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NUTRITIONAL EVALUATION OF SOME FORAGE PLANTS FROM KHUSHAB, PAKISTAN IN TERMS OF SEASONAL VARIATIONS

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Abstract

Seasonal variations have been shown to influence the nutritional quality as well as chemical compositions of forage species. In this direction, this study critically investigated the changes in the proximate composition of various forage species during different sampling seasons (summer, autumn, winter &spring) in Khushab, Pakistan. During different samplings seasons 10 samples were collected in each season. Proximate analysis method was used for the study of crude protein, crude fibers, moisture contents, ether extract, ash and dry matter. The mean dry matter, moisture, crude protein, crude fibre, ether extract, ash and nitrogen free extract substance (NFES) contents in different sampling seasons were ranged from 89.87% to 94.15%, 5.84% to 10.13%, 16.23% to 19.04%, 14.49% to 16.64%, 0.77 % to 0.83%, 20.19% to 20.54%
and 29.42% to 31.01%, respectively. Higher dry matter, crude fiber, ash and NFES percentage were found during summer sampling season, higher moisture and ether extract content were found during spring season and higher crude protein content was found during winter season. ***Keywords:*** *Forage, proximate analysis, Pakistan.*

INTRODUCTION

Ruminants are mainly dependent on forages as these are essential to maintain their health and production at various stages of their development and growth. In developed countries, sufficient grazing land is available, so ruminants can get adequate amount of green grasses during grazing seasons and when it is not possible in other season they are supplied with silage and other high- quality conserved forages. Conversely, green forages are not abundantly available in some developing countries, so ruminants are mainly supplied with low quality forages (LQF) like cereal straws. The longevity and production are adversely affected when ruminants are reared with poor quality forage. To get more production from these ruminants it is necessary to enhance the utilization of these LQF. It may be possible to increase the nutritive value of these low-quality forages through either biological or chemical procedures (Chaudhry, 1998).

During the last five decades many studies were done to improve the quality of these forages by using different biochemical treatments. But improving the quality of forages by using these treatments was not always successful. Supplementation is another tool to improve the quality of LQF by adding nutrients that otherwise is low in these forages (Khandaker*et al.*, 1998; Muetzel*et al.*, 2003; Chaudhry, 2008). Supplements increase the utilization of LQF, but the

requirement for these supplements is more than their availability in many developing countries (Devendra and Sevilla, 2002).

Spices which have long been safely used for human consumption could be tested as alternative supplements to enhance forage utilization and reduce nutrient wastage from ruminant livestock. Recently several researchers have used some plant extracts to manipulate rumen fermentation (Cardozo *et al.*, 2004; Busquet*et al.*, 2005; Patra *et al.*, 2006a). But obtaining these extracts from plants will be costly as the extraction process will require expensive instruments and the farmers from developing countries will not be able to afford such technology. Besides, only a small quantity of these plants will be available as extracts and the rest of such plants will be unused and wasted. Furthermore, the whole spices may contain some other useful components that can differ from their small amounts of extracts and these also can have more desirable impacts on degradability and fermentation. Therefore, it is necessary to chemically analyse these spices before testing their potential use as supplements for LQF consuming ruminants to enhance forage degradability.

Seasonal variations have been shown to influence the nutritional quality as well as chemical compositions of forage species, where appropriate remedial measures were not imposed (George *et al.*, 2005). Hence, this work critically investigated the changes in the proximate composition of various forage species during different sampling seasons (summer, autumn, winter &spring).

Materials and Methods Study Site

"Livestock Experiment Station Chak No. 61/Mb, Khushab" was selected for the present investigation. Khushab is a district of Punjab province of Pakistan (Figure 1). District Khushab is home to the Heavy Water and Natural Uranium Research Reactor, a critical part of the Pakistan's Special Weapons Program, which has come under much heated scrutiny**.** Khushab with 102,793 habitants is located in Pakistan - roughly 100-mile (or 160 km) South-West of Islamabad, the seat of the Pakistani government. The co-ordinates of Khushab District are 32.2883° N, 72.2831° E.

