Connecting nutritional facts with the traditional ranking of ethnombotanically used fodder grasses by local farmers in Central Punjab of Pakistan

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The local farmers of Central Punjab, Pakistan have been using indigenous grasses as vital components of ruminant diets, but little is reported about their nutritional potential. Hence this study investigated nutritive potential of a selection of ethnombotanically important fodder grasses. Multiple nutritional parameters (proximate components, fibre fractions), secondary metabolites (phenolics, tannins) and in vitro digestibility values were determined. Furthermore, the legitimacy of ethnombotanical knowledge of local inhabitants about these grasses was also verified. The results suggested that majority (77%) of these grasses can be regarded as good quality fodders because of their high protein (169 g/kg) and good digestibility (457 g/kg) with moderate fibre (≤ 602 g/kg), lignin (≤ 50 g/kg) and secondary metabolites (total phenols ≤ 87 g/kg, total tannins ≤ 78 g/kg, condensed tannins ≤ 61 g/kg). Pearson correlation between nutritional parameters indicated that in vitro digestibility values were positively correlated with crude proteins (IVDMD, r = + 0.83 and IVOMD, r = + 0.83 respectively) and negatively correlated with fibre (NDF, r = − 0.91), ADF, r = − 0.84 and ADL, r = − 0.82) contents. Moreover, a positive relationship was identified between ethnombotanical knowledge and laboratory findings for studied grasses. Spearman correlation test showed that ranking of grasses based on ethnombotanical preferences were highly correlated (r values) with the laboratory results for CP (0.85), NDF (− 0.76), ADF (− 0.72) and ADL (− 0.62). The resilient complementarities between ethnombotanical preferences and nutritive analysis authenticate farmer’s traditional knowledge, which needed to be aligned with the corresponding scientific data. Farmers can use these findings for appropriate fodder selection and development of precise supplements for feeding ruminants within a sustainable and economically viable livestock industry for food security.

The Punjab province is the most developed, populous, and prosperous region of Pakistan, representing about 60% (110 Million) of the country’s total population. It has an area of 205,344 sq km and it is the 2nd largest province in area after Balochistan. This zone is playing a promising role in agricultural production. It contributes about 68% to the annual food grain production, whereas 51 million acres of land is cultivated and another 9.05 million acres are remained to be converted as a cultivable land in different parts of this province (https://punjab.gov.pk/about_punjab_economy). Central Punjab refers to the alluvial planes that are surrounded by the two rivers i.e. Jhelum and Sutlej. This region is one of the biggest and most developed areas of Punjab, comprising one of the most extensive canal irrigation system frameworks in the world. This made it permissible for boosted agricultural output and an immense increment in arable land. Among agribusiness this land not only involves

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production of staple grains (wheat, maize, rice) but also plays a significant part to supply feeds ingredients for the livestock industry.

This region is one of great providers of ruminant milk and meat in the country. However indigenous farmers or shepherds of rural areas still rely on traditional fodders in order to raise their animals. Diversified range of plants such as trees, shrubs, herbs and grasses are traditionally significant for their fodder value. Though all kind of florras are in use as fodders, the regional grasses are considered to be a more reliable fodder source for ruminant animals. This preference may be due to the fact that grasses are more palatable than other shrubby fodders for ruminants. As grasses are able to grow massively in various seasons around the year, their accessibility for ruminant feeding is more convenient. It is reported that 53% of total ruminant feed is composed of grasses. A recent ethnobotanical study of Central Punjab Pakistan had enlisted 53 valuable fodder grasses of this region. This study also represented an order of priority among ethnobotanically listed fodder grasses which was based on their utilization preferences by local shepherds and animal caretakers.

Though Harun et al. highlighted many grasses as potential fodders based on their use by indigenous people to feed animals, this ethnobotanical data requires scientific verification. Thus, nutritional exploration of suitable forage species is strongly recommended for sustainable growth and reproduction of livestock. Such kind of information will be supportive in planning to utilize these conventional fodders more appropriately to eliminate the nutritional inadequacies in animal feeding practices. Around the globe the nutritional evaluation of ethnobotanical fodder and forages has been in practice in 2017. In 2017, six ethnobotanically used grass species of western Maharashtra district Ahmednagar, India were nutritionally evaluated. It was reported that these grasses had relatively good levels of protein which could be a desirable contributor to an animal diet. However sometimes they are rich in silica which can negatively affect feed digestibility. Another nutritional study conducted in 2004 in North Sumatra, Indonesia revealed that Cynodon plectostachyus was the most nutritious fodder among customarily used grass species. Similarly in Bangladesh the nutritive value of three fodder grass species (Bracharia mutica, Echinocloa crus-galli and Hymenachne pseudointerrupta) at different stages of maturity was evaluated. This study declared the studied grasses as a suitable fodder to feed ruminant animals.

Nutritional evaluation of these ethnobotanically used fodders grasses is a global trend. However, in Pakistan only a few studies had been conducted in this regard and most of these were confined to Himalayan grassland, highlands of Baluchistan and the desserts of Cholistan. Moreover, with relevance to Central Punjab, Pakistan we found limited nutritional data about ethnobotanically used fodder grasses. Sultan et al. evaluated the nutritional capacitances of ten commonly used irrigated grasses (i.e. Panicum antidotale, Sorghum halepense, Pennisetum purpureum, Vetiveria zizanioides Cymbopogon citrates, Cenchrus ciliaris, Leptochloa fusca, Chloris gayana, Cynodon dactylon and Panicum colunum). Ahmed et al. also nutritionally investigated two traditionally used fodder grasses (Eragrostis pilosa, Dactyloctenium aegyptium) of district Sargodha. In another study five indigenous grasses (C. ciliaris, L. fusca, C. gayana, C. dactylon and P. colunum) of Faisalabad and Sargodha were subjected to proximate analysis and their order of preference was established on the basis of nutritional facts. While these studies recommended many grasses as a valuable part of ruminant feed, they did not satisfactorily represent all the nutritional facts of a diversified range of ethnobotanically used fodder grasses in Central Punjab Pakistan.

