NUTRITIONAL STATUS AT PHENOLOGICAL STAGES IN SOME NOXIOUS WEEDS

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ABSTRACT

Most weeds are palatable and of acceptable quality for animal feed in some areas. Weeds can be high in nitrates so it is prudent to test these plants for feed quality as alternative fodder. Present study was aimed to investigate the nutritional potential of five noxious weed species (Solanum nigrum, Parthenium hysterophorous, Agertum conizoids, Ranunculus sclerosis and Brasssica nigra) at three phenological stages (vegetative, reproductive and post-reproductive), which are commonly grazed by livestock in Rajowal, district Okara. Whole weed plants (except root) were collected, cleaned, dried and then ground into powder form. Dried weed powder was used for proximate analysis. Resultsexhibited that S. nigrum, P. hysterophorous, A. conizoids, R. sclerosis and B. nigra have varying level of nutritional values at three phenological stages. These weed showed appreciable range of moisture (77.15-88.37 %), fibre (0.48-8.82%), fats (5.12-8.53%), protein (10.02-27.15%) carbohydrate (40.18-65.5%) and ash (22.71-10.3%) at three phenological stages It can be concluded that S. nigrum, P. hysterophorous, A. conizoids, R. sclerosis and B. nigra weeds are good source of nutrition, however, cannot be recommended as alternative to conventional fodder crops, as these weeds are reported to contain harmful compounds.

Key words: Nutrition, phenology, proximate analysis, weeds.

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INTRODUCTION

Weeds are unwanted plants competing with crops for water, space, nutrients, light and release allelochemicals into the rhizosphere (Khaliq *et al*., 2013; 2014ab). Weeds usually disturb the normal

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developmental growth of crops (Qureshi *et al*., 2009) and are also limiting factor for crop production by causing serious loss in yield of grains, seeds and fruits etc. (Abbas *et al*., 2010). In spite of all negative aspects, weedy species are important traditional medicine for treating several diseases (Lewu and Afolayan, 2009) and considered an ideal livestock feed compared to most available feed resources.

Weeds have been utilized as a source of livestock feeding in various regions of Pakistan. Nearly all the grass species and many dicotyledonous are grazed by cattle. Weed species like *Stellaria media*, *Taraxacum officinale*, *Malva neglecta*, *Medicago denticulate* and *Solanum nigrum* are used as vegetables. Many other species such as *Chenopodium album*, *Cymbopogon jwarancusa*, *Boerhaavia diffusa*, *Chrozophora oblique*, *Datura metel*, *Rhazya stricta*, *Vitex trifolia*, *Withania somnifera*, *Albizia lebbeck*, *Tamarix aphylla* etc., have been used as forage for cattle, fuel and as medicine for fever, stomach problems, respiratory tract infections, and high blood pressure by local communities in Balochistan central Punjab, Pakistan (Khan *et al*., 2013; Zereen *et al.,* 2013).

Since centuries, weeds are being used as forage of cattle both in settled and nomadic animal grower worldwide. Common weeds are a silent source of food and fodder for animals with high nutritional potential, antioxidant activity and medicinal importance. According to facts, a more than 50% of total fodder for livestock comes from weeds on the farms (Paulavon, 2003). However, some type of weeds plants grazing is among the important causes of economic loss and health issues in livestock. These plants as fodder or direct grazing affect animals and cause chronic illness, decreased weight gain, abortion, birth defects and death. In district Okara, villagers prefer to graze their animals on some toxic weeds. Therefore, aim of present study was to estimate the nutritional values of these common weeds which are being used as the fodder/or grazing animals in Rajowal, district Okara, Pakistan.

MATERIALS AND METHODS

Experimental work was performed in Plant Biotechnology and Organic Food Lab, Pakistan Council Scientific and Industrial Research (PCSIR), Lahore. Following methodology was adopted to carry out present study.

Sample Collection and Preparation

Five weeds (*Solanum nigrum, Parthenium hysterophorous, Ageratum conizoids, Ranunculus sceleratus* and *Brassica nigra*), were collected, from surrounding of Rajowal, district Okara. Collected whole plant (except root) sample at three phonological stages (vegetative, reproductive and post reproductive), was thoroughly washed, sun dried and grounded. The powder sample of each weed was stored in sterilized seal bags at room temperature for further use.

