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Effect of Spraying Berseem Green Fodder by Tryptophan and Pyridoxine Solutions on Crop Yield and Chemical Composition

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Abstract: This work aimed to investigate the impact of spraying solution of tryptophan or pyridoxine at different levels (0, 50, 75 and 100 mg/L water) of supplementation on crop yield of green berseem fodder and their chemical composition at different cut stages. Another target of the study is to improve their crop yield as value-added in animal feed and improving their chemical composition. The results showed that the dietary treatments had significantly effect ($P<0.05$) on the forage yield. The forage yield data seemed to be curve-linear where the third cut stage of Green Berseem Fodder (GBF) recorded the highest value of forage yield followed by the fourth cut stage (3.589 ton/ hectare) then second cut stage. Spraying GBF by pyridoxine recorded the highest value of forage yield comparing to that sprayed by tryptophan. There were significant ($P<0.05$) interaction between source and level (S x L) of supplementation; source and cuts (S x C); level and cuts (L x C) and source, level and cuts (S x L x C) on forage yield of GBF. Except for EE content, the other values of chemical analysis, energetic and nutritive values and cell wall constituents that include (NDF, ADF, ADL, hemicellulose and cellulose) were significantly affected by source of supplementation. Spraying berseem by tryptophan solution significantly ($P<0.05$) increased DM, OM, NFE, GE, DE, ME, NE, TDN, hemicellulose contents compared to spraying berseem by pyridoxine. Meanwhile, spraying berseem by pyridoxine solution significantly ($P<0.05$) increased moisture, CP, CF, ash, DCP, NDF, ADF, ADL and cellulose contents in comparison with spraying berseem by tryptophan solution. The main effect of level of supplementation had significantly affect ($P<0.05$) on all parameters of chemical analysis, energetic and nutritive values and cell wall constituents. Increasing level of spraying significantly ($P<0.05$) increased CP content, but it significantly ($P<0.05$) decreased NFE content compared to control (Zero mg/L). Spraying BGF by high level (100 mg/L) recorded the highest values of energetic values (GE, DE, ME and NE) and nutritive values (TDN and DCP percentages). Meanwhile, cell wall constituents includes (NDF, ADF, ADL, cellulose) significantly ($P<0.05$) decreased with increasing the level of supplementation. Cut stages had significantly affecting ($P<0.05$) on all parameters of chemical analysis, energetic and nutritive values and cell wall constituents. There were significant ($P<0.05$) interactions between source and level of supplementation (S x L); source and cut stages (S x C); level of supplementation and cut stages (L x C) and source, level of supplementation and cut stages (S x L x C) on all parameters of chemical analysis; energetic & nutritive values and cell wall constituents. The main effects of supplementation source had significant effect on total carbohydrates, total soluble sugars, chlorophyll a, phenolic compounds and flavonoids. However, the other parameters of photosynthetic pigments was not affected ($P>0.05$) by the source of the supplementation. Spraying BGF by pyridoxine solution significantly ($P<0.05$) increased the total carbohydrates, total soluble sugars, chlorophyll a phenolic compounds and flavonoids in comparison with the spraying BGF by tryptophan. Meanwhile, the other determined components of photosynthetic pigments that include (polysaccharides, chlorophyll b, carotenoids and total pigments) were

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in the near value among the two sources of supplementation (tryptophan and pyridoxine). Level of supplementations and Berseem cut stages had significant effect ($P<0.05$) on all determined nutrients of photosynthetic pigments of BGF. Their were significant ($P<0.05$) interactions among (S x L), (S x C), (L x C) and (S x L x C) for all parameters determined of photosynthetic pigments of BGF.

Key words: Tryptophan · Pyridoxine · Berseem · Crop Yield · Chemical Composition · Photosynthetic Pigments

INTRODUCTION

Berseem (*Trifolium alexandrinum* L.) called "Egyptian clover" is an annual, cool season forage crop grown in various parts of Egypt. Berssem gives several cuttings during its growing season and supplies nutritious and juicy forage for animals [1]. Normally four to six cuttings of berseem are taken [2]. It is fed either green or in hay form, when seasonal conditions permit.