Therefore, the current research was conducted as an extension of our previous studies to generate a nutritional database for the previously identified ethnobotanically used fodder grasses of Central Punjab Pakistan. We anticipate that this information and its subsequent use may help the herdsman and range managers to determine the incorporation of suitable grasses in animal diets for improved animal health and performance. Moreover, this study aimed to highlight possible complementarities between the fodder grass rankings based on ethnobotanical knowledge, preferences and nutritional compositions. The outcomes of this study could be used as a benchmark to exploit local fodders more efficiently as a nutritional resource to optimize animal production in an affordable and a sustainable manner.

Results
Nutritional composition. The overall nutritional compositions of studied grasses are presented in Table 1. A wide range of nutritional values were observed among selected grasses under this study. The moisture content of various grass species used for feeding livestock varied between 336 and 798 g/kg. Around 70% of the studied grasses contained more than 600 moisture g/kg. The maximum moisture content was observed in Echinocloa crus-galli, whereas minimum moisture was found in Cenchrus setiger. It is well-known that dry matter (DM) and moisture contents have inverse relationship to each other. Therefore, the grass species with peak moisture content had lowest DM i.e. 202 g/kg and species with minimum moisture possessed the maximum amount of DM i.e., 664 g/kg. Moreover, the ash and organic matter (OM) contents also have reverse relationship among them i.e., minimum ash with greater OM and vice versa. Hence current nutritional results reported the lower ash values in Eragrostis minor (7 g/kg) with upmost OM content (993 g/kg). Whereas Setaria verticillata exhibited the greater ash content (115 g/kg) with lowest OM content (885 g/kg). In regards to the fat content, Acrachne racemosa expressed the maximum value (46 g/kg) while Agrostis gigantean showed the least i.e., 20 g/kg. However, the crude protein (CP) contents in grasses ranged from 41 to 164 g/kg. The lower CP was observed in Arundo donax while higher CP was observed in Eragrostis minor. Interestingly these two species (Arundo donax and Eragrostis minor) exhibited exactly inverse results for NDF. Eragrostis minor reported least NDF (429 g/kg) whereas Arundo donax showed the highest value (798 g/kg). This showed a considerable negative association between CP and NDF contents of grasses. However, in terms of ADF, Zea mays showed the smallest value (247 g/kg) and maximum value was observed in Setaria verticillata (603 g/kg). The utmost ADL content was reported in Arundo donax (153 g/kg) although undermost ADL content was in Bracharia reptans i.e. 35 g/kg.
| Binomial name                        | ERG | Nutritional composition (g/kg) | Secondary metabolites (g/kg) | In vitro digestibility (g/kg) |
|-------------------------------------|-----|-------------------------------|-----------------------------|------------------------------|
| Agrostis gigantea Roth             | A   | M  624 376 60 940 20 146     | NDF 562 290 39 251 272     | IVMD 63 66 42 25 551 524     |
| Avena sativa L.                     | B   | DM 645 355 78 922 35 123     | ADF 528 378 53 326 150     | JVOMD 51 48 45 461 455       |
| Bromus japonicus Thunb             | A   | OM 633 367 68 932 30 140     | ADL 548 333 33 300 214     |                             |
| Dactylis glomerata L.              | C   | Fat 613 387 89 911 36 43     | CE 796 603 107 496 193     |                             |
| Lolium temulentum Linn             | C   | CP 573 427 91 909 41 53      | HC 737 550 78 472 187      |                             |
| Phalaris minor Retz                 | A   | NDF 675 325 65 935 35 129    | In vitro 527 364 36 328 162|                             |
| Poa annua L.                       | B   | CP 553 447 85 915 34 102     | 50 37 31 484 479           |                             |
| Poa infirma Kunth                  | B   | CP 693 307 86 914 33 95      | 53 618 373 45 328 264      |                             |
| Polygono monopelosum (L.) Desf     | B   | CP 536 464 76 924 33 98      | Desmostachya bipinnata (L.)|                             |
| Arundo donax L.                    | A   | CP 638 362 64 936 32 41      | Leptochloa panicea (L.)    |                             |
| Phegmites australis (Cav.) Trin. ex Steud | C | CP 713 287 74 926 42 116     | Desf 604 377 50 327 226    |                             |
| Aristida adscensionis Linn         | B   | CP 760 240 98 902 35 66      | Bothriochloa bladhii L.    |                             |
| Arrachne racemosa (B. Heyne ex Roth) | O | CP 713 287 112 888 46 59   | Phalaris minor Retz         |                             |
| Cynodon dactylon (L.) Pers         | A   | CP 601 399 65 935 29 161     | Saccharum bengalense L.    |                             |
| Dactyloctenium acegyptium (L.) Wild | A | CP 594 406 62 938 33 137     | Brachiaria ramosa (L.)     |                             |
| Desmostachya bipinnata (L.) Stapf  | A   | CP 645 355 71 929 37 127     | Brachiaria reptans L.      |                             |
| Elesine indica (L.) Gaertn          | A   | CP 645 355 63 937 38 159     | Cenchrus biflorus L.       |                             |
| Enneapogon persicus Boiss          | B   | CP 540 400 69 931 34 47      | Cenchrus ciliaris (Boiss.) |                             |
| Eragrostis japonica (Thunn.) Trin  | B   | CP 579 421 58 942 34 99      | Cenchrus setiger (Boiss.)  |                             |
| Eragrostis minor Host              | A   | CP 596 404 7 993 30 164      | Aristida adscensionis Linn |                             |
| Eragrostis pilosa (L.) P. Beauv     | B   | CP 619 381 66 934 33 108     | Leptochloa panicea (L.)    |                             |
| Leptochloa panicea (Retz.) Ohwi    | C   | CP 755 245 102 898 42 72      | Desmostachya bipinnata (L.)|                             |
| Tetrapogon villosa Desf            | C   | CP 689 311 71 929 40 100     | Heteropogon vivaceus (L.) |                             |
| Apluda mutica L.                   | C   | CP 675 325 80 920 41 63      | Digitaria ciliaris (Retz.) |                             |
| Bothriochloa bladhii (Retz.) S.T. Blake | A | CP 647 353 66 934 30 156  | Digitaria ciliaris (Retz.) |                             |
| Brachiaria ramosa (L.) Stapf        | B   | CP 613 387 71 929 31 98      | Setaria viridis (L.) P. Beauv |                             |
| Brachiaria repitans (L.) C.A.Gardner & C.E.Hubb | A | CP 653 347 58 942 34 142     | Digitaria ciliaris (Retz.) |                             |
| Cenchrus biflorus Roxb             | B   | CP 677 323 68 932 23 104     | Digitaria longiflora (Retz.)|                             |
| Cenchrus ciliaris L.               | A   | CP 524 476 57 943 24 158     | Echinochloa colona (L.)     |                             |
| Cenchrus ciliaris M.               | A   | CP 624 376 58 942 26 137     | Echinochloa colona (L.) Link |                             |
| Cenchrus setiger Vahl              | C   | CP 337 663 63 937 44 98      | B 746 254 60 940 38 103    |                             |
| Crysopogon australis (Boiss.) Stapf | A | CP 590 410 62 938 27 136     | Echinochloa crus-galli (L.) P. Beauv |                             |
| Crysopogon australis (Boiss.) Stapf | A | CP 590 410 62 938 27 136     | Heteropogon contortus (L.) P. Beauv |                             |
| Imperata cylindrica (L.) Raeschel  | A   | CP 640 360 58 942 30 140     | Imperata cylindrica (L.) Raeschel |                             |
| Ochthochloa compressa (Forsk.) Hilo | C | CP 597 404 70 930 41 72      | Imperata cylindrica (L.) Raeschel |                             |
| Panicum antidotale Retz             | B   | CP 580 420 67 933 38 15      | Imperata cylindrica (L.) Raeschel |                             |
| Paspalium distichum L.             | B   | CP 546 454 66 944 36 109     | Imperata cylindrica (L.) Raeschel |                             |
| Pennisetum orientale Rich          | B   | CP 627 354 58 944 36 109     | Imperata cylindrica (L.) Raeschel |                             |
| Saccharum bengalense Retz           | A   | CP 613 387 58 944 30 132     | Imperata cylindrica (L.) Raeschel |                             |
| Saccharum spontaneum L.             | A   | CP 656 344 53 947 24 126     | Imperata cylindrica (L.) Raeschel |                             |
| Setaria pumila (Porr) Roem. & Schult | A | CP 663 337 66 934 30 146     | Imperata cylindrica (L.) Raeschel |                             |
| Setaria verticillata (P. Beauv)     | C   | CP 661 339 115 885 40 53      | Imperata cylindrica (L.) Raeschel |                             |
| Setaria viridis (L.) P. Beauv       | A   | CP 515 485 59 941 36 149     | Imperata cylindrica (L.) Raeschel |                             |
| Sorgium bicolor (L.) Moench        | A   | CP 599 401 62 938 27 140     | Imperata cylindrica (L.) Raeschel |                             |
| Sorgium halepense (L.) Pers         | A   | CP 697 303 66 934 29 160     | Imperata cylindrica (L.) Raeschel |                             |
| Zea mays L.                         | A   | CP 634 366 63 937 29 164     | Imperata cylindrica (L.) Raeschel |                             |