Proximate Analysis

Estimation of moisture, ash, protein, carbohydrate and fat, was done by following methods;

Moisture content

Each weed sample was transferred and distributed evenly to a depth in the crucible for determination of the moisture content. Sample was heated at 110°C in hot air oven and weighed at five minutes intervals until a constant weight was obtained. The difference in dry and initial weight was calculated as the moisture content. Same experiment was repeated three times for precision and percent moisture for the all samples were calculated. Moisture content was calculated according to Ranganna (1986);

$$\text{\% of moisture content} = \frac{W1 - W2}{W1 - W} \times 100$$

Where, W = Weight of the empty crucible; W1 = Weight of crucible + sample; W2 = Weight of crucible + dried sample

Total ash

Oven dried weed sample was incinerated at 550°Cin muffle furnace until free from carbon. The crucibles were cooled to room temperature and weighed. Percentage of total ash was calculated with reference to air dried substance (Ranganna, 1986).

$$\text{\% of ash content} = \frac{W1 - W}{W2 - W} \times 100$$

Where, W = Weight of the empty crucible; W1=Weight of sample heated at 550°C; W2 = Weight of crucible + sample

Determination of Protein

Digestion of sample

Plant sample of 1gm (w1) was taken in the Kjeldahl flask. Selenium oxide (0.5g) was added into flask followed by 20mL of conc. H_2SO_4 for plant material digestion. Mixture was boiled at 350°C for about 5-6 hours until it become transparent. The digested material was transferred to the volumetric flask to make the volume up to 100 mL and carried out for distillation.

Steam Distillation

Digested material (5mL), 10mL of 40% NaOH solution added in distillation Kjeldahl flask to liberate ammonia. Vapors were collected in a 100mL beaker, containing 10mL of 2% boric acid with methyl red dye. The distillation was continued color of boric acid turned yellow. The boric solution was used for titration.

Titration

Distilled boric acid was titrated against standard N/70 HCl till pink color appeared. Protein content was calculated as mention Ranganna (1986);

$$\% \text{ Nitrogen} = \frac{\{\text{sample titre} - \text{blank titre}\} \times \text{Normality of HCL} \times \text{Volume madeup of the digestion} \times 100 \times 14}{\text{aliquot of the digest taken} \times \text{Weight of sample taken}(w1) \times 1000}$$

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25 \text{ (Factor for calculations)}$$

Determination of crude fibre

Plant material (1 g) and 100mL of 1.25% H_2SO_4 was added in a round bottom flask, condenser was attached and boiled for 30 minutes. Sample residues was filtered with the muslin cloth followed by washing with hot water and was transferred to the flask again separately. A 100mL of 1.25% NaOH was added to each sample residues and set the apparatus for boiling again for 30 minutes. Residues were filtered using ash less filter paper followed by washing with hot water. Sample residue was left at room temperature and later oven dried over night at 50°C. Dried plant residue was weighed and put it in muffle furnace at 550°C for 5 hours. Ash of plant sample was cooled in desiccators for an hour, weighed again and the difference in the two weights of the sample was noted down and fibre was calculated with formula used by Akubugwo *et al*. (2007);

$$\% \text{ Crude fibre} = \frac{\text{Loss in weight of sample}}{\text{initial weight of sample}} \times 100$$

Determination of crude fat

Dried plant material (1g) was taken in thimble. Dip the thimble in 100 ml n-hexane for 24 hours. After that removed n-hexane and remained the thimble to dry at room temperature till n-hexane evaporated and then for 1 hour in oven at 100°C. Cooled and weighed the sample. The difference in the weights gives the n-Hexane soluble material present in the sample (Ranganna, 1986).

$$\% \text{ Crude fat} = \frac{\text{Wt. of n} - \text{Hexane soluble material}}{\text{Wt. of sample}} \times 100$$

Determination of carbohydrates

Total carbohydrate in plant sample was estimated according to Akubugwo *et al.* (2007) by following formula;

$$\text{carbohydrate } (\% \text{ dw}) = \{100 - \text{Fibre } (\%) - \text{Protein } (\%) - \text{fat } (\%) - \text{Ash } (\%)\}$$

Statistical analysis

The data were statistically analyzed as means ± SE and one-way analyses of variance (ANOVA) by Costat to evaluate significant difference between nutritional values in selected weeds.

