



## Research Article

# Effect of Bio Extract on the Forage Quality of Berseem

Saima Bibi\* and Nazir Ahmad

Department of Animal Nutrition, the University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan.

**Abstract** | The study evaluated the impact of bio-extracts (Phenolic compounds) and commercial tannic acid on the nutrient composition and in sacco digestibility of berseem fodder in three non-lactating ruminally cannulated Holstein cows at the University of Agriculture Peshawar, Pakistan. The cows were fed a diet of 90% fodder and 10% concentrate on a dry matter basis. Five Plant-derived phenolic compounds and commercial tannic acid at concentration of 1.5%, 3.0% and 4.5% were used. The phenolic extracts were prepared by treating plants in hot water, allowing biological activity at room temperature and then filtering. These extracts were then applied to berseem fodder to assess changes in nutrient composition. The treatment decreased neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) while increasing in sacco digestibility of the fiber. Significant variations ( $P < 0.001$ ) were observed in DM, CP, NDF, ADF, ADL and in sacco digestibility between pre and post treatment samples. The CP content of berseem ranged from 16.4% to 22.2% of DM, with highest in berseem treated with seed extracts phenolic compounds and 4.5% tannic acid and the lowest in untreated berseem. DM content varied from 23.1% to 26.9% with the highest in berseem silage treated with Bakayan seeds and 4.5% tannic acid. Significant differences ( $P < 0.001$ ) were found in NDF, ADF, ADL contents, with the lowest values in Bakayan seeds plus 4.5% tannic acid treatment. pH varied significantly from 4.70 to 5.20. The in sacco digestibility showed significant differences with DM disappearance ranging from 39.5% to 59.3% after 36 hours of incubation, CP disappearance from 52.4% to 82.3%, NDF from 49.6% to 69.9% and ADF from 39.8 to 73.5%. Treated berseem generally had higher digestibility. Among phenolic compound treatment, Bakayan seed with 4.5% tannic acid had highest CP (22.2%) and lowest NDF (33.3%), ADF (23.5%) and ADL (5.9%). It also had the highest in sacco disappearance rates for DM (59.3%), NDF (69.9%), ADF (73.5%) and the lowest disappearance of CP (52.4%). The results show that phenolic and tannic treatments effectively improve berseem's nutritive value and digestibility.

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**\*Correspondence** | Saima Bibi, Department of Animal Nutrition, the University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan;

**Email:** dr.saima56@gmail.com

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**Keywords** | Phenolic compound, in Sacco digestibility, Berseem, Acid detergent fiber, Neutral detergent fiber



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## Introduction

Pakistan faces two scarcity periods of fodder availability each year, from May to July and

December to January, which mainly impact the livestock productivity. The forage source is deficient by 27.29 million tons of total digestible nutrients (TDN) and 1.68 million tons of digestible protein

(Sarwar *et al.*, 2002). Livestock production is classified into two systems: the rural household, where animals are integrated in rural subsistence economy using home-grown fodder and crop residues, and large herds retained on rangelands. Most of the livestock in Pakistan remain undernourished with low productivity and poor fertility except for peri-urban and commercial rural farming. Improving forage productivity is essential for optimizing the livestock production. The existing fodder system relies on summer crops like *Sorghum bicolor*, *Zea mays*, *Pennisetum americanum*, *Cyamopsis teragonoloba*, *Sorghum* hybrids and *Vigna unguiculata* while winter crops include *Trifolium alexandrinum*, *Avena sativa*, *Brassica sp.*, *Trifolium resupinatum*, *Medicago sativa*, *Hordeum vulgare* and *Lolium multiflorum*. Punjab produces 80% of the country's fodder, Sindh 13%, Khyber Pakhtunkhwa province 4% and Baluchistan province 2%, which hardly meet 50% of the animal's requirement (Sarwar *et al.*, 2002). A substantial fodder deficiency occur in winter (November to January) due to the slow growth of berseem and in (June to July) when maize is in early growth stage. Berseem, the major winter fodder, planted alone or mixed with barley, oats and Brassica. During optimum climatic condition (March to May) Berseem is harvested periodically and fed to animals.

Fodder preservation is obtained by lowering the pH through bacterial anaerobic fermentation which produces organic acid like acetic acid, lactic acid and butyric acid. Berseem, a high yielding and highly nutritious, multi cut fodder can be efficiently ensiled to fulfill the gap of the green fodder availability during the scarcity periods. In these periods, animals are mostly feed with wheat straw which has lesser nutrition. The surplus berseem in the active growth period could effectively be conserved as silage and later mixed with the wheat straw. This blend enhances the nutritional value of the diet thus improving animal health and performance.