Forage Samples

The available forage samples were collected from the same site by using sterilized apparatus. These forages were that on which the Buffalo Graze. Composite fodder samples were collected that were being served to the buffaloes at the time of blood sample collection. Traditionally mixed fodders (at least two fodder species) are served to the buffaloes. The collected forage samples during different seasons were shown in Table 1.

During different samplings seasons 10 samples were collected in each season. A total 40 forage samples were collected during all samplings. The collected forage samples were washed with distilled water and diluted HCl to remove dust particles and other contaminants. These samples were dried in air and placed in oven at 50 ^{0}C temperature for 15 days to remove all the moisture contents.

Proximate Analysis

This method was used for the study of crude protein, crude fibers, moisture contents, ether extract, ash and dry matter. Proximate Analysis Procedure including the percentage of moisture content, crude protein, ash contents and crude fiber in the sample were determined by AOAC (1990).

Dry matter detection

The amount of dry matter was investigated by using the formula:

Dry matter in percentage = 100 - moisture amount

Moisture contents detection

The amount of moisture was also found by using the following formula:

$$\text{Moisture amount} = \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample after drying}} \times 100$$

Crude protein detection

Crude protein was detected by taking sample of 1g in the flask and digested it with sulphuric acid and potassium sulphate. Boil the sample till it became transparent. Dilute the sample by adding distilled water. 10 ml of this sample was taken and distilled it with 50 mg of zinc and sodium hydro oxide. Add methyl red as indicator and titrated it with sulphuric acid until light pink color appeared. The amount of protein is measured by the amount of acid used.

Crude fibers detection

Crude fibers were measured with the help of a method of acid base digestion. 1.25% of diluted sulfuric acid and 1.25% of sodium hydroxide used. Put the sample in a beaker. 200 mL of sulfuric acid was added. For half an hour boiled the sample and chilled the sample and filter them by using filter paper. The material was washed three times by using distilled water. The material was then transferred in to the beaker and again digested by using 200 ml of sodium hydroxide, boil it for 30 minutes, cooled and then filtered to obtain residues of the sample, washed three times by using 25 mL of ethanol. This material was dried by putting it in to the oven, cooled and weight. The difference between the weights of the sample was the contents of crude fibers.

Ether Extract

A dried sample (2 g) extracted with petroleum ether (4°C-60°C) in Soxhlet apparatus to remove the ether soluble component present in it. The extracted material was dried to a constant weight in an oven at 70°C.

Ash Detection

Take a sample of 1g, burn it at 600 °C, burn all the organic contents. Ash contents were investigated by using formula:

$$\text{Ash \%} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Net Energy: (crude protein x 4) + (ether extract x 8.7) + NFES

Nitrogen free extractable substance (NFES)

100 – (Crude protein + mineral matter + ether extract + crude fiber)

Statistical Analysis

Data for different attributes were subjected to a statistical analysis using the SPSS (Statistical Package for Social Sciences) software, and one-way analysis of variance (ANOVA). Statistical significance between the mean was tested at 0.05, 0.01 and 0.001 level of probability as suggested by Steel *et al.* (2006).

Results and Discussion Dry Matter (%)

The analysis of variance showed significant ($p<0.001$) result of dry matter (%) in all sampling seasons (Table 2). The mean dry matter contents in different sampling seasons were ranged from 89.87% to 94.15% (Figure 2). Higher dry matter percentage was found during summer sampling season and lower was observed during spring sampling season (Table 3). The percentage values of dry matter in different seasons were summer (94.151%), autumn (94.03%), winter (90.391%) and spring (89.87%). The order of the dry matter percentages in all the forage samples during investigations were detected as summer>autumn>winter>spring. The percentage dry matter contents in forage samples showed consistent pattern of decrease along the sampling seasons.

Dry matter (DM) refers to material remaining after removal of water, and the moisture content reflects the amount of water present in the feed ingredient. The nutrients in feeds, required by the animal for maintenance, growth, pregnancy, and lactation are part of the DM portion of the feed. The DM concentration presented in this study was greater than the values as recommended by Torres (1983). These values were lower than the values already demonstrated by Ahmad *et al.* (2010) in Punjab, Pakistan. The DM value of the forages in this study was higher than the values determined by Olugbemi*et al.* (2010) and Mutayoba*et al.* (2011). The DM values reported by these researchers ranged from 87.2% to 93.7%.