**Mean values**

| Mean | MD 632 368 69 931 33 108 | NDF 602 381 54 327 221 | 68 59 40 457 463 | **Mean** |

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and *Eragrostis minor* followed by *Tetrapogon villosus* and reduce the fibre digestion and DM intake in ruminant animals. Trations of dietary fats can not only suppress the rumen microbial (protozoa and fungi) activity but also it can *Setaria pumila*, *Cenchrus setiger* were reported in 12–85 g/kg, respectively (Table 1). Among all the tested grass species, the maximum secondary metabolites were reported in *Cenchrus setiger*, *Phragmites australis*, *Acraechne racemosa*, *Lolium temulentum*, *Apluda mutica*, *Tetrapogon villosus* and *Setaria verticillata*. Conversely, the lowest values were recorded in *Cenchrus ciliaris* followed by *Cenchrus pennisetiformis*, *Chrysopogon aukheir*, *Eleusine indica* *Chrysopogon zizanioides*, *Setaria viridis* and *Eragrostis minor*.

**In vitro digestibility.** Current digestibility analysis reported mean values of 457 g/kg and 463 g/kg for in vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) respectively. The highest IVDMD and IVOMD were observed in *Eragrostis minor* (660 and 661 g/kg respectively) whereas the minimum values were observed in *Dactylis glomerata* (175 and 179 g/kg respectively) (Table 1).

**Discussions**

As indicated in Table 1, the average moisture and DM contents were within the range for good quality fodders. However, it also depended upon the time, stage and weather of harvesting. The reported DM contents of *Cenchrus ciliaris*, *Cynodon dactylon*, *Apluda mutica*, *Setaria pumila*, *Saccharum spontaneum*, *Desmostachya bipinnata* and *Pennisetum orientale* were found to be much higher in comparison to the values presented by Sultan et al. who analyzed some grasses from Northern regions of Pakistan. The probable reasons for this variation are disparities in the land geography, climate, soil composition and sampling times which can affect the corresponding nutritional variations in forages. However, in comparison to the current study, Manzoor et al. and Sultan et al. reported lower DM values for some grasses (*Cenchrus ciliaris*, *Cynodon dactylon*, *Panicum antidotale*, *Sorghum helenepe* and *Chrysopogon zizanioides*) from the same geographical range. These lower values were possibly due to differences in soil fertility level, phenological states of grasses, and sampling or analytical procedures.