Protein in forages often is poorly utilized by ruminants due to extensive degradation in the rumen. Excess nitrogen from the degraded protein is absorbed from the rumen as ammonia NH<sub>3</sub> and excreted in the urine as urea (Tabbaco *et al.*, 2006). In ruminant nutrition, the ability of tannins to form complex with proteins negatively affects the rumen fermentation. These protein-tannin complexes prevent microbial growth and enzyme activity by binding to microbes that degrade the rumen cell wall (Jones *et al.*, 1994;

McAllister *et al.*, 1994; Bae *et al.*, 1993; Min *et al.*, 2002). Tannin also binds with feed proteins and reducing their availability to the rumen microorganisms (Tanner *et al.*, 1994). Ruminant feces, a principal by-product of forage degradation by rumen microbes, have higher levels of fiber nitrogen (Somda and Powell, 1998). Tannin-protein complexes are broken by the low pH in the abomasum while the proteins are degraded by the pepsin (Perezmalonado *et al.*, 1995) making amino acids for the animals (Poncet and Remond, 2002). The nitrogen in ruminant feces consists of (a) endogenous N from microorganisms or microbial products of the gastrointestinal tract (b) N originating from the walls of digestive tract itself; and (c) undigested fiber N (Min *et al.*, 2002).

No concrete measures have been taken so far to ensure uninterrupted livestock feeding and year-round supply of quality green fodder (Sarwer *et al.*, 2002). One option is to improve current production by introducing high yielding varieties and optimizing fodder production. In this study berseem and alfalfa were treated with phenolic extracts from various trees such as tannin, for silage making. Research has shown that applying tannic acid to clovers can prevent its degradation in rumen as well as increases its digestibility. Within the first hour, more than 45% of protein loss in clovers can be prevented through tannin application, allowing the fodder to be conserved as silage for use during hot summer months, which can improve the performance of the dairy animal. Therefore, the current study was designed to improve the nutritive value and digestibility of berseem to meet the feeding requirements of dairy animals and increase their production in Pakistan.

## Materials and Methods

The present study was conducted at the Department of Animal Nutrition, The University of Agriculture Peshawar. The theme of the experiment was to extract phenolic compounds (Tannin) from various plant species (seeds and hulls etc.) and then it was applied on berseem leguminous fodder in order to protect plant protein from microbial degradation in the rumen. It is estimated that about 70% protein is lost in the rumen when clovers are fed to animal through microbial degradation. The tannin containing plants materials (leaves, fruits hulls etc.) were collected and oven dried at 60 °C for 72 hours. The dried samples were ground to 1 mm particle size with the Thomas Wily Mil at the

Department of Animal Nutrition. Then the ground powder was treated with (water: Methanol) mixture for 48 hours to extract the phenolic compound. Different phenolic compounds were dispensed on berseem ensiled for qualitative changes. Phenolic compounds (Tannin) were dispensed at harvest after chopping at the desired moisture content 65% and ensiled for a month. Solutions of commercially available tannic acids (0, 1.5% 3.0%, and 4.5% v/w) were also used for fodder qualitative changes.

The plant species sources which were used for phenolic extractions (a) Hulls of pomegranate (*Punica granatum*) (b) Seeds of Bakayen (*Melia azedarach*) (c) Seeds of sorghum (*Sorghum bicolor*) (d) seeds of tea plant (*Camellia sinensis*) (e) Walnuts hulls (*Juglans regia*) (f) Commercial Tannic Acids.

Preparation of solution and its application on berseem Solution was prepared 5-10% (w/v) in water. The mixtures of plants with solvent were treated in hot water and kept at room temperature to allow biological activities for releasing phenols. Solutions were filtered through fourth-fold quality cloths and the supernatant was collected. The filtrate was further boiled to evaporate Methanol for maximum concentration. Application of extract on forage dry matter (Berseem) for qualitative changes (DM losses, CP, NDF and ADF etc.) were compared in pre- and post-ensiled materials.

#### Preparation of silage

The treated and untreated sample of berseem properly chopped 0.5 to 1 mm manually with the help of chop cutter at the laboratory of Department of Animal Nutrition. The chopped control berseem plant kept in the plastic bottle about 1000 g capacity. While for the treated berseem dispenses phenolic extracts on the berseem fodder with desire treatments and was filled in plastic bottles for a month. The silo was made airtight by covering with a rubber ring between bottle and the cover. The silo was transferred to freezer until the chemical analysis.

#### Chemical analysis

Dry matter content of the fresh samples was determined by oven drying at 100 °C till constant weight (International Organization for Standardization (ISO, 1999). The remaining fresh samples were divided into two portions, one portion (~500 g) was air dried at 60°C (ISO, 1999) and

another part was frozen as a backup. The air-dried samples were then grinded by Thomas willey mill and pass from 1mm sieve, and was stored in clean dry bottles and properly labelled. Ash after incineration at 550 °C (ISO, 1978), CP (N × 6.25) according to Kjeldal method (ISO, 2005), NDF, ADF and ADL according to Van Soest (1991) was determined and in sacco digestibility according to method of Orskov and McDonald (1979).

#### Statistical analysis

Data on the effect of phenolic compound on DM losses, PH, *In Sacco* digestibility and CP digestibility in untreated and treated with phenolic compound extracted through water of Berseem silage.

$$Y_{ij} = \mu + P_i + \epsilon_{ija}$$

Where;  $Y_{ij}$  is the response of treatment (phenolic compound);  $\mu$  is the overall mean,  $P_i$  is the effect of phenolic compound on the forage quality of Berseem,  $\epsilon_{ij}$  is the random error associated with response of treatment.