RESULTS AND DISCUSSION

Results exhibited that moisture, ash and fibre percentage (%) were significantly different at three phonological stages in *S. nigrum* (Figure 1). However, reduction in moisture, fibre, and ash contents was recorded with majority of *S. nigrum* plants; whereas, proteins, fats and carbohydrates contents were higher at majority. Higher protein, (27.15%), fats (6.03%) and carbohydrates (50.21%) contents were recorded at post-reproductive stage followed by vegetative stage. Whereas, maximum moisture (88.37%), fat (6.03%) and ash (20.36%) were found in vegetative stages.

In case of *P. hysterophorous*, minimum difference between moisture at three phonological stages was recorded (Figure 2). However, reduction in moisture, ash and fibre was found with majority of plant. *P. hysterophorous* has maximum percent moisture while fibre was found in very low quantity at three phonological stages. Though, protein, fat and carbohydrates contents were increased with majority; whereas, post-reproductive stage had maximum protein (22.56%), fats (6.37%) and carbohydrates (59.17%) followed by vegetative stage. While, maximum carbohydrates (64.95%) were found at reproductive stage of *P. hysterophorous*.

Present results revealed reduction in fibre, moisture and ash percentage (%) in all stages of *A. conizoids* (Figure 3). Ontherotherhand, protein, fat and carbohydrate contents were showed increasing trend with maturity. Post-reproductive stage has maximum protein, %fat and carbohydrates followed by vegetative stage. Maximum moisture and fibre were 84.93% and 8.82% found in vegetative stages while maximum ash was found 22.16% found in reproductive stage. Maximum fat, protein and carbohydrates were 8.53%, 23.44% and 46.8% found in post-reproductive stage respectively.

In the present study, results concluded that *R. sceleratus*hasmaximum protein (14.45%) and fat (8.28%) at post-reproductive stage followed by vegetative stage (Figure 4). However the difference between theses contents was very low. Maximum moisture and ash were 82.07% and 13.34% found at vegetative stages. Maximum carbohydrates were 65.5% found in vegetative stage followed by minimum 52.31% in post-reproductive stage. Fibre (%) was low in *R. sceleratus* at three phonological stages.

Present study exposed minimum difference in ash, moisture and fibre percentage (%) between three phonological stages of *Brassica nigra* (Figure 5). Maximum moisture was 87.47% at reproductive stage. The percentage values of ash and fibre (16.54%, 2.42%) were found maximum at post-reproductive stage. While maximum protein (25.63%), fat (7.9%) and carbohydrates (52.58%)

were present at post-reproductive stage followed by vegetative stage. Max carbohydrates (56.3 %) were found at reproductive stage *B. nigra.*

Results indicated that moisture was significantly different, ranges from (77.15%-88.37%) at three stages in all weed samples (Figure 6). The present study directed that %moisture was maximum (88.37%) at vegetative stages followed by minimum (77.15%) at post-reproductive stage in all studied weed samples. *B. nigra* has maximum moisture (87.22%, 87.47%, and 87.28%) while *R. sceleratus* has minimum moisture (82.07%, 81.19%, and 77.41%) at three phonological stages respectively.

In current present study the ash was significantly varies from (22.71%-10.3%) at three phonological stages from each other (Figure 7). Ash content was recorded minimum (13.34%, 13.30% and10.3%) in *R. sceleratus*and maximum ash (21.75%, 22.16% and 15.51%) was found at all three phonological stages of *A. conizoids* respectively. There was an increase in ash from vegetative stage followed by post-reproductive stage in all studied weed samples.

In case of fat, the result revealed that there was a significant increase from vegetative stage to post-reproductive stage in all weed samples (Figure 8). *A. conizoids*has highest (8.53%) fat at post-reproductive stage followed by W4, W5, W2 and W1. Whereas at reproductive stage, maximum %fat was present in W5 (7.58%) followed by W4, W3, W2 and W1. In the present study, the minimum fat (5.12%, 5.13% and 6.03%) was shown at vegetative, reproductive and post-reproductive stage in W1.

The protein was significantly different at three stages in all weed samples (Figure 9). The protein consents were maximum at post-reproductive stage and minimum at vegetative stages in all studied weed samples. The minimum protein (10.02%) was found at vegetative stage of W2 while maximum protein (27.15%) was estimated at post-reproductive stage of W1. Maximum protein (22.79%, 22.97% and 27.15%) was also recorded at three growth stages of W1. While W4, showed minimum protein level (12.63%, 14.03% and 14.15%) was at three phenological stages.