It is worthwhile to know the amount of total ash in different feeds because it contains different types and amounts of macro, micro and trace minerals which are needed for ruminant animal’s health. This information can help us to use suitable forages for feeding animals to satisfy their mineral requirements with or without supplements. Conversely, high mineral contents in animal feeds may dilute the quantity of other available nutrients to animals. Moreover, if animal fed with excessively high mineral contents, these will not only affect the digestibility but also might cause the accumulation and crystallization which ultimately lead to urinary restrictions or infections. In the current study mean ash and OM contents were found to be within the satisfactory limits (Table 1). Sultan et al. and Rafay et al. stated much higher ash values for *Cenchrus ciliaris*, *Cynodon dactylon*, *Setaria pumila*, *Saccharum spontaneum*, *Pennisetum orientale*, *Cymbopogon jwarancusa*, *Ottochloa compressa* and *Panicum antidotale*. Variation in soil and other habitat features might have affected the ash content of plants. However, the current ash values were also found to be lower than the reported values from similar agro climatic zone. These lower ash values inferred the probable less soil contamination of these samples.

The current study reported that the average fat content was 33 g/kg which is within acceptable limits for ruminant diets (Table 1). This amount reasonably satisfies the ruminant’s fat requirement. In fact, high concentrations of dietary fats can not only suppress the rumen microbial (protozoa and fungi) activity but also it can reduce the fibre digestion and DM intake in ruminant animals.

This study found that out of 53 studied fodder grass, 40 species possessed fairly moderate to good CP range i.e. 169–95 g/kg. Generally, the upper limit of CP values in present analysis were found to be relatively higher than the maximum CP content of some previously studied grass species from similar zone. These disparities in CP content were probably due the difference in their growth stages and soil fertility at the time of sampling. However comparable CP values were reported by other researchers, who obtained variable CP contents (up to 170 g/kg) of several tropical grasses around the globe. The ruminant’s protein demand varies according to its age or growth stage, such as the higher CP levels (150–160 g/kg) are especially good for lactating cows (up to 170 g/kg) of several tropical grasses around the globe. The ruminant’s protein demand varies according to its age or growth stage, such as the higher CP levels (150–160 g/kg) are especially good for lactating cows because during lactation amino acids demand is higher for additional metabolic functions and synthesis of milk protein. Current results revealed that *Dichanthium annulatum*, *Bothriochloa bladhii*, *Eleusine indica*, *Sorghum helenepe*, *Cynodon dactylon*, *Zea mays* and *Eragrostis minor* were especially good in this aspect because of their higher CP content. However, for non-lactating ruminants comparatively lower protein intake is recommended. Therefore, measurement of CP is a supportive tool in order to assure that animal is ingesting sufficient proteins as per its body requirement. As intake of excessive proteins or consumption of poor quality proteins are both economically detrimental.

The recommended critical value for CP content is 70 g/kg. Results showed that CP of *Ottochloa compressa* (72 g/kg) was at borderline; whereas remaining 12 grasses possessed CP lower than the critical value (Fig. 2). They showed decreasing CP contents from 60 to 40 g/kg and their descending order was arranged as *Aristida*.
temulentum > Digitaria longiflora > Cymbopogon jwarancusa > Lolium temulentum > Setaria verticillata > Heteropogon contortus > Leptochloa panacea > Enneapogon persicus > Dactylis glomerata > Arundo donax. By comparing these results with ethnobotanical priority groups of fodder grasses (previously reported by Harun et al.1 it was shown that two (A. donax and H. contortus) of those grasses belonged to the high priority group (A) and three (A. adscensionis, E. persicus, D. longiflora) were from moderate priority group (B) whereas rest of the seven grasses were from the low priority grass group (C). The presence of greater number of fodder grasses with lower CP in group C declares a supportive evidence for ethnobotanical knowledge of local inhabitants of the study area. The intake of this kind of protein deficient diet will not only result in the loss of appetite but also reduce the cellulolytic activity of rumen microbes which slow down the fibre digestion59. Low feed intake and poor digestion reduce the food efficiency which can cause underprivileged growth and development of livestock40,41. Therefore, for healthy animal productivity uninterrupted supply of feed, as lower the concentration of fiber in feed it become easier to digest which results in good energy value24. The results predicted an interesting relationship between CP and fibre contents as indicated in Fig. 1. This figure showed NDF varying from 670 g/kg to 780/kg was considered to be high enough to limit DM intake and digestibility. Lignin content is also negatively correlated with fodder palatability42,53 because it has negative affect on OM digestion39. Low feed intake and poor digestion reduce the food efficiency which can cause underprivileged growth and development of livestock40,41. Therefore, for healthy animal productivity uninterrupted supply of CP is mandatory42,43. Although 77% of studied grasses showed sufficient levels of CP which recommend them as good protein sources for maintaining healthy livestock, they still cannot compete with high CP containing legumes44,45. The differences between grass and legumes for CP contents are quite significant; hence a blend of grass and legume in ruminant diet is more recommendable44,46.