## Results and Discussion

The chemical composition, dry matter (DM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), ash and PH and in Sacco digestibility i.e., digestible crude protein (DCP), digestible neutral detergent fibre (DNDF), digestible dry matter (DDM) and digestible acid detergent fiber (DADF) of berseem treated with different biological and chemical extract are listed in Table 1. There was large difference found in the chemical composition and in Sacco digestibility of the berseem treated with different biological and chemical extract. The difference in the DM was 23.1 to 26.9%, the difference in the CP was 22.2 to 16.4%, the difference in the NDF content was 55.1 to 33.3%, ADF was 36.9 to 23.5%, ADL was 7.3 to 5.91%, pH was 5.20 to 4.70% and ash was 19.9 to 10.2 were recorded in this experiment. Similarly, variation was found in the in Sacco digestibility with different interval 2h, 12h and 36h of berseem treated with different biological and chemical extract.

Table 2 showed the dry matter, chemical composition and PH of untreated and treated with different biological extract and chemical tannic acid 1.5%, 3.0% and 4.5% separately of berseem. For improving the quality of

**Table 1:** Chemical composition and in sacco digestibility of berseem treated with different Phenolic compound extracted through water and commercial tannic acids.

| Bio extract                                                                              | Parameter | Percentage | Mean value | Lowest value | Highest value |
|------------------------------------------------------------------------------------------|-----------|------------|------------|--------------|---------------|
| Berseem treated with different biological and chemical extract                           | DM        | %          | 25.5       | 23.1         | 26.9          |
|                                                                                          | CP        | %          | 18.6       | 16.4         | 22.2          |
|                                                                                          | ADF       | %          | 30.9       | 23.5         | 36.9          |
|                                                                                          | NDF       | %          | 44.0       | 33.3         | 55.1          |
|                                                                                          | ADL       | %          | 6.80       | 5.91         | 7.30          |
|                                                                                          | pH        |            | 4.90       | 4.70         | 5.20          |
|                                                                                          | Ash       | %          | 13.3       | 10.2         | 19.9          |
| In Sacco digestibility of berseem treated with different biological and chemical extract | DM        | 2          | 8.70       | 8.30         | 9.10          |
|                                                                                          | CP        | hours      | 14.7       | 12.8         | 16.2          |
|                                                                                          | NDF       |            | 9.20       | 8.90         | 9.80          |
|                                                                                          | ADF       |            | 8.40       | 7.50         | 9.00          |
|                                                                                          | DM        | 12         | 24.3       | 18.5         | 29.9          |
|                                                                                          | CP        | hours      | 46.7       | 38.5         | 52.2          |
|                                                                                          | NDF       |            | 36.0       | 25.8         | 39.7          |
|                                                                                          | ADF       |            | 34.8       | 22.9         | 41.8          |
|                                                                                          | DM        | 36         | 48.1       | 39.5         | 59.3          |
|                                                                                          | CP        | hours      | 73.9       | 52.4         | 82.3          |
|                                                                                          | NDF       |            | 62.0       | 46.9         | 69.9          |
|                                                                                          | ADF       |            | 62.2       | 39.8         | 73.5          |

DM: dry matter; CP: crude protein; CFat: crude fat; ADF: acid detergent fibre; NDF: neutral detergent fibre; ADL: acid detergent lignin.

berseem fodder through increasing the CP content and digestibility meanwhile decreasing the content

of NDF, ADF and ADL. There was large significant difference ( $P < 0.001$ ) existed in the DM, nutritional value and pH of berseem treated with different biological phenolic compound extracted with water. The mean value of DM was (25.5%), the highest value of DM (25.9%) was recorded for the berseem treated with Bakayin seeds while the lowest value (23.1%) of DM was recorded for the control untreated berseem. The CP value of berseem fodder was increased ( $P < 0.001$ ) with the treatment of biological phenolic compound and commercial tannic acids. The mean value of CP was (17.6%), the highest value of CP (18.9%) was recorded for the berseem treated with Bakayin seeds while the lowest value (16.4%) of CP was recorded for the control/untreated. The mean value of ash was (13.3%), the highest value of Ash (19.9%) was recorded for the berseem treated with Tannic acid 3.0% while the lowest value (10.2%) of ash was recorded for the berseem treated with Bakayin seeds. The mean value of Cfat was (2.1%), the highest value of Cfat (2.31%) was recorded for the berseem treated with Walnut hulls while the lowest value (1.98%) of Cfat was recorded for the berseem treated with Bakayan seeds extract. The value of NDF, ADF and ADL of berseem were decreased ( $P < 0.001$ ) with the treatment of biological phenolic compound and commercial tannic acids. The mean value of NDF was (44.0%), the highest value of NDF (55.1%) was recorded for the control/untreated berseem while the lowest value (34.2%) of NDF was recorded for the berseem treated with Bakayin seeds extract. The mean value of ADF was (30.9%), the highest value

**Table 2:** Dry matter content, chemical composition and PH of untreated and treated with different biological extract and chemical Tannic acid used separately of berseem.