Fibre percentage (%) has decreased significantly from vegetative to post-reproductive stages in weed samples (Figure 10). Maximum fibre (8.82%, 8.07% and 7.07%) was estimated in W3 and minimum (1.01%, 1.005% and 0.89%) in W4 at three phonological stages respectively. So it is concluded that W3 has much fibrous than other weed samples. The carbohydrate contents of weed samples were significantly different from each other at three phonological stages (Figure 11). *P. hysterophorous* has maximum carbohydrates (64.56%) at reproductive and post-reproductive (59.17%) stages; while *R.*

sceleratus has maximum (65.5%) and *A. conizoids* contains minimum carbohydrates (40.92- 46.8%) at vegetative stage.

Livestock is an important sector of agriculture, depends upon quality forage for the growth of this industry. Animal productivity depends upon quantity and nutritive quality of vegetation available to grazing animals. Sultan *et al*., (2007) suggested that there is a need of 13.5 and 110.3 m tons of crude proteins and total digestible nutrients for maintaining livestock in year however, the present feed resources only provide 40% and 75% of crude protein and total digestible nutrients. Many studies have also assessed the nutritional value of wild plants in natural rangelands (Khan *et al*., 2002; Khan *et al*., 2005) and in cultivated fodder species.

Maximum ash (20.36%) and crude fibre (3.61%) at vegetative stage and maximum protein content (22.97%), and carbohydrates (50.39%) at reproductive stage were recorded in S. nigrum. Akubugwo *et al.* (2007) also reported ash (8.05-10.18%), crude fibre (6.29-6.81%), protein content (17.63- 24.90%), and carbohydrate (53.51 and 55.85%) in leaves and seed samples of S. nigrum. In a similar study, Hussain *et al.* (2010) observed a progressively decline in ash content towards maturity in some plants. Similar results have been found in present study, where minimum ash (10.3%) was found in post-reproductive stage followed by maximum ash (22.71%) at vegetative stage in *R. sceleratus*.

In selected weeds moisture percentage was also recorded from 77.15 - 88.37% with a significant variation. Similar finding were documented by Hussain *et al.* (2010) in different weeds species. In another study, high moisture content was also recorded in green leafy vegetables (Das *et al*., 2009), parallel with the present findings where maximum moisture (88.37%) was found in vegetative stage of *S. nigrum*.

Plant material is divisible into fibrous and non-fibrous fractions. In ruminants, fibre fractions that provide energy are important as celluloses and hemicelluloses are easily digestible. Present results revealed maximum crude fibre (8.82%) in vegetative stage of *A. conizoids* followed by minimum (0.48%) in post-reproductive stage of *B. nigra*. Similarly, Hameed and Hussain (2015) also reported that the crude fibre contents decreased from vegetative stage to post reproductive stages in some plants of Solanaceae. However, fibre contents in tested weed species were low.

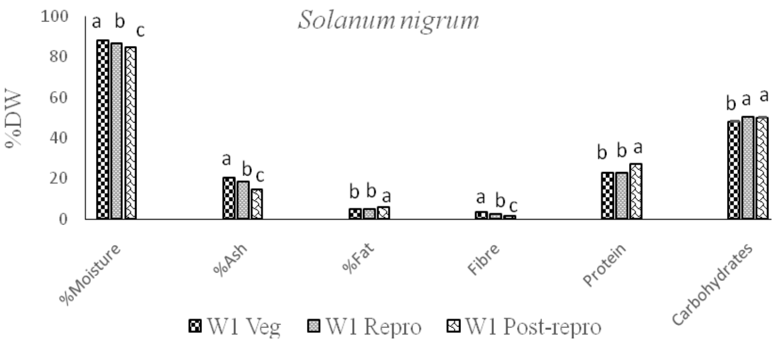

Figure 1. Nutritional status of *Solanum nigrum* (W1) at three phonological stages (vegetative, reproductive, post-reproduc.)