Moisture Contents (%)

The percentage moisture contents in forage samples varied significantly ($P<0.001$) by all sampling seasons (Table 2). The mean moisture contents in various sampling seasons were ranged from 5.84% to 10.13% (Figure 3). The maximum moisture contents (%) were noticed in forage samples collected during spring sampling season and the minimum moisture contents

(%) were noticed during summer sampling season (Table 3). The percentage values of moisture contents in different seasons were summer (5.84%), autumn (5.96%), winter (9.61%) and spring (10.13%). The order of the moisture percentages in all the forage samples during investigations were noticed as spring>winter>autumn>summer. The percentage moisture contents in forage samples showed consistent pattern of increase along the sampling seasons.

The observation during present study as much lower than the values obtained by Little (1967) while working in unfertilized medium in Sudan. Similar trend was found during earlier study by Oduro *et al.* (2008). The variation in the nutritional values will differ for a wide range of reasons, such as cultivated regions, growing conditions, and nature of soil, seasonal changes, genetically different cultivars, storage conditions or due to the period of analysis (McCance and Widdowson 1992; Imeh*et al.*, 2002).

Crude Protein (%)

The results of the present study showed that the crude protein (CP) (%) in forage affected significantly (P<0.001) by various sampling seasons (Table 2). The mean CP contents in different sampling seasons were ranged from 16.23% to 19.04% (Figure 4). The higher CP contents (%) were observed in forage during winter sampling season and the lower CPcontents (%) were found during autumn sampling season (Table 3). The percentage values of CP concentration in various seasons were summer (18.87%), autumn (16.24%), winter (19.04%) and spring (17.93%). The order of the investigated CP percentage in all forage samples were determined as winter>summer>spring>autumn. The percentage CP in forage samples explicit inconsistent pattern of increase and decrease along the various sampling seasons.

The CP contents presented in this study are above the critical value (7.0%) as reported by Minson (1971). Kiatoko *et al.*, (1982) also reported the range of normal mean protein values as 8.45-9.4% for the seasons. This agrees with Ganskopp and Bohner (2001) who reported high CP contents in various food stuffs. All organisms need protein for maintaining growth and reproduction. The deficiency of proteins leads to reduced appetite, low feed intake and poor food efficiency that in turn results in poor growth and development of animals and humans (Anonymous, 1981, 1985). The present results agree with the results of Vallentine (1990), Holeche*et al.* (1998) and Robles and Boza (1993), who also reported high CP contents in different plants. For healthy productivity a continuous supply of CP is required (Holeche*et al.*, 1998). The CP value reported by Olugbemi*et al.* (2010) was lower (27.44%) than the value obtained in this study (16.23%-19.04%). Plants need more nitrogenous food for vegetative growth and therefore they efficiently store protein in early stages of growth, which is later consumed during flowering and fruiting followed by dormant phase whereby their nutritional status reduces. During this period, dry spell from May to October is characteristic for the area (Durrani and Hussain, 2005). This is the time when besides reduction in overall availability of food (Hussain and Durrani, 2007), plants also become poor in quality including CPcontents. The present study showed that all forage samples generally contained adequate CP content.

Crude Fiber (%)

The statistical analysis showed that the crude fiber (%) in forage affected significantly (P<0.001) by different sampling seasons (Table 2). The mean crude fiber quantities in different sampling seasons were ranged from 14.49% to 16.64% (Figure 5). The maximum crude fiber contents (%) were found in forage during summer sampling season and the minimum crude fiber contents (%) were found during autumn sampling season (Table 3). The percentage values of crude fiber concentration in sampling seasons were summer (16.64%), autumn (14.49%), winter (15.67%) and spring (15.53%). The order of the crude fiber percentage contents in all the forage samples during the present research were observed as summer>winter>spring>autumn. The percentage crude fiber contents in forage samples showed inconsistent pattern of increase and decrease along the different sampling seasons.

