requirements. The *in situ* digestion trial was 45 days long. Initial 15 days were adjustment phase, which helped in proper microbial development in the rumen and the following 30 days were meant for the *in situ* incubation in the rumen.

Digestion kinetics of these grasses were measured by *in situ* technique. Nylon bags having size of 10x25 cm with an average pore size 60 µm were used for determination of rate, lag and extent of disappearances of dry matter (DM) and neutral detergent fiber (NDF) from the grasses samples in the bags. The size of grasses particles was kept 2 mm by Wiley mill. Each bag contained 5 g of sample. These bags were placed in the rumen in a reverse sequence and all bags were removed at the same time to reduce variation associated with the washing procedure. For each grass, 3 bags were inserted. Fermentation time for each bag was 0, 1, 2, 4, 6, 12, 24, 36, 48 and 72 h. After removing these bags from the rumen, the bags were washed by running tap water. These bags were then dried in forced air oven at 60^{o}C till constant weight. The dried residues had been transferred into bottles and stored for analysis. Digestion coefficients of DM and NDF had been determined.

2.2. Estimation of digestibility, lag time, rate and extent of disappearance

Rate of disappearance of DM and NDF were determined by subtracting the indigestible residue i.e. the 72 hours residue from the amount in the bag at each point and then regressing the natural log (Ln) of that value against time. Then lag time and extent of digestion of DM and NDF were determined. Digestibility of DM and NDF was measured at 48 h.

$$\text{Lag time (h)} = (\text{Ln } 100) - \text{Intercept} / \text{Rate of digestion}$$

Extent of DM and NDF disappearance was determined at 72 hours of incubation. The lag time is the initial phase during which either no digestions occur or it occurs at a greatly reduced rate. Lag time was determined by the procedure as described by Sarwar *et al*. [9].

2.3. Chemical analysis

Proximate analysis of the grasses was done according to AOAC [12]. The DM was calculated by keeping the samples in forced air oven till constant weight. The crude protein (CP) was measured by Kjeldhal apparatus. Ash was calculated by burning the samples in furnace while organic matter (OM) was calculated by difference. The NDF, acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by the method as described by Van Soest *et al*. [12]. The Ca and Mg were determined by titration method. The Na and K were measured by Flame photometer. The P was estimated by atomic absorption spectrophotometery. Gross energy (GE) of grasses was measured by using bomb calorimeter [13].

Statistical analysis

The data collected was analyzed by using analysis of variance technique (ANOVA) in a completely randomized design and means were compared by least significant difference [14].

3. Results and Discussion

3.1. Chemical composition

The chemical composition of experimental grasses is presented in Table 2-a and 2-b. The dry matter (DM) contents varied from 11.38% for C. gayana to 38.42% for V. zizanioides. The organic matter (OM) contents ranged from 85.01 for P. antidotale to 90.16% for C. ciliaris. Similarly, Sultan et al. [15] analyzed marginal land grasses and reported that DM content varied from 23.8 to 36.9% at early bloom stage, whereas, at mature stage, it varied from 34.7 to 43.9%. Whole plant of Cenchrus ciliaris (Dhaman grass) and Panicum antidotale (Malai/Blue panic grass), showed that they contained OM 90.72 and 91 % respectively (Mushtaque, 2004). According to Rahim et al., [16] the DM content in free grazing rangeland grasses at early bloom varied from 23.8% (Desmostachya bipinnata) to 36.9% (Andropogon squarrosus).

Highest (14.28%) crude protein (CP) was observed for P. antidotale and lowest (7.90%) for P. colunum. The highest value is contrary to the study of Ramirez et al. (2004) who evaluated different grasses and found highest value of CP as 12% and lowest value as 6%. The highest value also contradicts with that of Sultan (2007) who found that the CP at early bloom stage of marginal land grasses varied from 6.2% (Andropogon squarrosus.) to 11.4% (Cynodon dactylon). The value of CP differs either due to age of plant or maybe due to seasonal effect. Dittberner and Olson (1983) concluded that B. gracilis (aerial fresh, immature) had CP values of 11% in spring but 6% in winter. Soil fertility can be another cause of change in protein value in the forages. Increasing nitrogen fertilization caused an increase in the protein content from 7.9 to 15.2% in the Brome grass hays and from 12.6 to 20.1% in the reed canary grass hays. At the higher levels of nitrogen fertilization on Brome grass there was also an increase in the GE and EE content while the fiber and nitrogen-free extract decreased .