Fibre is often regarded as the negative index of nutritive quality47,48. It is negatively related to digestibility of feed, as lower the concentration of fibre in feed it become easier to digest which results in good energy value24. Fibre can be determined in terms of NDF and ADF contents but the NDF method is considered to be most reliable one for measuring the total cell wall a fibre contents in a feed29. Forage digestibility and ruminant’s consumption capacity largely influenced by its NDF content49. Its value in animal feed is quite critical and a feed is considered to be of poor quality if it has NDF above 650 g/kg50. The current study revealed the mean NDF value of 602 g/kg which is under the critical limit but 12 species i.e., Arundo donax > Dactylis glomerata > Digitaria longiflora > Heteropogon contortus > Cymbopogon jwarancusa > Setaria verticillata > Leptochloa panacea > Lolium temulentum > Enneapogon persicus > Apluda mutica > Acraicia racemosa > Apluda mutica > Aristida adscensionis had NDF above the critical value (650 g/kg). However, Ottochloa compressa exhibited the NDF values that were very close to the critical value of 697 g/kg. The grasses with more than the critical NDF value indicated reduction in voluntary feed intake and feed conversion efficiency but lengthier rumination periods51. Norton37 stated that NDF varying from 670 g/kg to 780/kg was considered to be high enough to limit DM intake and digestibility. The results predicted an interesting relationship between CP and fibre contents as indicated in Fig. 1. This figure showed grasses with higher CP were lower in NDF.

NDF is also associated with other cell wall structural contents i.e., lignin, cellulose and hemicellulose52. Lignin content is also negatively correlated with fodder palatability42,53 because it has negative affect on OM digestibility53. The average ADL content was reported as 382 g/kg. One of the significant reasons in preference of grasses by ruminants is their digestible cellulose and hemicellulose contents. The microflora of ruminant’s digestive is capable to efficiently digest cellulose and hemicellulose of grasses52. The mean cellulose and hemicellulose in studied grasses were reported as 317 g/kg and 220 g/kg, respectively. However, digestibility of cellulose is inversely proportional to the amount of lignin. As fodder matures its lignification increases with 100 g/kg which lessens the cellulose digestibility by about 60%. The lignin content in younger fodder is the 50 g/kg, which increases the cellulosic digestibility up to 80%55. The current results exhibited that 75% of species had lignin from 34 to 50 g/kg which represented their good cellulosic digestibility. However a few species with higher lignin content (Brachiaria ramosa, Phragmites australis, Ottochloa compressa, Apluda mutica, Dactylis glomerata, Lolium temulentum, Leptochloa panacea, Digitaria longiflora, Heteropogon contortus, Cymbopogon jwarancusa, Enneapogon persicus, Arundo donax, Setaria verticillata, Acraicia racemosa, Aristida adscensionis) negatively affected the cellulose digestion in ruminants. On comparison with previous studies relatively higher fibre values (NDF, ADF, ADL, cellulose and hemicellulose) were found in the findings stated by Rafay et al.50 and Sultan et al.24. The soil fertility and season of fodder harvesting are important influencing factors for variation in fibre contents56-58.

Figure 1. Comparative illustration of the CP and NDF contents of ethnobotanical grasses (Yellow NDF bars showing above critical value > 650 g/kg while red CP bars showing below critical value < 70 g/kg).
Secondary metabolites like phenolics and tannins are known as anti-nutritional factors because of their negative effects on ruminant health especially if consumed in large amounts. The significant possible damages of secondary metabolites are such as reduction in immune function, growth and reproduction impairments, which ultimately leads to animal morbidity and mortality. By considering these effects, quantification of secondary metabolites is appraised to be essential in order to make appropriate fodder selection. According to reports, tannins at 60–120 g/kg DM in animal diet can lower the efficiency of animal digestive system and ultimately its productivity as well. However, few other researchers reported that low levels of these secondary metabolites positively affected ruminant health. Like condensed tannins between 20 and 50 g/kg DM are essentially required for efficient utilization of nitrogen and healthy weight maintenance. On the basis of recommended acceptable ranges of secondary metabolites, the studied grass species had been categorized into three groups i.e. high (> 100 g/kg), moderate (50–100 g/kg) and low (< 50 g/kg). Grass species ranked as low and moderate groups are within the satisfactory limits for animal health. These results suggested that 86% of studied grass species possessed fewer secondary metabolites than their suggested toxic levels which indicated that those were potentially of good quality as animal fodders. Certain levels of phenols in these species are beneficial as antioxidative, anti-inflammatory, anti-diabetic, anti-allergy, anti-microbial and gastro or hepato-protective activities. Moreover, sufficient tannin concentrations facilitate to increase the digestion proficiency, milk production, wool growth and animal’s ovulation rate. Additionally, specific quantity of these secondary metabolites has positive ecological effects such as improvement of soil quality and nutrient cycles. However, grasses belong to the high secondary metabolite category (Cenchrus setiger, Phragmites australis Acrachne racemosa, Lolium temulentum, Apluda mutica, Tetrapogon villosus and Setaria verticillata) are not considered good for ruminant’s health. Panhwar stated that the excessive intake of secondary metabolites (anti nutrients) could affect the digestibility of vital nutrients which not only lessened the palatability but could also be fatal. It had been reported that higher dosage of phenols may reduce the bone mineralization and could also cause disturbance in cholesterol or estrogen levels. Whereas greater amounts of tannins could result in reduction of protein digestion and absorption which eventually depress the voluntary feed intake (VFI). 

As reported above, numerous nutritional parameters can indicate the potential feed values for providing particular nutrients. However, the real feed value for animal can only be determined by finding the digestion of those nutrients. However, the digestibility of fodder or forages were strongly affected by the seasonal variations. Kallah et al. and Megersa et al. suggested that on average 450 g/kg digestibility of forage/fodder was sufficient to maintain an optimal animal performance. The findings of Van Soest suggested that 400–700 g/kg digestibility for grasses were found in tropical and subtropical lands. Noguiera also acclaimed 400–527 g/kg digestibility for grasses. For more understanding, ethnobotanical grasses were further classified into 3 clusters i.e. high (< 450 g/kg), medium (400–450 g/kg) and low digestible grasses (> 400 g/kg) (Fig. 3). Results reported greater number of grass species (n = 29) in high digestible cluster followed by low (n = 13) and medium (n = 11) digestibility groups. Minor in vitro DM digestibility indicates the presence of anti-nutritional factors within those fodders which can obstruct the activity of microbial activities in rumen.