| Berseem plus biological extract | DM                 |                    |                      | Chemical composition |                    |                    |                    | pH                 |
|---------------------------------|--------------------|--------------------|----------------------|----------------------|--------------------|--------------------|--------------------|--------------------|
|                                 | Ash                | CP                 | Cfat                 | NDF                  | ADF                | ADL                |                    |                    |
| Bakayin seeds                   | 25.9 <sup>a</sup>  | 10.2 <sup>b</sup>  | 18.9 <sup>a</sup>    | 1.98 <sup>e</sup>    | 34.2 <sup>e</sup>  | 24.4 <sup>d</sup>  | 6.12 <sup>c</sup>  | 4.60 <sup>c</sup>  |
| Tannic acid 3.0%                | 25.2 <sup>ab</sup> | 19.9 <sup>c</sup>  | 18.9 <sup>a</sup>    | 2.05 <sup>cde</sup>  | 36.8 <sup>d</sup>  | 26.6 <sup>cd</sup> | 6.34 <sup>bc</sup> | 4.81 <sup>b</sup>  |
| Pomegranate hull                | 25.6 <sup>ab</sup> | 10.4 <sup>b</sup>  | 18.1 <sup>abc</sup>  | 2.11 <sup>bcd</sup>  | 40.9 <sup>cd</sup> | 26.9 <sup>cd</sup> | 6.55 <sup>bc</sup> | 4.91 <sup>b</sup>  |
| Tannic acid 4.5%                | 25.7 <sup>ab</sup> | 14.2 <sup>ab</sup> | 17.5 <sup>abcd</sup> | 2.08 <sup>cde</sup>  | 40.9 <sup>cd</sup> | 29.8 <sup>c</sup>  | 6.87 <sup>b</sup>  | 4.90 <sup>b</sup>  |
| Walnut hull                     | 25.1 <sup>ab</sup> | 11.0 <sup>b</sup>  | 17.5 <sup>abcd</sup> | 2.31 <sup>a</sup>    | 43.4 <sup>c</sup>  | 31.5 <sup>b</sup>  | 6.98 <sup>ab</sup> | 4.49 <sup>c</sup>  |
| Sorghum seed                    | 25.3 <sup>ab</sup> | 14.6 <sup>ab</sup> | 17.4 <sup>bcd</sup>  | 2.00 <sup>de</sup>   | 43.4 <sup>c</sup>  | 32.0 <sup>b</sup>  | 7.02 <sup>ab</sup> | 4.97 <sup>ab</sup> |
| Tannic acid 1.5%                | 24.9 <sup>b</sup>  | 11.8 <sup>b</sup>  | 17.2 <sup>bcd</sup>  | 2.18 <sup>b</sup>    | 49.5 <sup>b</sup>  | 34.5 <sup>ab</sup> | 7.11 <sup>a</sup>  | 4.86 <sup>b</sup>  |
| Tea seed                        | 23.5 <sup>c</sup>  | 13.3 <sup>ab</sup> | 16.7 <sup>cd</sup>   | 2.22 <sup>ab</sup>   | 51.4 <sup>ab</sup> | 35.4 <sup>a</sup>  | 7.21 <sup>a</sup>  | 5.00 <sup>ab</sup> |
| Berseem                         | 23.1 <sup>c</sup>  | 14.3 <sup>ab</sup> | 16.4 <sup>d</sup>    | 2.16 <sup>bc</sup>   | 55.1 <sup>a</sup>  | 36.9 <sup>a</sup>  | 7.31 <sup>a</sup>  | 5.22 <sup>a</sup>  |
| SEM                             | 0.30               | 0.11               | 0.16                 | 0.02                 | 0.61               | 0.35               | 0.08               | 0.03               |
| Significance                    | ***                | ***                | ***                  | ***                  | ***                | ***                | ***                | **                 |

SEM, standard error of the means; \*\*\*,  $P < 0.001$ ; DM: dry matter; CP: crude protein; CFat: crude fat; ADF: acid detergent fibre; NDF: neutral detergent fibre; ADL: acid detergent lignin.

**Table 3:** *In Sacco* disappearance of untreated and treated with different biological phenolic compound extracted through water and commercial Tannic acid of berseem.