The values observed in the present study were similar with the value observed by Holeche*et al.*, (1998). Seasonal variation affects the crude fiber contents (Azim *et al.*, 1989). Crude fibers are less nutritional than cell contents due to its slow digestibility. The range of crude fiber in the present study was greater when compared to other plants (Ashraf *et al.*, 1995).

As the plants became older, the crude fiber tended to increase for all plants, but for some plants more than others. The crude fiber contents found in the present study was ranged from 16.45 to 18.15% which is lower from the recommended value (31.6%) reported by Morrison (1961). Therefore, adequate amount of crude fiber was present for the proper growth and need of peoples in this studied land. Though the crude fiber contents of the sample was observed to be a little higher compared to results of Esenwah and Ikenebomeh (2008), andOmafuvbe*et al.* (2004).

Ether Extract (%)

According to the analysis of variance the percentage ether extract concentrations in forage samples affected non-significantly (P>0.05) by sampling seasons (Table 2). The mean ether extract contents in different sampling seasons were ranged from 0.77 % to 0.83% (Figure 6). The higher ether extract contents (%) were found in forage samples collected during spring sampling season and the lower ether extract contents (%) were observed during autumn sampling season (Table 4). The percentage values of ether extractcontents in different seasons were summer (0.78%), autumn (0.83%), winter (0.800%) and spring (0.77%). The order of the observed ether extract percentage in all the forage samples were determined as autumn>winter>summer>spring. The percentage ether extract in forage samples showed inconsistent pattern of increase and decrease along the various sampling seasons.

The present values were higher than the values observed by Martinez (1994). Ether extract is the lipid component and the energy derived from it is utilized by the animal for body maintenance and production. The higher value of ether extracts in some of the tested samples is an indication of higher energy level for the humans (Babayemi and Bamikole, 2006; Odedire and Babayemi, 2008) and this is a major form of energy storage in plants which is being utilized by the humans for body maintenance and production. All plant parts have nutritional qualities which when used in the right proportions could be of tremendous benefit to the body. Further studies will concentrate more on the use of the extracts of these plants in laboratory animals to determine their metabolic effects. The values obtained for ether extract were within the ranges of reported values (Ifon and Bassir, 1980).

Ash (%)

According to the data of analysis of variance it was observed that the ash percentage concentrations in forage were differed non-significantly (P>0.05) by sampling seasons (Table 2). The mean ash contents in different sampling seasons were ranged from 20.19% to 20.54% (Figure 7). The higher ash contents (%) were observed in forage during summer sampling season and the lower ash contents (%) were found during autumn sampling season (Table 4). The percentage values of ash concentration in various seasons were summer (18.87%), autumn (16.24%), winter (19.04%) and spring (17.93%). The order of the ash percentage concentrations in all the forage samples during the present research were observed as winter>summer>spring>autumn. The percentage ash contents in forage samples showed inconsistent pattern of increase and decrease along the different sampling seasons.

Ash contents play important role in promoting balanced growth of humans. Similar to the present findings, Kilcher (1981) reported that ash contents of food progressively decline with advancing maturity. However, Azim *et al.* (1989), Wahid (1990) and Liu (1993) observed high ash contents with increasing degree of maturity of plants. In the present study, it was also recorded higher ash contents in all plant samples. This increase or decrease of ash contents at all sites by different plants may be due to variation in soil and other habitat features that need to be explored. The ash content as shown in which is an indication of the presence of ash % is within the ranges of reported values (Amata and Lebari, 2011; Hassan and Umar, 2006).

Nitrogen Free Extract Substance-NFES (%)

In the current research it was observed that the NFES (%) in forage affected non-significantly (P>0.05) by different sampling seasons (Table 2). The mean NFES quantities in various sampling seasons were ranged from 29.42% to 31.01% (Figure 8). The highest NFES content (%) was found in forage during summer sampling season and the lowest NFES content (%) was found during winter sampling season (Table 4). The percentage values of NFES concentration in sampling

seasons were summer (31.01%), autumn (29.86%), winter (29.43%) and spring (30.83%). The order of the NFES percentage concentrations in all the forage samples during present investigation were found as summer>spring>autumn>winter. The NFES concentration was lower than the critical values established by Ahmad *et al.* (2010) at all sites. The mean values of nitrogen free extract during current study are almost close to the values reported by Hussain (1985).