Correlation between studied nutritional parameters. Evitayani et al. also predicted similar negative relationship between CP and fibre contents while studying nutritive value of tropical forages in North Sumatra, Indonesia. This clearly indicates that if any species possessed high protein content then its NDF, ADF, ADL and cellulosic contents must be lower. Especially the negative connection between CP and NDF contents was...
also supported by Ronquillo et al.81 and Mlay et al.82. Whereas with the exception of hemicellulose, all other studied fibre contents (NDF, ADF, ADL and cellulose) exhibited significant positive relationship with each other.

Many factors influenced the digestibility of a particular fodder. Harper and McNeill49 described that forage digestibility was negatively influenced by NDF contents whereas Arif et al.83 argued about the positive influence of CP on digestibility values of fodder/forages. As mentioned in Table 2, IVDMD and IVOMD values were positively correlated with CP (r = 0.856*** and r = 0.856*** respectively) and negatively correlated with NDF (r = −0.914***), ADF (r = −0.844*** and r = −0.844*** respectively) and ADL (r = −0.820***). These results can be interpreted as that by increasing the crude protein the digestibility amplified, however increase in cell wall content lessened the digestibility of a particular fodder. These correlation findings were in line with the previous findings21,24,84,85. These results were also concordant with the report of Van Soest77 who stated negative association between NDF and digestibility of various feeds. Lichtenberg and Hemken86 specified that DM digestibility was decreased by 3–4 units with per unit increase in lignin content. However, poor digestibility fodder grasses can be improved by adding urea supplements into an animal diet85. Some other studies also supported the fact that by addition of urea as a supplement can improve the digestibility of low quality forages87,88.

Moreover, it also had been observed that digestibility was inversely proportional to secondary metabolite contents (anti-nutritional factors). As shown in Table 2. The IVDMD and IVOMD values showed negative association with TP (r = −0.422 and −0.424 respectively), TT (r = −0.442 and −0.447 respectively) and CT (r = −0.528 and

**Table 2.** Pearson correlations (r) (alongside their significance levels at *, **, ***) between crude proteins (CP) and overall fibre content neutral detergent fibre (NDF), acid detergent fibre (ADF), acid lignin fibre (ADL), cellulose (CEL), hemicellulose (HCL), total phenol (TP) total tannins (TT), condensed tannins (CT), in vitro dry matter digestibility (IVDMD), in vitro organic matter digestibility (IVOMD) (g/kg) of studied ethnobotanical fodder/forage grasses (n = 53). Here *, ** and *** represent significance at P < 0.05, P < 0.01 and P < 0.001 respectively.

| Items | CP | NDF | ADF | ADL | CE | HC | TP | TT | CT | IVDMD | IVOMD |
|-------|----|-----|-----|-----|----|----|----|----|----|-------|-------|
| CP    | −0.856*** | −0.786*** | −0.819*** | −0.735*** | 0.009 | −0.477*** | −0.495*** | −0.607*** | 0.832*** | 0.827*** |
| NDF   | 0.893*** | 0.859*** | 0.855*** | 0.045 | 0.442** | 0.458** | 0.488*** | −0.914*** | −0.914*** |
| ADF   | 0.849*** | 0.989*** | −0.409** | 0.419** | 0.418** | 0.489*** | −0.844*** | −0.844*** |
| ADL   | 0.761*** | −0.144 | 0.361** | 0.368** | 0.405** | 0.405** | −0.818*** | −0.820*** |
| CE    | −0.461** | 0.413** | 0.410** | 0.486*** | 0.486*** | −0.806*** | −0.805*** |
| HC    | −0.035 | −0.001 | −0.096 | 0.020 | 0.020 | 0.020 | 0.021 |
| TP    | 0.987*** | 0.845*** | −0.422** | −0.424** | 0.005*** | −0.424** | 0.099*** |
| TT    | 0.857*** | 0.845*** | −0.424** | −0.447** |
| CT    | −0.528*** | −0.526*** |
| IVDMD | 0.999*** |
−0.526 respectively). Njidda89 supported this fact that low level of tannins resulted in good IVDMD of fodders. This is because those tannins in NDF and ADF firmly bound to cell wall and cell protein and thus resulted in lower digestibility values51. Whereas CT had also been notorious in order to lower the digestibility values54.

**Correlation between ethnobotanical knowledge and laboratory findings.** After obtaining the nutritional results, all the studied grasses were ranked in order of priority with reference to CP, NDF, ADF, ADL, IVDMD and IVOMD contents. These nutritional rankings were compared with the ethnobotanical preferences ranking of fodder/forage grasses established earlier in the previous study of Harun et al.1 (Figs. 4, 5, 6, 7, 8).

Figure 4. Positive correlation (r) between ethnobotanical preferences ranking of fodder grass versus order of priority based on CP content (high to low).

Figure 5. Negative correlation between fodder grass rankings based on ethnobotanical preference ranking versus ranks based on NDF contents (high to low).

Figure 6. Negative correlation between fodder grass rankings based on ethnobotanical preference ranking versus ranks based on ADF contents (high to low).
The results demonstrated strong correlations between laboratory findings and ethnobotanical knowledge about studied forages/fodder grasses, perceived by local respondents. Figure 4 showed a strong positive association ($r = +0.8$) between ranking based on CP and ethnobotanical preferences. This means that grasses which ranked as a high priority by local communities also possessed sufficient quantities of proteins and fodder grasses with low priority were below the critical CP value (>70 g/kg). However, as CP and fibre contents were inversely proportional to each other, ranking order based on NDF, ADF and ADL exhibited negative correlation ($r = −0.768$, $r = −0.726$, $r = −0.625$ respectively) with the ethnobotanical preferences of fodder grasses (Figs. 5, 6, 7). This can be inferred that a fodder/forage grass with high fibre content is least preferable by the local farming communities of Central Punjab Pakistan. Also, the ranking based on the digestibility results of fodder grasses positively correlated ($r = +0.876$) with the ethnobotanical rankings of these grasses (based on the experiences of local people) (Fig. 8).