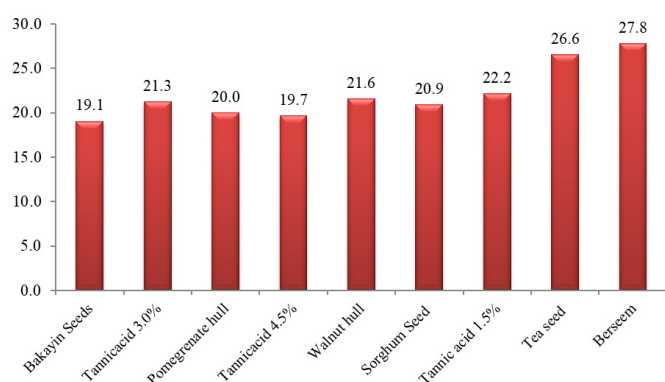
| Incubation time (h)            | Untreated control | Pomegranate hull    | Walnut hull         | Sorghum seed        | Tea seed            | Bakayin Seeds      | Tannic acid 1.5%    | Tannic acid 3.0%    | Tannic acid 4.5%   | SEM  | Significance |
|--------------------------------|-------------------|---------------------|---------------------|---------------------|---------------------|--------------------|---------------------|---------------------|--------------------|------|--------------|
| <b>Dry matter</b>              |                   |                     |                     |                     |                     |                    |                     |                     |                    |      |              |
| 2                              | 8.31              | 8.52                | 8.92                | 8.45                | 8.35                | 9.12               | 8.75                | 8.99                | 9.02               | 0.20 | NS           |
| 4                              | 9.55 <sup>e</sup> | 11.5 <sup>d</sup>   | 13.6 <sup>c</sup>   | 12.8 <sup>cd</sup>  | 9.59 <sup>e</sup>   | 17.8 <sup>a</sup>  | 14.3 <sup>bc</sup>  | 16.2 <sup>abc</sup> | 17.3 <sup>ab</sup> | 0.37 | ***          |
| 8                              | 15.9 <sup>c</sup> | 17.7 <sup>bc</sup>  | 17.3 <sup>bc</sup>  | 18.9 <sup>b</sup>   | 16.1 <sup>c</sup>   | 21.5 <sup>a</sup>  | 19.4 <sup>ab</sup>  | 20.5 <sup>ab</sup>  | 22.4 <sup>a</sup>  | 0.41 | ***          |
| 12                             | 18.5 <sup>c</sup> | 22.6 <sup>d</sup>   | 23.1 <sup>cd</sup>  | 25.4 <sup>bc</sup>  | 19.2 <sup>e</sup>   | 29.9 <sup>a</sup>  | 25.3 <sup>bc</sup>  | 26.8 <sup>abc</sup> | 27.9 <sup>b</sup>  | 0.48 | ***          |
| 24                             | 34.5 <sup>c</sup> | 38.3 <sup>bc</sup>  | 36.7 <sup>c</sup>   | 39.1 <sup>abc</sup> | 34.9 <sup>c</sup>   | 43.8 <sup>a</sup>  | 39.1 <sup>abc</sup> | 40.2 <sup>abc</sup> | 44.5 <sup>ab</sup> | 1.01 | ***          |
| 36                             | 39.5 <sup>e</sup> | 45.2 <sup>cd</sup>  | 44.9 <sup>cde</sup> | 47.8 <sup>cd</sup>  | 42.5 <sup>de</sup>  | 59.3 <sup>a</sup>  | 48.2 <sup>bc</sup>  | 51.6 <sup>ab</sup>  | 53.8 <sup>b</sup>  | 1.22 | ***          |
| <b>Crude protein</b>           |                   |                     |                     |                     |                     |                    |                     |                     |                    |      |              |
| 2                              | 16.2 <sup>a</sup> | 14.3 <sup>bcd</sup> | 14.9 <sup>abc</sup> | 15.1 <sup>ab</sup>  | 15.8 <sup>b</sup>   | 12.8 <sup>d</sup>  | 14.6 <sup>abc</sup> | 15.2 <sup>ab</sup>  | 13.3 <sup>cd</sup> | 0.35 | ***          |
| 4                              | 26.8 <sup>a</sup> | 24.2 <sup>ab</sup>  | 25.3 <sup>ab</sup>  | 24.9 <sup>ab</sup>  | 26.3 <sup>a</sup>   | 18.3 <sup>d</sup>  | 23.4 <sup>bc</sup>  | 24.7 <sup>ab</sup>  | 21.2 <sup>c</sup>  | 0.47 | ***          |
| 8                              | 38.9 <sup>a</sup> | 33.5 <sup>b</sup>   | 35.4 <sup>b</sup>   | 35.7 <sup>b</sup>   | 38.1 <sup>ab</sup>  | 27.3 <sup>c</sup>  | 34.5 <sup>b</sup>   | 34.2 <sup>b</sup>   | 33.6 <sup>b</sup>  | 0.66 | ***          |
| 12                             | 52.2 <sup>a</sup> | 45.8 <sup>c</sup>   | 48.1 <sup>b</sup>   | 48.1 <sup>b</sup>   | 51.4 <sup>ab</sup>  | 38.5 <sup>d</sup>  | 46.7 <sup>abc</sup> | 47.5 <sup>b</sup>   | 41.7 <sup>cd</sup> | 1.03 | ***          |
| 24                             | 71.2 <sup>a</sup> | 53.4 <sup>b</sup>   | 55.7 <sup>ab</sup>  | 59.8 <sup>ab</sup>  | 59.1 <sup>ab</sup>  | 41.3 <sup>d</sup>  | 58.7 <sup>ab</sup>  | 53.2 <sup>b</sup>   | 48.3 <sup>c</sup>  | 1.22 | ***          |
| 36                             | 82.3 <sup>a</sup> | 62.5 <sup>bc</sup>  | 65.5 <sup>abc</sup> | 66.3 <sup>ab</sup>  | 75.3 <sup>a</sup>   | 52.4 <sup>e</sup>  | 65.2 <sup>abc</sup> | 62.1 <sup>bc</sup>  | 59.9 <sup>cd</sup> | 2.01 | ***          |
| <b>Neutral detergent fibre</b> |                   |                     |                     |                     |                     |                    |                     |                     |                    |      |              |
| 2                              | 8.92              | 9.12                | 9.14                | 9.1                 | 8.99                | 9.77               | 9.13                | 9.03                | 9.17               | 0.22 | NS           |
| 4                              | 12.5 <sup>d</sup> | 14.3 <sup>c</sup>   | 16.2 <sup>b</sup>   | 15.2 <sup>bc</sup>  | 13.5 <sup>cd</sup>  | 18.5 <sup>a</sup>  | 16.8 <sup>abc</sup> | 17.5 <sup>ab</sup>  | 18.2 <sup>ab</sup> | 0.38 | ***          |
| 8                              | 16.7 <sup>c</sup> | 20.6 <sup>cd</sup>  | 22.4 <sup>bc</sup>  | 21.5 <sup>bc</sup>  | 18.9 <sup>de</sup>  | 25.3 <sup>a</sup>  | 23.4 <sup>abc</sup> | 24.7 <sup>ab</sup>  | 25.1 <sup>a</sup>  | 0.47 | ***          |
| 12                             | 25.8 <sup>c</sup> | 37.2 <sup>ab</sup>  | 38.3 <sup>ab</sup>  | 37.7 <sup>ab</sup>  | 30.7 <sup>abc</sup> | 39.1 <sup>a</sup>  | 37.5 <sup>ab</sup>  | 38.2 <sup>ab</sup>  | 39.7 <sup>a</sup>  | 0.70 | ***          |
| 24                             | 37.5 <sup>c</sup> | 48.3 <sup>ab</sup>  | 49.2 <sup>ab</sup>  | 47.6 <sup>ab</sup>  | 45.3 <sup>b</sup>   | 51.2 <sup>ab</sup> | 49.9 <sup>ab</sup>  | 51.4 <sup>ab</sup>  | 52.9 <sup>a</sup>  | 1.27 | ***          |
| 36                             | 46.9 <sup>d</sup> | 61.2 <sup>abc</sup> | 64.3 <sup>ab</sup>  | 60.9 <sup>bc</sup>  | 54.9 <sup>cd</sup>  | 69.9 <sup>a</sup>  | 64.7 <sup>ab</sup>  | 65.9 <sup>ab</sup>  | 69.6 <sup>a</sup>  | 1.87 | ***          |
| <b>Acid detergent fibre</b>    |                   |                     |                     |                     |                     |                    |                     |                     |                    |      |              |
| 2                              | 7.53              | 8.22                | 8.37                | 8.47                | 8.11                | 8.87               | 8.34                | 8.56                | 8.96               | 0.19 | ***          |
| 4                              | 10.6 <sup>d</sup> | 14.5 <sup>c</sup>   | 18.7 <sup>ab</sup>  | 17.5 <sup>b</sup>   | 12.5 <sup>cd</sup>  | 20.6 <sup>a</sup>  | 17.9 <sup>b</sup>   | 19.8 <sup>ab</sup>  | 21.7 <sup>a</sup>  | 0.40 | ***          |
| 8                              | 14.6 <sup>f</sup> | 20.8 <sup>de</sup>  | 22.8 <sup>cd</sup>  | 23.6 <sup>cd</sup>  | 18.7 <sup>e</sup>   | 28.9 <sup>a</sup>  | 24.8 <sup>bc</sup>  | 26.3 <sup>ab</sup>  | 28.4 <sup>a</sup>  | 0.49 | ***          |
| 12                             | 22.9 <sup>e</sup> | 32.5 <sup>c</sup>   | 35.4 <sup>c</sup>   | 36.2 <sup>c</sup>   | 27.8 <sup>d</sup>   | 41.8 <sup>a</sup>  | 36.7 <sup>bc</sup>  | 38.9 <sup>ab</sup>  | 41.2 <sup>a</sup>  | 0.78 | ***          |
| 24                             | 31.5 <sup>e</sup> | 44.5 <sup>d</sup>   | 45.1 <sup>cd</sup>  | 49.8 <sup>abc</sup> | 35.4 <sup>e</sup>   | 53.9 <sup>a</sup>  | 48.6 <sup>bcd</sup> | 51.7 <sup>ab</sup>  | 53.5 <sup>a</sup>  | 1.12 | ***          |
| 36                             | 39.8 <sup>e</sup> | 62.3 <sup>c</sup>   | 63.9 <sup>bc</sup>  | 65.5 <sup>bc</sup>  | 48.3 <sup>d</sup>   | 73.5 <sup>a</sup>  | 66.2 <sup>abc</sup> | 68.1 <sup>abc</sup> | 71.8 <sup>ab</sup> | 1.76 | ***          |