Conclusion

There is a need general evaluation about crude protein, crude fibers, moisture contents, ether extract, ash and dry matter. It is important to check the health status of the animals that feed on these forages and we should add some mineral mixture in the diet of the animals

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Table 1. The collected forage samples during different seasons

Samplings	Season	Common Name	Scientific name
1st	Summer	Maize Jowar	*Zea mays Sorghum bicolor*
2nd	Autumn	Bajra Guar	*Pennisetumglaucum* *Cyamopsistetragonoloba*
3rd	Winter	Barseeem Lucerne	*Trifoliumalexandrinum Medicago sativa*
4th	Spring	Sarsoon Barseem	*Brassica campestris Trifoliumalexandrinum*

Table 2. Analysis of variance for proximate data of forages at various sampling seasons.

Source of Variation (SOV)	Mean Squares		P-Value
	Sampling Season	Error	
Dry Matter (%)	158.232	10.991	***
Moisture (%)	158.232	10.991	***
Crude Protein (%)	51.392	5.566	***
Crude Fiber (%)	23.111	3.152	***
Ether Extract (%)	0.019	0.008	**ns**
Ash (%)	0.896	2.754	**ns**
NFES (%)	17.205	18.752	**ns**
Degrees of Freedom (df)	3	36	----

P <0.001= ***, P >0.05=ns

Table 3. Descriptive Analysis for Dry Matter, Moisture, Crude Protein and Crude Fiber Concentrations in Forage Influenced by Different Seasons

Parameters	Dry Matter (%)	Moisture (%)	Crude Protein (%)	Crude Fiber (%)
Mean	92.111	7.890	17.932	15.585
Std. Error±	0.350	0.350	0.237	0.175
Median	93.335	6.665	17.830	15.660
Mode	94.140[a]	5.250[a]	14.990[a]	15.660[a]
Std. Deviation	3.835	3.835	2.593	1.912
Minimum	82.750	2.550	11.390	11.360
Maximum	97.450	17.250	26.410	19.820

a. Multiple modes exist. The smallest value is shown

Table 4. Descriptive Analysis for Dry Matter, Moisture, Crude Protein and Crude Fiber Concentrations in Forage Influenced by Different Seasons

Parameters	Ether Extract (%)	Ash (%)	NFES (%)
Mean	0.800	20.372	30.288
Std. Error±	0.008	0.150	0.395
Median	0.811	20.280	29.030
Mode	0.789[a]	19.850	27.840[a]
Std. Deviation	0.090	1.645	4.326
Minimum	0.611	15.850	18.590
Maximum	0.977	23.950	39.580