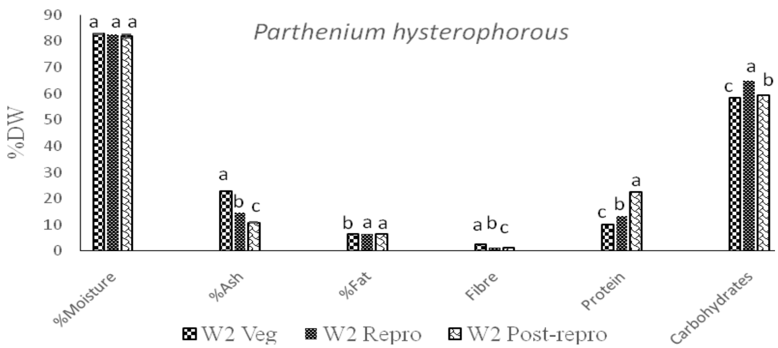

Figure 2. Nutritional status of *Parthenium hysterophorus* (W2) at three phonological stages (vegetative, reproductive, and post-reproductive).

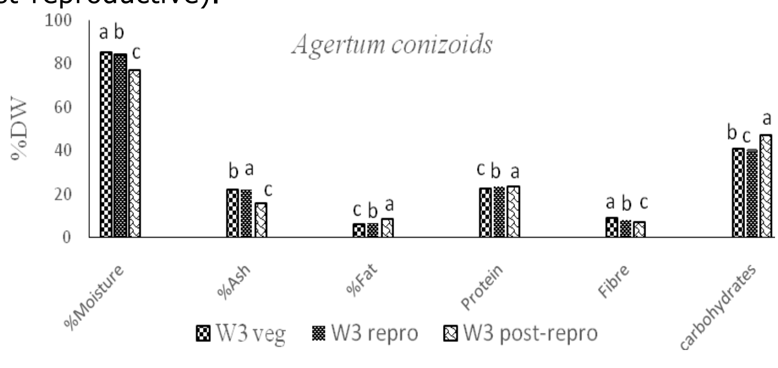

Figure 3. Nutritional status of *Ageratum conizoids*(W3) at three phonological stages (vegetative, reproductive, and post-reproductive).

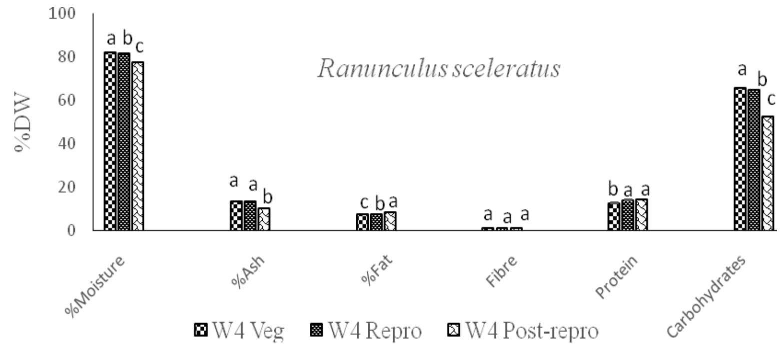

Figure 4. Nutritional status of *Ranunculus sceleratus* (W4) at three phonological stages (vegetative, reproductive, and post-reproductive).

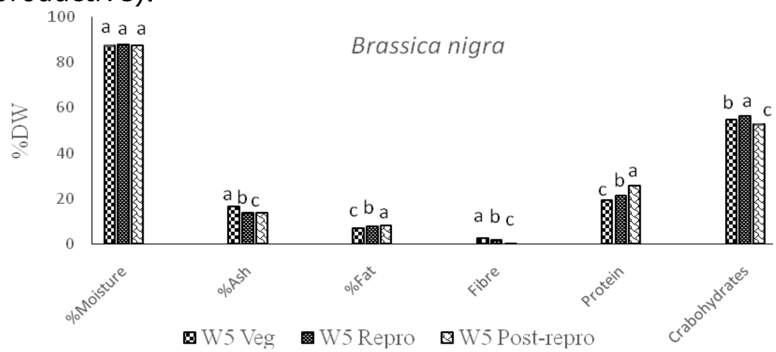

Figure 5. Nutritional status of *Brassica nigra* (W5) at three phonological stages (vegetative, reproductive, and post-reproductive).