NS; non-significant, SEM, standard error of the means; \*\*\*,  $P < 0.001$

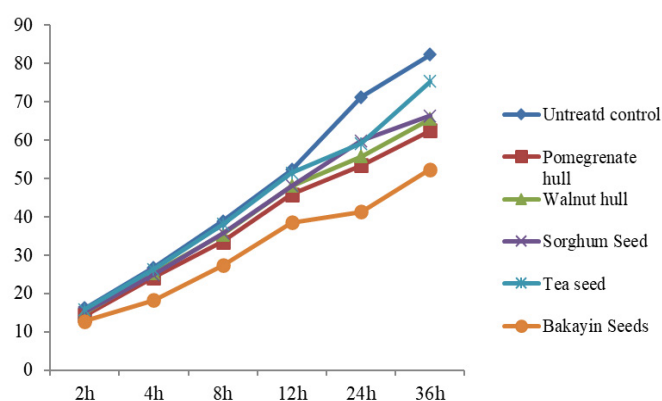
of ADF (36.9%) was recorded for the control/untreated berseem while the lowest value (24.4%) of ADF was recorded for the berseem treated with Bakayin seeds. The mean value of ADL was (6.80%), the highest value of ADL (7.30%) was recorded for the control/untreated berseem while the lowest value (6.10%) of ADL was recorded for the berseem treated with Bakayin seeds. The PH value of berseem fodder was increased ( $P < 0.001$ ) with the treatment of biological phenolic compound and commercial tannic acids. The mean value of pH was (4.90%), the highest value of pH (5.22%) was recorded for the untreated berseem while the lowest value (4.60%) of pH was recorded for the berseem treated with Bakayan seeds extract.