a. Multiple modes exist. The smallest value is shown

Figure 1. Geographical Location of Research Site

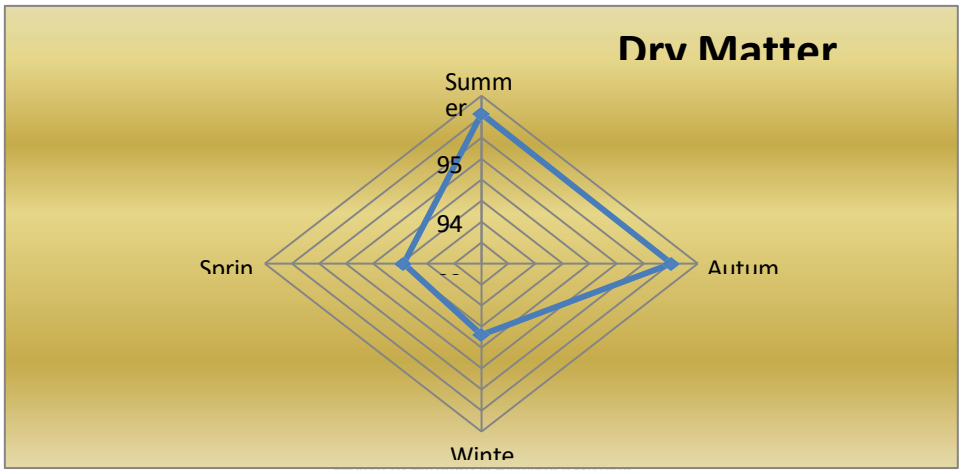

Figure 2. Fluctuations in dry matter (%) values in forage at different seasons

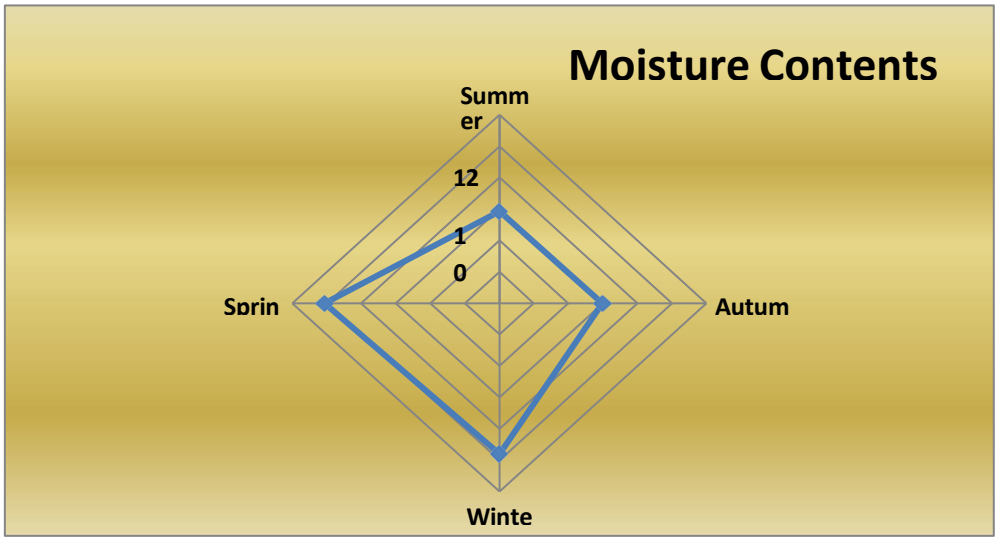

Figure 3. Fluctuations in moisture (%) values in forage at different seasons

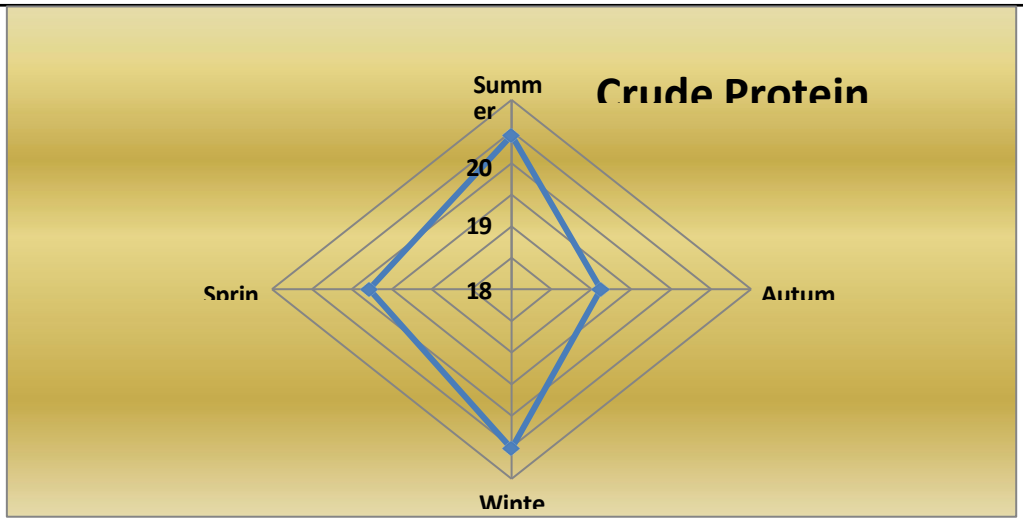

Figure 4. Fluctuations in crude protein (%) values in forage at different seasons