As noted by [3-6] they reported that Berseem clover with the scientific name of (*Trifolium alexandrinum* L.) is used in resent decades and is so popular for farmers because of its fast growth, high number of harvests and fresh forage production with good quality and quantity.

The results of researches shows that rate of berseem production depends on sowing date, climate condition, soil fertility, shrub height, the number of harvests and variety. For example in Mazandaran province after rice harvest with three number of harvests 55-70 t/ha, in Gilan province with the same number of harvests 20-30 t/ha and in Khuzestan province 100t/ha fresh forage is produce [5]. Regarding to the important role of this plant in production cycle of dairy and protein substance, the roles in fertility preservation and the plant coverage of soil, one of the most important objectives of Egyptian authorities of agricultures is production and performance increase of forage crops.

Zlatic and Dumanovsky [7] noted that clover (*Trifolium* spp.) is one of the most important legume fodder crops and has been called the king of fodder. It is widely used as a green fodder for all livestock. Dried clover is also an important poultry feed. Due to its desirable qualities, it is suggested that it might be the cheapest supplementary protein source in livestock rations as recorded by [8].

The newly reclaimed sandy soil generally exposed to a combination of a biotic stresses as nutrient deficiency, the available water is water, fluctuation of temperature and increased irradiance. Thus different strategies used for improving plant tolerance to these adverse conditions. Selection of tolerant cultivars, using of optimum cultural practices and using of natural compounds (amino acids, vitamins, antioxidants) as seed soaking or as foliar treatment of plant are among these strategies (using of various growth regulating substances and its precursors have an important influence on plant growth and yield via its effect on different biochemical and physiological processes of plants. Amino acids are among these precursors of growth promoting substances [9].

Dawood and Sadak [10] reported that amino acids are an effective tool for increasing plant productivity. Because amino acids are organic nitrogenous compounds they considered as the building units in biosynthesis of proteins via amino acids polymerization. Such as of these amino acids is tryptophan and pyridoxine that has an important role on different plants as it improve various biochemical processes such as regulation of plant growth and differentiation through increasing water and different nutrients availability.

Rai [11]; Hussein *et al.* [12] and El Karamany *et al.* [13] noted that under different a biotic stress such as drought or salinity stress, tryptophan play an amazing role as ion transport regulator, modulating of stomatal opening and as an osmolyte.

Chen *et al.* [14] found that tryptophan is a precursor of the plant growth regulator auxin (Indole acetic acid IAA) and melatonin in higher plants.

The effect of exogenous treatment of tryptophan on different plants under normal growth conditions are recorded by many authors, as example, Dawood and Sadak [10] tabulated that tryptophan or benzyl adenine increased growth and yield of canola plant, Abbas *et al.* [15] confirmed this stimulatory role on chickpea plant. Meanwhile, Bakry *et al.* [16] mentioned that tryptophan treatment under water deficit conditions improved growth and yield of quinoa plant.

So, this work aimed to investigate the impact of incorporation both tryptophan and pyridoxine at different levels (0, 50, 75 and 100 mg/ L water) of supplementation on crop yield of green berseem fodder and their chemical composition at different cut stages in a trial to increase its crop yield as value-added in animal feed and improving their chemical composition.

MATERIALS AND METHODS

This study was carried out in Co-operation work among Field Crops Department, Division of Agriculture Researches, National Research Center, Dokki, Giza, Egypt and Animal Production Department, Division of Agriculture Researches, National Research Center, Dokki, Giza, Egypt.

The present work aims to studying the impact of incorporation of both tryptophan and pyridoxine at different levels (0, 50, 75 and 100 mg/ L water) of supplementation on green berseem fodder (GBF) quantity (crop yield) and chemical composition at different cut stages (1-4 cuts).

Two field experiments were carried out during winter seasons of 2015/2016 and 2016/2017 in Researches and Production Station of National Research Centre (NRC), Al-Nubaria District, Al-Behaira Governorate, Egypt.

The experimental soil before sowing was analyzed according to Chapman and Pratt [17]. Soil texture was sandy and its characteristics are shown in Table (1).