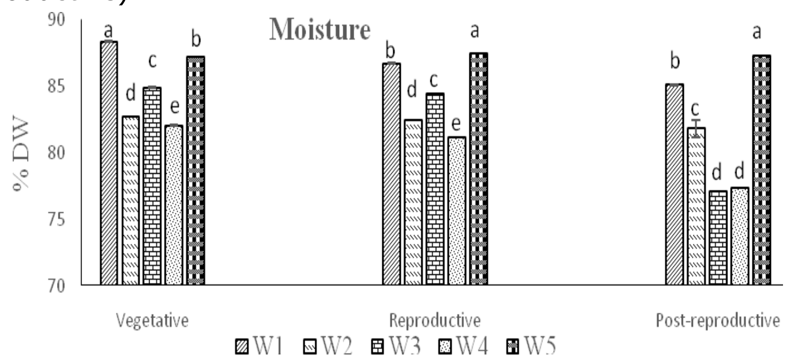

Figure 6. The percent moisture at three phonological stages (vegetative, reproductive and post-reproductive) in *Solanum nigrum* (W1), *P.hysterophorus* (W2), *A. conizoids* (W3), *R. sceleratus* (W4) and *B. nigra* (W5).

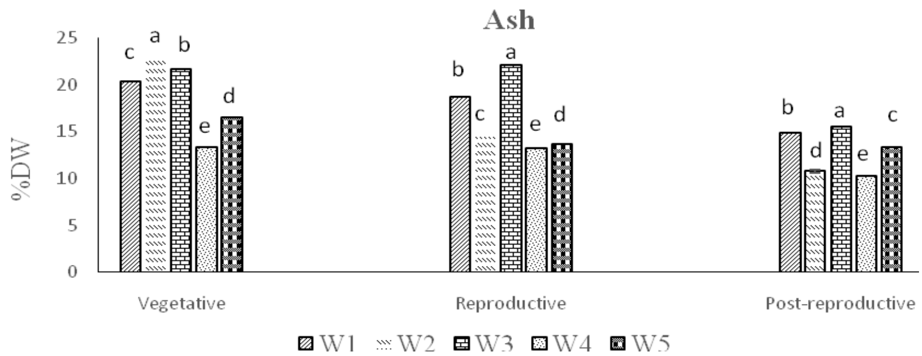

Figure 7. Ash at three phonological stages (vegetative, reproductive and post-reproductive in *S. nigrum* (W1), *P. hysterophorus* (W2), A*. conizoids* (W3), *R. sceleratus* (W4) and and *B. nigra* (W5)

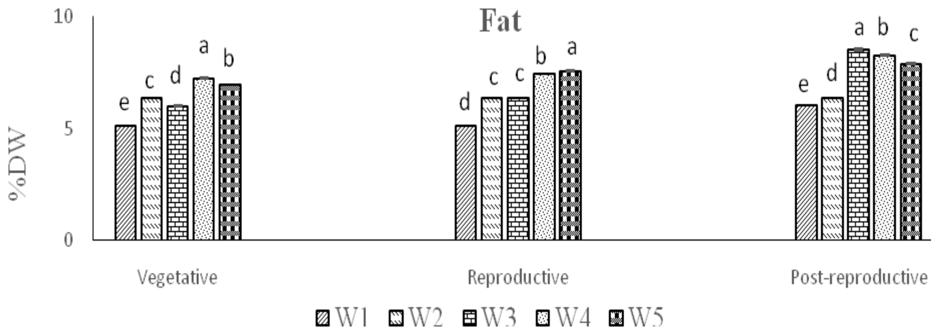

Figure 8. Fat at three phonological stages (vegetative, reproductive and post-reproductive in *S. nigrum* (W1), *P. hysterophorus* (W2), *A. conizoids* (W3), *R. sceleratus* (W4) and *B. nigra* (W5).

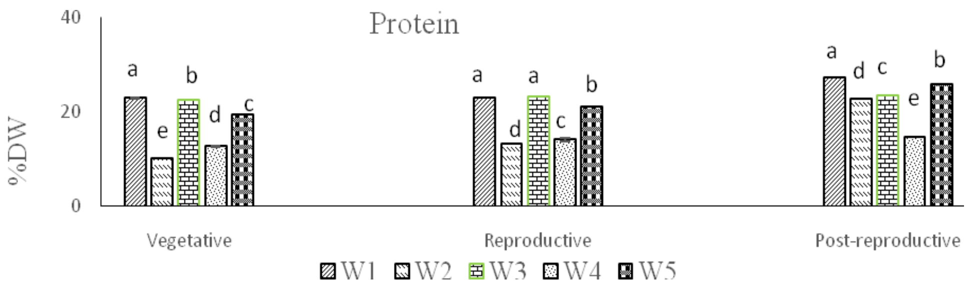

Figure 9. Protein at three phonological stages (vegetative, reproductive and post-reproductive in *S. nigrum* (W1), *P. hysterophorus* (W2), *A.conizoids (*W3), *R. sceleratus* (W4) and *B.nigra* (W5).