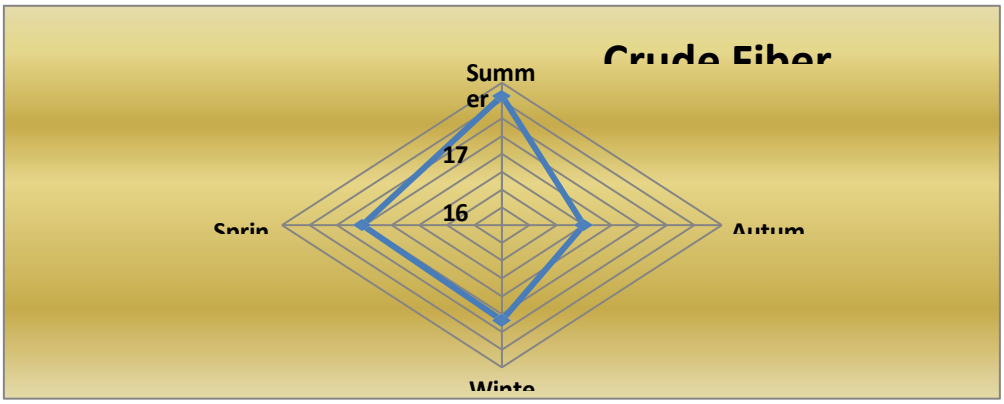

Figure 5. Fluctuations in crude fiber (%) values in forage at different seasons

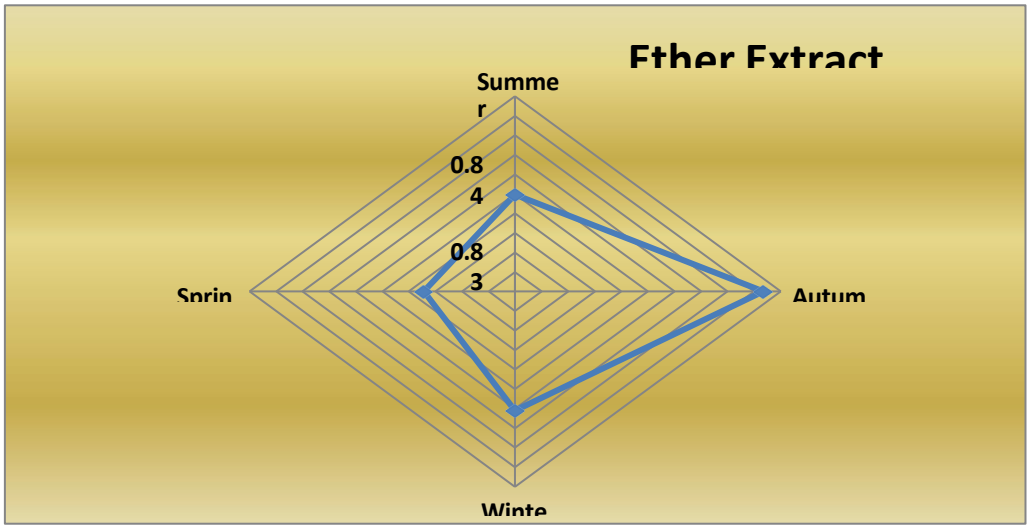

Figure 6. Fluctuations in ether extract (%) values in forage at different seasons