Maximum neutral detergent fiber (NDF) was found 73.4% in V. zizanioides and minimum 53.1% in P. antidotale. Highest acid detergent fiber (ADF) was 46.3% and lowest ADF value was 17.5% for C. ciliaris and P. purpureum, respectively. Bourquin et al., [17] reported 72.4% NDF and 43.8% ADF in the Orchard grass. The difference in the observations of NDF and ADF values maybe due to age of maturity of grasses. Borreani et al. [17] studied the nutritive value of Sulla (Hedysarum coronarium L.) at vegetative and seed set morphological stages. They reported that NDF contents increased from 20 to 61 % with advancing maturity. However, Kramberger and Klemen [18] found that Cerastium holosteoides grass harvested in summer contained significantly higher NDF and ADF values as compared to those grasses harvested in spring.

Highest hemicellulose value was found in V. zizanioides (50.2%) and lowest value was calculated in C. citrates (21.0%). On contrary, Sultan et al. [15] examined the hemicellulose percentage of marginal land grasses at early bloom stage which varied between 18% (Digitaria sanguinalis) to 36% (Andropogon squarrosus). Whereas, Moore and Hatfield [19] studied that most of the tropical grasses contain average hemicellulose 35.4%.

The value of acid detergent lignin (ADL) varied from 8.5% (for V. zizanioides) to 3.0% (for C. dactylon). Sultan et al [15] found that the lignin contents of marginal land grasses at early bloom varied between 2.8% to 4.6%. At mature stage lignin contents varied between 3.4% to 5.7%. The cell wall lignin increased in leaves (45 to 60 %) and stem (55 to 70 %) with advancing grass age (Kilcher and Troelson, 1973). Brown et al., [20] reported that the soil fertility could also influence grass lignin concentration. According to Mbwile and Uden, [21] lignin in forages mainly affected by age of the plant and the season of the cut.

Maximum ash contents were determined 14.99% for P. antidotale and minimum were 9.84% for C. ciliaris. This figure matches to the reported value of ash for Rhodes grass which was calculated 16 % [22]. Khanam et al. [23] calculated ash contents 13.4% for Echinochloa crusgalli (a variety of Swank grass). On the other hand, Sultan et al. [15] examined the ash contents of marginal land grasses at early bloom varied from 6.2% (Panicum turgidum) to 10.0% (Pennisetum orientale).

Table 2-a: Chemical composition of grasses at pre-bloom stage (%)

Botanical Names	**DM**	**OM**	**CP**	**NDF**	**ADF**	**HC**	**ADL**	**Ash**
P. antidotale	25.25	85.01	14.28	53.1	29.0	25.5	3.5	14.99
S. halepense	17.75	87.71	9.51	62.4	24.0	38.4	5.0	12.05
P. purpureum	11.88	86.88	8.19	56.5	17.5	39.0	6.1	13.12
V. zizanioides	38.42	86.40	9.84	73.4	22.9	50.2	8.5	13.60
C. citrates	36.61	87.38	8.71	56.3	35.3	21.0	5.0	12.62

DM, OM, CP, NDF, ADF, HC and ADL stand for dry matter, organic matter, crude protein, nutrient digestible fiber, acid detergent fiber, hemi cellulose and acid detergent lignin respectively.

Table 2-b: Chemical composition of grasses at pre-bloom stage (%)

Botanical Names	DM	OM	CP	NDF	ADF	HC	ADL	Ash
C. ciliaris	21.75	90.16	11.13	67.5	46.3	21.2	4.8	9.84
L. fusca	18.75	85.30	9.00	66.5	43.1	23.4	6.6	13.70
C. gayana	11.38	89.91	9.34	63.0	40.5	22.5	3.5	10.09
C. dactylon	28.36	88.44	10.53	70.7	42.2	28.5	3.0	11.56
P. colunum	22.41	86.39	7.90	63.3	41.5	21.8	4.3	13.41

DM, OM, CP, NDF, ADF, HC and ADL stand for dry matter, organic matter, crude protein, nutrient digestible fiber, acid detergent fiber, hemi cellulose and acid detergent lignin respectively.

3.2. Gross Energy

Gross energy (GE) value of the grasses is presented in Table 3. The GE value of grasses ranged from 3654.28 Kcal/kg for *P. antidotale* to 2799 Kcal/kg for *C. citrates*. A wide range in predicted metabolizeable energy maybe due to different agronomic conditions at different farms[23].