Results of crosstab analysis reported strikingly positive associations between the clusters of grasses based on laboratory results (in vitro digestibility and secondary metabolites) and ethnobotanical ranking groups of high, medium and low priority. Secondary metabolites analysis also showed an affirmative association with ethnobotanical knowledge. Crosstab analyses clearly declared that grasses ranked in high and medium ethnotibotanical priority groups were comparable to low and medium secondary metabolites ranking groups (Table 3). Additionally, those grasses which were least preferred ethnotobationally lie in the category of high secondary metabolites (anti-nutrients) group. This can be explained as the grasses of high and medium ethnotobatical fodder priority possessed secondary metabolites (total phenols, total tannins and condensed tannins) below the critical limit whereas grasses with least ethnotobatical fodder priority contained comparatively higher amount of secondary metabolites. Moreover, Crosstab analysis of in vitro digestibility and ethnobotanical ranking groups of grasses showed that out of 25 high priority ethnotobatical grasses, 23 grasses were also positioned in high priority group based on the in vitro digestibility values. Similarly, out of 11 low priority ethnotobatical grasses group, 8 grasses were also placed in low priority group of in vitro digestibility (Table 4). The study revealed that the ethnotobatical knowledge of local shepherds and animal caretakers was quite consistent with the nutritional data of studied grasses. The current findings are in agreement with the reported data of Keba et al.10, Dhungana et al.11, Talore12, Rakib-Uz-Zaman et al.90 and Rodrigues et al.91 who supported the positive association between laboratory results.
and ethnobotanical knowledge. Therefore, traditional knowledge should not be ignored and must be used as an
approach for better understanding of nutritive potential of local fodder/forage resources under predominant
environmental conditions. However, the laboratory based nutrient compositions are needed to formulate nutri-
tious diets to optimize the use of local feed resources to promote livestock health and the environment.

Conclusions
Grasses of Central Punjab Pakistan had been used as animal fodder for centuries but unfortunately little infor-
mation about their nutritional worth was available. This study not only provided a nutritional profile of studied
grasses but also made an attempt to validate the ethnobotanical knowledge of local inhabitants of Central Pun-
jab Pakistan about these fodder grasses. It can be concluded that a strong positive correlation existed between
ethnobotanical preferences of fodder grasses and nutritional facts of particular species. Nutritional results con-
firmed that those grasses which ranked superior by the local inhabitants of the study area also contained higher
CP levels than those species which were ranked as inferior. Moreover, the grasses perceived for low palatability
possessed great levels of structural fibres (NDF, ADF and ADL) and secondary metabolites (anti-nutrients).
The Pearson correlation studies also showed that grasses with higher proteins had low fibre content and good
digestibility. It can be summarized that good quality fodders were high in protein and digestible nutrients, but
low in fibre and lignin. The strong complementarities between ethnobotanical preferences and nutritive analysis
reflected the reliability of ethnobotanical knowledge of local farmers and shepherds. However, it is recommended
to integrate these conventionally used fodders into modern feeding systems. The good quality grasses can be
directly incorporated into ruminant diets whereas grasses with low nutritional quality can be improved by using
biochemical processing before making either silage or hay or can be mixed with either good quality forages or
supplements before feeding the animals. These results are valuable in making appropriate fodder selection and
supplement development that will match livestock requirements which consequently can support economical
livestock performance. However, further studies are required to evaluate their mineral composition; feed intake
and animal’s ability to efficiently utilize these ethnobotanical feed resources for sustainable animal production
in low to moderate animal input systems.

Methods
Study area and its main features. The study was conducted in Central Punjab region of Pakistan which is
regarded as the subtropical continental low land (Fig. 9). Normally temperature of this region remains hot but
also shows significant variation between summers and winters. On average, the summer temperature ranged
between – 2° and 45 °C whereas in winters it can drop down to – 10 °C. The mean annual rainfall of this area
is 46 cm. This region consists of 19 cities which are categorized under 3 agro-ecological zones i.e., Northern
irrigated zone, Sandy deserts zone and Barani zone. For the current study Northern irrigated zone was selected
because the dominant grasses in this zone were a result of irrigation by using an extensive canal system and a
good precipitation. Most of the time ad libitum grazing was in practice but cut and carry system for mixing
specific forages with other feed types was also used in these regions 4.

Sampling of fodder grasses. For sample collection the rural areas from Northern irrigated zone were
actually targeted because of their reliance around feeding conventional grasses for raising their ruminant ani-

| Ethnobotanical ranking groups | Secondary metabolites based grasses categories | Total |
|------------------------------|---------------------------------------------|-------|
|                              | High | Medium | Low  |
| High (A)                     | 0    | 4      | 21   | 25   |
| Medium (B)                   | 0    | 14     | 3    | 17   |
| Low (C)                      | 7    | 4      | 0    | 11   |
| Total                        | 7    | 22     | 24   | 53   |

Table 3. Crosstab analyses between secondary metabolites based grass categories and ethnobotanical ranking
groups of studied ethnobotanical fodder grasses.

| Ethnobotanical ranking groups | In vitro digestibility based grasses categories | Total |
|------------------------------|---------------------------------------------|-------|
|                              | High | Medium | Low  |
| High (A)                     | 23   | 0      | 2    | 25   |
| Medium (B)                   | 5    | 9      | 3    | 17   |
| Low (C)                      | 1    | 2      | 8    | 11   |
| Total                        | 29   | 11     | 13   | 53   |

Table 4. Crosstab analyses between In vitro digestibility’s based grasses categories and ethnobotanical ranking
groups of studied ethnobotanical fodder grasses.
mals. As the literature reported, the grasses were more palatable before their maturity. Therefore, representative samples of each of the 53 specimens from different parts of the same field were collected at their pre bloom stage through repeated field visits during this study. These samples represented the same fodder grasses as previously reported by Harun et al.\(^1\). Representatives of collected fodder grass sample was taxonomically identified by using specimen identification guidelines in the herbarium of Lahore College for Women University, Lahore and the Quaid i Azam University, Islamabad. Moreover two online plant databases i.e. flora of Pakistan (http://www.efloras.org/index.aspx) and flora of India (https://sites.google.com/website/eflorasofindia/) and various grass flora identification keys\(^92\) were also used in fodder grass identification process. However, remaining samples were washed to remove contaminants or dust particles, shade dried and finely ground (Ultracentrifugal mill ZM 200). All the samples were kept inside airtight polythene bags until further nutritional analysis as described below.