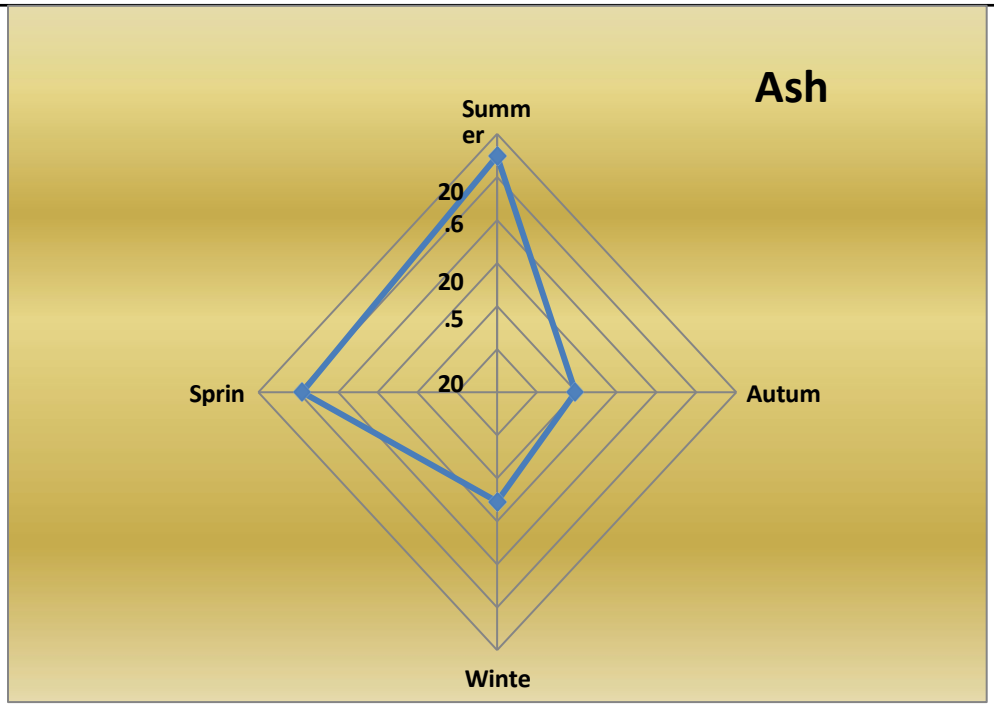

Figure 7. Fluctuations in ash (%) values in forage at different seasons

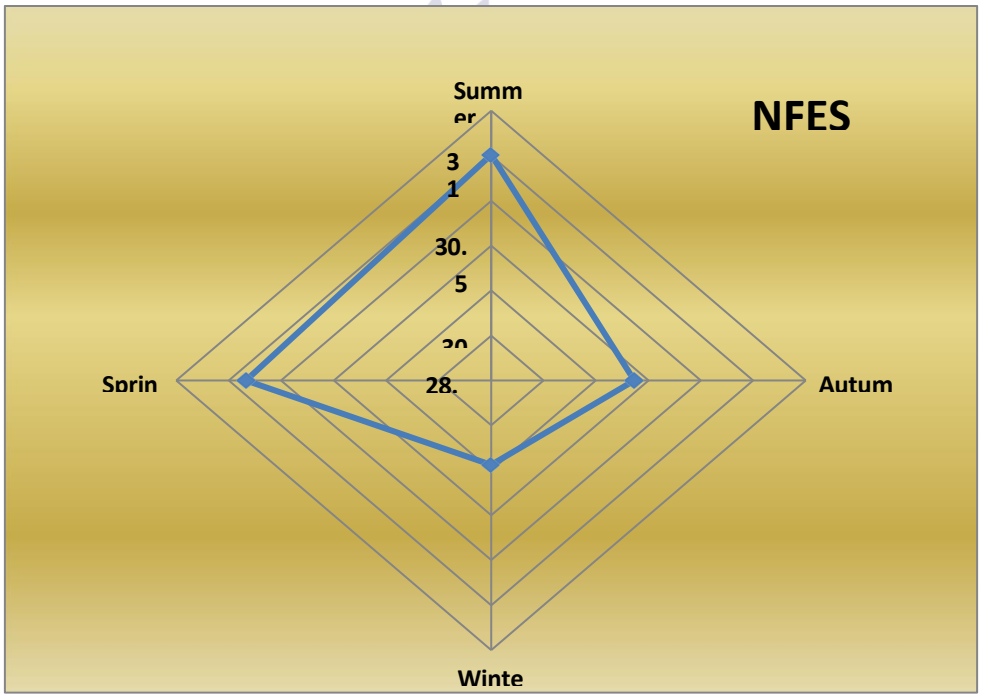

Figure 8. Fluctuations in NFES matter (%) values in forage at different seasons