Table 3: Gross energy of grasses at pre-bloom stage

Name of grass	Gross energy (kcal/kg)
P. antidotale	3654.28
S. halepense	3096.4
P. purpureum	3233
V. zizanioides	3135
C. citrates	2799
C. ciliaris	3201.4
L. fusca	2812.1
C. gayana	3412.2
C. dactylon	2994.6
P. colunum	2990.3

3.3. Mineral contents

The mineral values of grasses are shown in Table 4-a and 4-b. The *C. gayana* contained maximum Ca (4 g/kg DM), whereas, minimum (1.6 g/kg DM) Ca was noticed in both *C. ciliaris* and *C. dactylon*. Minison [24] reported Ca level from 3.1g/kg DM to 19.8 g/kg DM and the mean was 6.3 g/kg. On the other hand Ca level in the diet of livestock to fulfill its maintenance and production requirements should remain within the range of 1.7 to 4.2 g/kg DM. Huston *et al*. [25] also found lower values of Ca and P (1.4 and 1.0 g/kg DM in early and in late summer, respectively) in *P. hallii*.

The value of Mg varied from 0.84 g/kg for *L. fusca* to 0.264 g/kg for *C. ciliaris*. The Mg concentration at maturity varied from 0.02 g/kg to 0.40 g/kg [16]. Whereas, Skerman & Riveros (1990) concluded that Mg content of tropical grasses varied from 0.4 to 9.0 g/kg. Value of P ranged maximum in both *P. purpureum* and *C. gayana* (0.59 g/kg DM) and minimum value was recorded in *C. ciliaris* (0.14 g/kg DM). This figure confirms the findings of Rahim *et al* [16] who determined P concentration in marginal land grasses at early bloom stage varied from 0.13 DM to 0.50 g/kg DM. In another study, the P content in tropical grasses varied from 0.2 to 0.6 g/kg DM and this variation might be due to available P in the soil.

Maximum value of Na was observed in *L. fusca* which was 10.5 g/kg while minimum Na was observed in both *V. zizanioides* and *C. citrates* which was 2.5 g/kg DM. The amount of K varied from 27.2 to 11.6 g/kg (*P. colunum* to *L. fusca* respectively). Humphreys [25] reported that K concentration varied in tropical

grasses from to 12 g/kg. Rahim *et al*. [16] found that K concentration in marginal land grasses at early bloom varied from 3.3 g/kg to 9.5 g/kg. The K concentration in these grasses at maturity varied from 2.5 g/kg to 7.5 g/kg.

Table 4-a: Mineral profile of grasses at pre-bloom stage (g/kg of DM)

Botanical Names	Ca	P	Mg	Na	K	Ca:P ratio
P. antidotale	2.4	0.52	0.67	7.5	26.1	4.62
S. halepense	3.2	0.30	0.48	5.0	20.0	10.67
P. purpureum	2.8	0.59	0.67	5.0	26.1	4.67
V. zizanioides	2.4	0.29	0.72	2.5	16.6	8.28
C. citrates	2.4	0.37	0.69	2.5	19.4	6.49

Table 4-b: Mineral profile of grasses at pre-bloom stage (g/kg of DM)

Botanical Names	Ca	P	Mg	Na	K	Ca:P ratio
C. ciliaris	1.6	0.14	0.26	10.0	12.7	10.67
L. fusca	3.6	0.22	0.84	10.5	11.6	16.36
C. gayana	4.0	0.59	0.72	5.0	17.7	6.78
C. dactylon	1.6	0.22	0.34	7.5	16.6	7.27
P. colunum	2.0	0.44	0.43	5.0	27.2	4.55

3.4. In situ nutrient digestibility of different grasses at pre-bloom stage at 48 hours fed to buffalo bull

The DM digestion kinetics is presented in Table 5. Highest digestibility value was observed for *P. antidotale* (74.2%) while the lowest was for *V. zizanioides* (29.5%). The DM digestibility of Vetiver grass was found 46.09% in Dongshan goat (Liu *et al*., 2003). Bortolo *et al*. [26] observed forage DM digestibility values from 41.8 to 59.3% for *Coastcross*-1 grazed by sheep. The decline in digestibility value is due to the increase of cell wall proportion and lignin concentration [12]. Digestibility of vegetative Rhodes grass, as with other C_4 grasses, is limited by its high NDF content and the chemical bonds between its cell wall polysaccharides, lignin and phenolic acids [27]. Gohl [27] reported that fiber digestibility of *C. ciliaris* was 76.2% at early bloom and it decreased to 71.6% with enhanced maturity. Whereas, Mushtaque [28] found the digestibility in whole plant of *C. ciliaris* were 58.7%.