Experimental soil ploughed twice and divided to plots 3 × 7 m, then made rows 20 cm^2 between. Egyptian clover cultivar (Meskawy) was inoculated with the appropriate (*Rhizobium trifolii*) in a commercial product produced by Ministry of Agriculture, Egypt. The recommended agricultural practices were applied. Pre-sowing, 360 kg/ hectare of calcium super-phosphate (15.5% P2O5) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at a rate of 180 Kg/ hectare in five equal doses before the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} irrigation. Potassium sulfate (48.52 % K_2O) was added in two equal doses of 120 kg/ hectare, before the 1^{st} and 3^{rd} irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days.

The applied tryptophan or pyridoxine used in the present work was supplied from Sigma Chemical. Berseem plants were sprayed twice with tryptophan or pyridoxine at (50, 75 or 100 mg/ L) while control plants were sprayed with distilled water during vegetative growth at 30 and 45 days after sowing. Four cuts were taken from each of the two seasons. The first cut was obtained 60 days post seeding date, the second cut was obtained after 50 days from the first one, while the third one was taken after 40 days from the second cut and the fourth was taken after 40 days from the third cut.

Determine the Fresh Forage Yields: Fresh forage yield of clover Berseem determined in m^2 for each of the subsequent four cuts, in each experimental plot recorded and estimated in ton /hectare in the two growing seasons.

Chemical Analyses: Different samples of unsprayed green Berseem fodder (USGBF) and sprayed green Berseem fodder (SGBF) that sprayed by tryptophan or pyridoxine at different levels that mentioned above were collected at different cuts and primary moisture was recorded through out processing drying for the samples at 60 ºC for 48 hours (air dried) and air dried samples were kept in clean paper bags until the carrying the chemical analyses.

Biochemical Determinations: Photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) in fresh leaves were determined as the method described by Moran [18]; total carbohydrates was determined according to Dubois *et al.* [19]; total soluble sugars were extracted by the method of Homme *et al.* [20] and measured by the method of Yemm and Willis [21]; polysaccharides were determined according to Naguib [22]; phenolic content was measured as the method described by Zhang and Wang [23] and flavonoids contents were determined by the method of Chang *et al.* [24].

Calculations: Energetic values composed of gross energy (GE), digestible energy (DE), Metabolizable energy (ME) and net energy (NE), in addition to nutritive values of both total digestible nutrients (TDN) and digestible crude protein (DCP) were also calculated according to different equations that concluded or described by NRC [25].

Analytical Procedures: Chemical analysis of USGBF and SGBF includes moisture, ash, crude protein (CP), crude fiber (CF) and ether extract (EE) contents were determined according to AOAC [26] methods.

Crude protein determination involved the use of routine Kjeldhal nitrogen assay (N×6.25). Meanwhile, nitrogen-free extract (NFE) or carbohydrate content was determined by the difference using the following equation:

NFE content = 100 – [Moisture + CP + CF + EE + ash].

Table 1: Mechanical and chemical analysis of experimental soil

Sand %	Silt %	Clay %	pH	Organic matter %	$CaCO_3$ %	E.C. dS/m	Soluble N, ppm	Available P, ppm	Exchange-able K, ppm
91.2	3.7	5.1	7.3	0.3	1.4	0.3	8.1	3.2	20

On the other hand, cell wall constituents including neutral detergent fiber (NDF), acid detergent fiber and acid detergent lignin (ADL) were determined according to Goering and Van Soest [27] and Van Soest *et al.* [28]. However, hemicellulose and cellulose contents were calculated by difference as follows:

Hemicellulose = NDF - ADF
Cellulose = ADF - ADL.

Calculations of Energetic and Nutritive Values: Gross energy (Kcal/ Kg DM) was calculated according to Blaxter [29] where, each g crude protein= 5.65 Kcal, g fat = 9.40 Kcal and g (crude fiber and carbohydrate) = 4.15 Kcal.

Digestible energy (Kcal/ kg DM) was calculated according to NRC [25] where, Digestible energy (DE) = gross energy x 0.76.

Metabolizabe energy (Kcal/ kg DM) was calculated according to NRC [25] (where, Metabolizable energy (ME) = digestible energy x 0.82.

Net energy (Kcal/ kg DM) was calculated according to NRC [25] (as follows Net energy (NE) = metabolizable energy x 0.56.