Table 3 summarized the data of disappearance of DM, CP, NDF and ADF from nylon bags are a function of ruminal incubation time. There was significant variation ( $P < 0.001$ ) existed in the disappearance of DM of berseem treated with different biological phenolic compound extracted through water and commercial tannic acids. Digestibility is the main indicator for the quality of fodder the fodder which is more digestible considers best quality fodder and vice versa. Figure 1 illustrate that disappearance of DM of treated berseem was found greater than that untreated berseem for all incubation time after 36h. The Bakayin seeds had greater 59.3% ( $P < 0.001$ ) disappearance of DM after 36h incubation time. Berseem fodder treated with commercial 4.5% tannic acid had the second

53.8% greater ( $P < 0.001$ ) value of DM disappearance while the untreated berseem had the lesser value 39.5% ( $P < 0.001$ ) of DM disappearance after 36h incubation time. There was significant variation ( $P < 0.001$ ) existed in the disappearance of CP of berseem treated with different biological phenolic compound extracted through water and commercial tannic acids. Disappearance of CP of treated berseem was found lesser than that untreated berseem for all incubation time after 36h. The Bakayin seeds had lesser 52.4% ( $P < 0.001$ ) degradation/disappearance of CP after 36h incubation time. Berseem fodder treated with commercial 4.5% tannic acid had the second 59.9% lesser ( $P < 0.001$ ) value of CP disappearance while the untreated berseem had the greater value 82.3% ( $P < 0.001$ ) of CP degradation/disappearance after 36h incubation time (Figure 2).



**Figure 1:** Showed the Dry matter loss of the berseem silage treated with different Phenolic compounds.



**Figure 2:** In sacco disappearance of CP of untreated and treated berseem silage with different biological extract after 36h incubation.

Significant variation ( $P < 0.001$ ) was found in the disappearance of NDF of berseem treated with different biological phenolic compound extracted through water and commercial tannic acids. Disappearance of NDF of treated berseem was found greater than that untreated berseem for all incubation time after 36h. The Bakayin seeds had greater 69.9% ( $P$

$< 0.001$ ) disappearance of NDF after 36h incubation time. Berseem fodder treated with commercial 4.5% tannic acid had the second 69.6% greater ( $P < 0.001$ ) value of NDF disappearance while the untreated berseem had the lesser value 46.9% ( $P < 0.001$ ) of NDF disappearance after 36h incubation time.

There was significant variation ( $P < 0.001$ ) existed in the disappearance of ADF of berseem treated with different biological phenolic compound extracted through water and commercial tannic acids. Disappearance of ADF of treated berseem was found greater than that untreated of berseem for all incubation time after 36h. The Bakayin seeds had greater 73.5% ( $P < 0.001$ ) disappearance of ADF after 36h incubation time. Berseem fodder treated with commercial 4.5% tannic acid had the second 71.8% greater ( $P < 0.001$ ) value of ADF disappearance while the untreated berseem had the lesser value 39.8% ( $P < 0.001$ ) of ADF disappearance after 36h incubation time. Generally, in Sacco digestibility disappearance of DM, CP, NDF and ADF of treated berseem increased threefold than untreated berseem at almost all ruminal incubation times.

Research in the past decade has established that forages are the major source of feed for dairy animals, and high-quality forages can be used to favourably increase dairy animal's production (Khan *et al.*, 2015). In ruminant nutrition, the ability of tannins to form complex with proteins of forages negatively affects the rumen fermentation. The phenolic compound interacts with feed proteins and decreases their availability to the rumen microorganisms (Tanner *et al.*, 1994). Ruminal tannin–protein complexes are broken by the low pH in the abomasum and the proteins are degraded by pepsin which limits the amino acids availability to the animals (Poncet and Remond, 2002).

The results of this study showed that bio extracts (phenolic compounds) markedly affect the nutrients composition and digestibility of berseem treated with different phenolic compound and commercial acids, thereby phenolic compounds present another opportunity to improve the nutrient composition of forages. There was significant difference existed in the content of DM of the berseem silage treated with different phenolic compound, varied from 23.1 to 25.9%. Similarly (Karamany *et al.*, 2021) reported large variation 89.9 to 90.9% in the content of DM of