**Nutritional analysis.** Parameters like moisture, dry matter, ash, proteins, fat, were evaluated by following the standard methods of AOAC\(^93\). Nitrogen content was determined by the advance MACRO CUBE System and obtained nitrogen value was multiplied by 6.25 to estimate the crude protein (CP) contents of these samples. However, for fat analysis advance micro-digester (MARS6) was used. Whereas neutral detergent fibre (NDF), acid detergent fibre (ADF), acid lignin fibre (ADL) were done by adopting the methodology prescribed by Van Soest et al.\(^48\). Cellulose and hemicellulose contents were calculated by the formulae Cellulose = ADF-ADL, Hemicellulose = NDF-ADF\(^94\).

**Secondary metabolites analysis.** The selected secondary metabolites i.e. total phenols (TP), total tannins (TT) and condensed tannins (CT) that can affect the fodder quality were also estimated by using the methodolody prescribed by Makkar et al.\(^94\). For the estimation of TP, TT and CT tannic acid and epigallocatechin gallate were used respectively to establish relevant standard curves.

**In vitro digestibility analysis.** The method of Tilley and Terry\(^95\) was adopted for digestibility test by using only the first stage of the 2 stage procedure. Rumen fluid (RF) was collected from 3 freshly slaughtered cattle (Aberdeen Angus breed) at a local abattoir (Linden Foods, Ltd.) of Newcastle upon Tyne, UK. Equal volumes of these RF were pooled and then mixed with the pre warmed buffer solution in a ratio of 1:2 and kept at 39 °C in a water bath (Gallenkamp UK Ltd) in order to maintain anaerobic conditions. Moreover, carbon dioxide was

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*Figure 9.* Illustration of sample collection sites i.e. Sargodha, Sialkot, Lahore, Gujranwala, Faisalabad, Okara, Sahiwal, Jhang, Sheikhpura, Nankana Sahib, Lahore, Okara, Gujranwala, Sheikhpura, Faisalabad, Sargodha, Jhang. The GIS version 10.8 software was used to draw this map. Basemap is added by choosing the online basemap option in ArcGIS. https://www.esri.com/en-us/arcgis/products/arcgis-pro/overview.
flushed into this buffered RF (BRF) and pH was adjusted around 7. For the incubation 0.5 g of each ground fodder grass sample was put inside the 50 ml capacity polypropylene tubes and 40 ml of the BRF was also dispensed into each tube. These tubes were sealed with the rubber stoppers (equipped with gas pressure discharge valves) and incubated in a water bath at 39 °C. The tubes were shaken manually few seconds thrice a day (morning, afternoon, evening). After 48 h the tubes were taken out and placed inside an ice filled bucket to stop the on-going fermentation. Later on the tubes were subjected to centrifugation (accuSpinTM3R) at 2000 rpm for 10 min. The insoluble residues were washed, dried, weighed and then ashed before estimating In vitro dry matter digestibility (IVDMD) and In vitro organic matter digestibility (IVOMD). IVDMD of samples were measured by drying the washed residues at 80 °C whereas IVOMD was estimated by formation of ash in the furnace at 550 °C. The calculations were done by following the formulae mentioned below and a blank sample of BRF was used for correction in dry matter residue weight.

$$\text{IVDMD} = \frac{\text{sample weight} - \text{corrected residue DM weight} \times 1000}{\text{sample DM}}$$

$$\text{IVOMD} = \frac{\left(\text{sample DM} - \text{corrected non} - \text{degradable OM weight}\right)}{\text{sample DM}} \times 1000$$

**Ranking of studied fodder grasses.** After obtaining laboratory results (nutritional, secondary metabolites and digestibility) the fodder grasses (n = 53) were ranked in descending order (high to low) according to results of each studied parameter. For each parameter, grass at rank 1 considered as most potential one whereas at rank 53 with the lowest potential. This ranking helped to correlate the laboratory findings with ethnobotanical preferences.

**Statistical analysis.** The obtained data was computed in the excel sheets and graphical illustrations were made for data analysis. Correlation between CP, NDF, ADF, ADL, CE and HC were inferred by using Pearson correlation method (r < 1; P < 0.05) through SPSS version 23. Moreover, the Spearman's rank correlation (r < 1; P < 0.05) was also used to examine possible relationships between laboratory results and ethnobotanical preferences of fodder and grazing grasses at P < 0.01. Crosstab method within descriptive statistics of SPSS was also employed for making comparisons between secondary metabolites based grass categories and ethnobotanical ranking of studied fodder grasses. Additionally, comparisons were made between In vitro digestibility based grass categories and ethnobotanical ranking of studied fodder grasses. Microsoft Excel was used to organize different data sets for creating trend lines and visual presentations.

**Data availability**
The datasets used and/or analyzed during the current study are available from the corresponding author on a reasonable request.

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Research was designed by S.S. and M.A. Laboratory analysis was performed by N.H. A.S.C. supervised this study in Newcastle University, UK and also supported the data analysis and manuscript writing. H.B., N.H. A.S.C. and Z.S. contributed in structuring and improving the scientific language of this article.

Competing interests
The authors declare no competing interests.

Additional information
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