Total digestible nutrients (%) was calculated according to NRC [25] where, Total digestible nutrients % = Digestible energy / 44.3.

Digestible crude protein (%) was calculated according to NRC [25] where, Digestible crude protein (%) = 0.85 X_1 – 2.5. Where X_1= Crude Protein% on DM basis.

Statistical Analysis: Data collected of chemical composition that includes {moisture, dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen-free extract (NFE) and ash}; cell wall constituents includes {neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), hemicellulose and cellulose}; energetic values includes {gross energy (GE), digestible energy (DE), metabolizable energy (ME) and net energy (NE)} and nutritive values includes {total digestible nutrients (TDN) and digestible crude protein (DCP), crop yield and biochemical determinations materials includes {total carbohydrates, total soluble sugars, polysaccharides, chlorophyll a, chlorophyll b, carotenoids, total pigments, phenolic compounds and flavonoids} were statistically analyzed as three factors-factorial analysis of variance using the general linear model procedure of SPSS [30]. Meanwhile, Duncan’s Multiple Range Test was used to examine the significance between means, Duncan [31].

The following model was used as the following:

$$Y_{ijkl} = \mu + S_i + L_j + C_k + (SL)_{ij} + (SC)_{ik} + (LC)_{jk} + (SLC)_{ijk} + e_{ijkl}$$

where:

Y_{ijkl} = Observation.

μ = The overall mean.

S_i = The effect of source of supplementations (S) for i = 1 to 2, 1 = tryptophan and 2 = pyridoxine.

L_j = The effect of levels of supplementations (L) for i = 1 to 4, 1 = Zero, 2 = 50 mg / L water, 3 = 75 mg / L water and 4 = 100 mg / L water.

C_k = The effect of green berseem fodder (GBF) cut stages (C) for j =1-4, 1= first cut stage of GFB, 2 = second cut stage of GFB, 3 = third cut stage of GFB and 4 = fourth cut stage of GFB.

$(SL)_{ij}$ = The interaction between source of supplementation (S) and levels of supplementations (L).

$(SC)_{ik}$ = The interaction between source of supplementation (S) and GBF cut stages (C).

$(LC)_{jk}$ = The interaction between levels of supplementations (L) and GBF cut stages (C).

$(SLC)_{ijk}$ = The interaction between source of supplementation (S); level of supplementations (L) and GBF cut stages (C).

e_{ijkl} = The experimental error.

RESULTS AND DISCUSSION

Fresh Forage Yield: Data presented in Table (2) showed that the dietary treatments had significantly affecting (P<0.05) on the forage yield. Third cut stage of green Berseem fodder (GBF) recorded the highest value of forage yield (3.663 ton/ hectare) followed by the fourth cut stage (3.589 ton/ hectare), then second cut stage (3.371 ton/ hectare), meanwhile the first cut stage recorded the lowest value of GBF (2.615 ton/ hectare).

On the other hand, with increasing the level of supplementations from tryptophan or pyridoxine solutions the quantities of forage yield from GBF were significantly (P<0.05) increased gradually. The corresponding values were 2.946, 2.990, 3.403 and 3.878 ton/ hectare for (zero, 50, 75 and 100 mg/L), respectively.

In addition to, spraying GBF by pyridoxine recorded the higher value of forage yield (3.538 ton/ hectare) in comparison with that sprayed by tryptophan (3.070 ton/ hectare).

Table 2: Main effects of dietary treatments on forage yield

Item	Forage yield (ton/ hectare)
Sources of supplementations	
Tryptophan	3.070^{b}
Pyridoxine	3.538^{a}
SEM	0.071
Levels of supplementations mg/L	
Zero mg/L	2.946^{d}
50 mg/L	2.990^{c}
75 mg/L	3.403^{b}
100 mg/L	3.878^{a}
Green Berseem fodder (GBF) cut stages	
First cut	2.615^{d}
Second cut	3.371^{c}
Third cut	3.663^{a}
Fourth cut	3.589^{b}

a,b,c and d: Means in the same colum within each treatments having different superscripts differ significantly (P<0.05). SEM: standard error of the mean