berseem treated with Tryptophan and Pyridoxine. In finding of our results (Mohsen *et al.*, 2011) reported large variation 86.7 to 87.1% in the content of DM of berseem treated with nitrogen fertilizer. Similarly (Myung and Kennelly, 1989) reported large variation 90.8 to 93.5% in the content of DM of rice straw treated with Tryptophan and Pyridoxine. There was significant difference existed in the content of Ash of the berseem treated with different phenolic compound, varied from 10.2 to 19.9%. Similarly (Karamany *et al.*, 2021) reported large variation 11.7 to 12.8% in the content of Ash of berseem treated with Tryptophan and Pyridoxine. In finding of our results (Mohsen *et al.*, 2011) reported large variation 14.5 to 15.8% in the content of Ash of berseem treated with nitrogen fertilizer. There was significant difference existed in the content of CP of the berseem treated with different phenolic compound, varied from 16.4 to 18.9%. Similarly (Karamany *et al.*, 2021) reported large variation 17.1 to 19.1% in the content of CP of berseem treated with Tryptophan and Pyridoxine. In finding of our results (Mohsen *et al.*, 2011) reported large variation 13.4 to 16.6% in the content of CP of berseem treated with nitrogen fertilizer. In support of our results (Myung and Kennelly, 1992) reported large variation 0.42 to 0.72% in the content of CP of Aspen sawdust treated with alkaline hydrogen peroxide and per acetic acid. Similarly (Myung and Kennelly, 1989) reported large variation 6.0 to 15.6% in the content of CP of rice straw treated with Tryptophan and Pyridoxine.

There was significant difference existed in the content of Cfat of the berseem treated with different phenolic compound, varied from 2.0 to 2.30%. Similarly, Karamany *et al.* (2021) reported large variation 2.46 to 3.51% in the content of Cfat of berseem treated with Tryptophan and Pyridoxine. In finding of our results (Mohsen *et al.*, 2011) reported large variation 2.45 to 265% in the content of Cfat of berseem treated with nitrogen fertilizer. There was significant difference existed in the content of NDF and ADF of the berseem treated with different phenolic compound, varied from 34.2 to 55.1% and 24.2 to 36.9% respectively. Similarly, Karamany *et al.* (2021) reported large variation in the content of NDF (42.2 to 44.3%) and ADF (26.6 to 30.8%) of berseem treated with Tryptophan and Pyridoxine. In finding of our results (Mohsen *et al.*, 2011) reported large variation in the content of NDF (74.8 to 83.1%) and ADF (40.0 to 51.1%) of berseem treated with

nitrogen fertilizer.

Significant variation was found in the content of ADL of the berseem treated with different phenolic compound, varied from 6.1 to 7.3%. Similarly, Karamany *et al.* (2021) reported large variation 4.96 to 5.50% in the content of ADL of berseem treated with Tryptophan and Pyridoxine. In finding of our results (Mohsen *et al.*, 2011) reported large variation in the content of ADL of berseem treated with nitrogen fertilizer.

Disappearance of DM, CP, NDF and ADF from nylon bags are a function of ruminal incubation time. There was significant variation ( $P < 0.001$ ) existed in the disappearance of DM of berseem treated with different biological phenolic compound extracted through water and commercial tannic acids, varied from 39.5 to 59.3%. The disappearance of CP of berseem varied from 62.4 to 82.3%, NDF was varied from 46.9 to 69.9 and ADF was varied from 39.8 to 73.5%. In support of our results (Myung and Kennelly, 1992) reported large variation in the disappearance of DM, 29.5 to 87.2%, NDF, 25.3 to 90.3% and ADL, 29.3 to 84.7% of Aspen sawdust treated with alkaline hydrogen peroxide and per acetic acid. Similarly (Myung and Kennelly, 1989) reported large variation in the disappearance of DM, NDF and ADF of rice straw treated with Tryptophan and Pyridoxine. The findings of this study are also in consensus with the results of the (Goel and Makkar, 2012), who found that tannin can sufficiently improve the quality of fodder thus optimizing the animal production.

## Conclusions and Recommendations

The treatment of berseem with phenolic extracts particularly from Bakayan seeds and commercial tannic acids improve the forage quality by reducing the protein degradation and enhancing fiber digestibility. Phenolic compound treatment decreases the concentration of NDF, ADF and ADL, while increases the digestibility of fodder. Among the Biological extract tea seeds extract treated berseem silage had the lowest value of CP (167%) and highest content of NDF (51.4), ADF (35.4) and ADL (7.21%), while had the highest content of *in sacco* disappearance of CP (75.3%) and lowest content of *in sacco* digestibility of NDF (54.9%) and ADF (48.3%). The biological extract from Bakayan seed treated silage had the highest value of CP (18.9%)

and lowest content of NDF (34.2), ADF (24.4%) and ADL (6.12%), while had the lowest content of *in sacco* digestibility disappearance of CP (52.4%) and highest of NDF (69.9%) and ADF (73.5%).

It is recommended to use Bakayan seeds extract to enhance the digestibility of berseem fodder. Commercial tannic acid at 3.0% and 4.5% also proved beneficial as an alternate to the natural sources of tannic acid. Further research on tannin-rich plants for their use in silage production is advised for optimizing livestock production.

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## Novelty Statement

The effect of bio extract and commercial tannic acid on the chemical composition and digestibility of berseem are analyzed for the first time.

## Author's Contribution

**Nazir Ahmad:** Conceptualization, supervision, data analysis, writing, and review and editing.

**Saima Bibi:** Methodology, lab analysis and original draft preparation.

Both the authors have read and agreed to the published version of the manuscript.

## Conflict of interest

The authors have declared no conflict of interest.

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