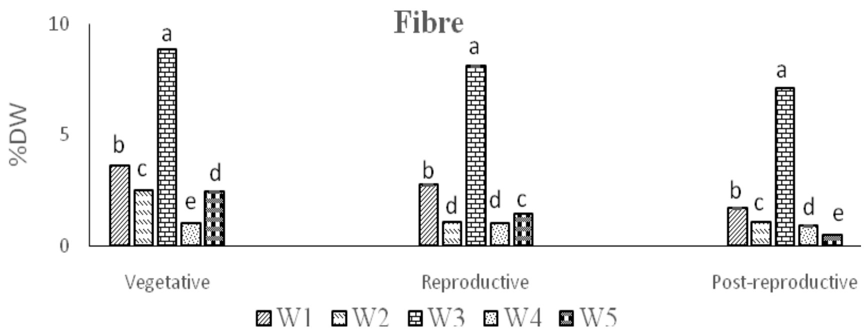

Figure 10.Fiber at three phonological stages (vegetative, reproductive and post-reproductive in *S. nigrum* (W1), *P. hysterophorus* (W2), *A. conizoids* (W3), *R. sceleratus*(W4) and *B. nigra* (W5)

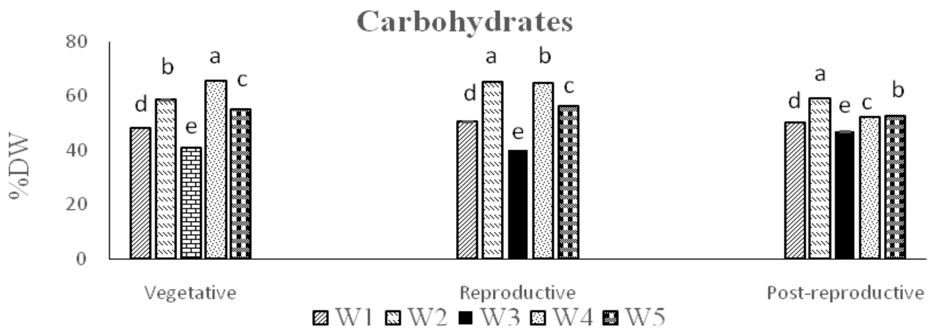

Figure 11. Carbohydrates at three phonological stages (vegetative, reproductive and post-reproductive in *S. nigrum* (W1), *P. hysterophorus* (W2), *A. conizoids* (W3), *R. sceleratus* (W4) and *B.nigra* (W5).

Ganskopp and Bohner (2001) revealed that crude protein, referring to all the nitrogenous compounds present in forage feed, is reliable source of overall nutritional status. They are directly related to digestibility, vitamin, calcium and phosphorus contents. These all features decline with low crude protein to almost deficient levels. Cheema *et al.* (2011) stated that differences in crude protein are due to differences of capability of plants to accumulate protein. These findings are in accordance to present study where, difference in amount of protein among the weed samples is recorded. Shah *et al.* (2009) also revealed that protein rich plants had 23%-33% protein and it is related to the present study where maximum protein is 27.15% in post-reproductive stage of S. nigrum and minimum 10.2 % in vegetative stage of *P. hysterophorous*.

During current investigation, an increasing trend of carbohydrates (%) ranges from 40.92-65.5-46.8-59.17% was recorded in vegetative reproductive to post-reproductive stages respectively. Similarly, Hameed and Hussain (2015) also concluded that the carbohydrates contents vary from 12.08-51.43% in vegetative stages, 8.38-67.17% in reproductive stages and 29.32-75.71% in post reproductive stages in some plants of family solanaceae. Holechek *et al.* (1998) stated that high level of carbohydrate in fodder plants are considered better than high lignin contents as they provide readily available energy and easily digestible. Its requirement cannot be ordinarily compensated by protein.