The NDF digestibility values of grasses are presented in Table 6. Highest digestibility value was observed for *P. antidotale* (67.96%) while the lowest was for *L. fusca* (27.37%). This study differs widely to that of Mushtaque, [28] who examined NDF digestibility was 56.4%. The difference in digestibility values is probably due to lignification due to maturity which interferes the digestion of structural carbohydrates by acting as a physical barrier to rumen microbial enzymes [19] . Van Soest [12] observed the existence of a negative curvilinear relationship between lignin content and NDF digestibility.

3.5. Dry matter digestion kinetics of grasses

Table 6: In situ dry matter digestion kinetics of different grasses fed to a cannulated buffalo bull

Name	DM Digestibility %	NDF Digestibility %
P. antidotale	74.2^{a}	67.96^{a}
S. halepense	72^{a}	63.81^{bc}
P. purpureum	70.4^{a}	64.69^{b}
V. zizanioides	29.5^{c}	27.78^{g}

C. citrates	69.2[a]	51.67[e]
C. ciliaris	73.3[a]	62.67[c]
L. fusca	32.8[c]	27.37[g]
C. gayana	48.5[b]	44.47[f]
C. dactylon	53[b]	50.70[e]
P. colunum	66.2[a]	57.27[d]
S.E	3.03	2.6

DM digestion kinetics are presented in table 6. In case of lag time, lowest was 0.03h for *C. citrates* and highest was 3.69h for *V. zizanioides*. Highest rate of disappearance was recorded (6.95%/h) for *C. ciliaris* whereas, lowest was recorded (5.05%/h) for *L. fusca*. The extent of digestion was maximum (78.2%) in case of *S. halepense* and minimum (36.5%) in case of *V. zizanioides*. Mushtaque [28] found the rate of disappearance and extent of digestion in whole plant of *C. ciliaris* were 4.5% and 70.3 %, respectively and Lag time was 0.5 h for dry matter of the grass. According to Cherney *et al*. [29] lag time associated with early harvest dates might be because of the high proportion of cell soluble material of the gasses.

3.6. NDF digestion kinetics of grasses

NDF digestion kinetics are presented in table 7. In case of lag time, lowest was 1.22 h for *V. zizanioides* and highest was 4.19 h for both *L. fusca* and *C. gayana*. Highest rate of disappearance was recorded (6.60 %/h) for *P. colunum* whereas, lowest rate was recorded (4.70 %/h) for *C. gayana*. The extent of digestion was maximum (70.56 %) in case of *P. purpureum* and minimum value (29.39 %) was in case of *V. zizanioides*. This study differs widely to that of Mushtaque, [28] who examined NDF rate of disappearance, extent of digestion were 3.8%/h, 68.9% respectively and lag time was 0.8 h for *C. ciliaris*. The difference in digestibility values is probably due to lignification due to maturity which interferes the digestion of structural carbohydrates by acting as a physical barrier to rumen microbial enzymes [19]. Van Soest [12] observed the existence of a negative curvilinear relationship between lignin content and NDF digestibility. Van Soest (1996) also observed a linear negative relationship between ADF content and fiber digestibility of forages growing during the spring, but not in those growing from mid-summer to autumn.

Table 7: *In situ* neutral detergent fiber digestion kinetics of different grasses fed to a cannulated buffalo bull

Name	**Lag time (h)**	**Rate (%/h)**	**Extent (at 72 h)**
P. antidotale	2.39[c]	6.56[a]	70.32[a]
S. halepense	1.97[d]	6.15[ab]	68.25[b]
P. purpureum	1.31[e]	6.02[ab]	70.56[a]
V. zizanioides	1.22[e]	6.25[ab]	29.39[g]
C. citrates	3.05[b]	6.19[ab]	57.42[d]
C. ciliaris	3.16[b]	6.15[ab]	69.22[ab]
L. fusca	4.19[a]	5.52[c]	31.77[f]
C. gayana	4.19[a]	4.70[d]	51.40[e]
C. dactylon	3.15[b]	5.87[bc]	56.45[d]
P. colunum	1.36[e]	6.60[a]	59.57[c]
SE	0.2	0.1	2.68

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