The use of weed plants as animal fodder and dietary supplements is very common practices in many parts of Pakistan like other regions of the world. However, it is now well-recognized that the concept of no adverse effects /or toxicity of these weeds incorrect. Like other noxious weeds, *S. nigrum*, *P. hysterophorous*, *A. conizoids*, *R. sceleratus* and *B. nigra* contain harmful effects such as reduce crop production, effects on livestock grazing, toxic and allelopathic impacts on native plants, negative impacts on human health and livestock. According to literature, genus Ageratum comprises thirty species that are not yet well-studies so far. The biological activity of *A. conyzoides* of pyrrolizidine alkaloids (PA), are potentially hepatotoxic, and characterized as haemorrhagic liver necrosis in animals, being rare in humans. Long-term exposure can cause hepatic megalocytosis and veno-occlusive disease, fatty liver degeneration and cirrhosis, and proliferation of the bile duct epithelium. Furthermore, some types of PA are genotoxic and carcinogenic in rodents (Bosi *et al*., 2013).

According to different studies, *R. sceleratus* is bitter and contains poisonous compounds which can be fatal to browsing animals. Young leaves of this weed cause inflammation of the mouth and digestive tract in animals. Leaves can also cause blisters and sores if rub or apply on skin. However there is no clear indication has been found on *B. nigra* in relation to harmful affect so far. However, B. oleracea (col silvestre) has been reported to causes intravascular hemolysis which are used as forage for ruminants. A non-protein amino acid S-methyl cysteinyl sulfoxide (SMCO) present in B. oleracea which reduces to dimethyl disulfide (a hemolysin) in the rumen and cause anemia (Duncan and Milne 1993). Intravascular hemolysis may be lethal in cattle which are very sensitive to the hemolytic effects of SMCO (Prache 1994). As we go on, exposure to *P. hysterophorus* has been reported to causes systemic toxicity in livestock (Gunaseelan, 1987). Different health related problems such as, alopecia, loss of skin pigmentation, dermatitis and diarrhoea has been reported in animals if feeding on this weed for longer period of time. The milk and meat quality of cattle, buffalo and sheep affect on consumption of this weed (Lakshmi and Srinivas, 2007). Furthermore, it cause liver and kidneys degeneration and later liver dehydrogenases in buffalo and sheep (Rajkumar *et al*., 1988). Significant immune system weakening has been reported in reduction in rat WBC count after oral treatment of Parthenium extract signifies its ability (Yadav *et al*., 2010).

As compare to other weeds *S. nigrum* ingestion can cause illness and complication such as rapid pulse and respiration, dark-coloured diarrhea followed by constipation, lack of rumination, pale mucous membranes, widely dilated pupils, low body temperature, oedema, in coordination, tremors and staggering movements (Cooper and Johnson 1984). This weed contains nitrate nitrogen (NO3-N) that can cause N03-N toxicity in livestock that resulting in a decrease in milk yield, abortion, impaired vitamin A and iodine nutrition, muscle tremors, staggering gait, rapid pulse, frequent urination, labored breathing, followed by collapse and coma, with or without convulsions. The levels of NO3-N apparently high when flowering stage approaches and then drop in later stage in life cycle of plant. However, nitrate toxicity in relation to all negative on animal health is still uncertain. However, different studies reporting that Solanum spp., are harmless as a food and fodder sources (Jagatheeswari *et al.,* 2013). However, like other noxious weeds, *S. nigrum*, *P. hysterophorous*, *A. conizoids*, *R. sceleratus* and *B. nigra* contain harmful chemicals and can cause health complications in livestock if use as fodder.

CONCLUSION

Weeds are an inevitable component of pastures and fields in Pakistan. Present investigation revealed that some toxic herbaceous weeds are potential fodder like high-quality maize. However, still not recommend for raising livestock on these plants. This study revealed that weeds have fibre contents equal like conventional fodder crop. Study also demonstrated that weeds satisfy the cruded protein (CP) needs of beef cattle. However, dairy cattle require more CP than beef cattle, goats, and sheep, so some weeds and forages may not meet their needs. It was also concluded that protein, fat and carbohydrate had the tendency to increase from vegetative to the post reproductive stage. According to results, various other nutritional parameters either increased or decreased with growing age of plant and seasonal changes. However, further study is needed to search out the real impact on health and productivity of these animals because toxicity of these weeds is well known. Although these weeds contains nutritional benefits like fodder crops but still health risks are associated with livestock to graze on *S. nigrum, P. hysterophorous*, *A. conizoids*, *R. sceleratus* and *B. nigra* for longer period of time.

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