Table 3: Results of ANOVA for forage yield

Item	Forage yield (ton/ hectare)
Main effects of	
Sources of supplementation (S)	*
Levels of supplementation (L)	*
Cut stages (C)	*
Interactions	
Sources of supplementation x levels of supplementation (S x L)	*
Sources of supplementation x cut stages (S x C)	*
levels of supplementation x cut stages (L x C)	*
Sources of supplementation x levels of supplementation x cut stages (S x L x C)	*

*: Significant at (P<0.05)

Table 4: Effect interactions between source & level of supplementation and green berseem fodder cut stages (S x L x C) on forage yield

Item			
Berseem Cuts	Sources	Levels mg/ L	Forage yield (ton/ hectare)
First cut (1st cut)	Tryptophan	0	2.223^{v}
		50	2.267^{u}
		75	2.340^{t}
		100	2.222^{v}
	Pyridoxine	0	2.223^{v}
		50	2.100^{w}
		75	3.123^{r}
		100	4.424^{d}
Second cut (2nd cut)	Tryptophan	0	3.122^{r}
		50	3.222^{p}
		75	3.152^{q}
		100	3.350^{m}
	Pyridoxine	0	3.122^{r}
		50	2.867^{s}
		75	3.600^{g}
		100	4.530^{c}
Third cut (3rd cut)	Tryptophan	0	3.320^{n}
		50	3.420^{k}
		75	3.510^{j}
		100	3.572^{h}
	Pyridoxine	0	3.320^{n}
		50	3.400^{l}
		75	4.100^{e}
		100	4.660^{b}
Fourth cut (4th cut)	Tryptophan	0	3.120^{r}
		50	3.342^{m}
		75	3.420^{k}
		100	3.524^{i}
	Pyridoxine	0	3.120^{r}
		50	3.300^{o}
		75	3.982^{f}
		100	4.740^{a}

a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t, u and v: Means in the same Colum having different superscripts differ significantly (P<0.05). SEM: standard error of the mean

Data of Tables (3 & 4) cleared significantly (P<0.05) interaction between source and level (S x L) of supplementation; source and cuts (S x C); level and cuts (L x C) and source, level and cuts (S x L x C).

These promotive effect of tryptophan are in harmony with those obtained by Dawood and Sadak [10] on canola; Bakry *et al.* [16] on quinoa and El-Awadi *et al.* [32] on chickpea. The positive effect of tryptophan or pyridoxine on yield components of clover plant might be due to the enhancing role of tryptophan or pyridoxine on cell division, increased endogenous phytohormones as auxins as noted by Abbas *et al.* [15] and improve uptake of nutrients and assimilation [33] in addition to increasing synthesis of proteins. Also, the forage yield in the present study within the same range that obtained by [34-38]. Furthermore, Hathout *et al.* [39]; Patel and Rajagopal [40]; Soleymani *et al.* [41] and Bakhoum *et al.* [42] noticed that dry forage yield of bio-organic + mineral fertilizers treatment increased from 1st to 2nd to 3rd cuts (1.143-2.026 and 3.093 ton/fad.). The second order of treatments recorded by the mineral fertilization treatments which produced 0.934, 1.838 and 2.746 ton/fad, for the successive three cuts of Egyptian berseem clover.

Main Effect of Supplementation Source, Level of Supplementation, Cut Sages and Their Different Interactions on Chemical Analysis, Energetic Values, Nutritive Values and Cell Wall Constituents: Data presented in Tables (5 and 6) except for EE content, the other values of chemical analysis, energetic and nutritive values and cell wall constituents that includes (NDF, ADF, ADL, hemicellulose and cellulose) were significantly affected by source of supplementation. Furthermore, spraying berseem by tryptophan solution significantly (P<0.05) increased DM, OM, NFE, GE, DE, ME, NE, TDN, hemicellulose contents comparing to spraying berseem by pyridoxine. Meanwhile, spraying berseem by pyridoxine solution significantly (P<0.05) increased moisture, CP, CF, ash, DCP, NDF, ADF, ADL and cellulose contents in comparison with spraying berseem by tryptophan solution. Mean while, EE content was not affected (P>0.05) by supplementation source.

As shown in Tables (5 & 6) the main effect of level of supplementation had significantly affecting (P<0.05) on all parameters of chemical analysis, energetic and nutritive values and cell wall constituents that includes (NDF, ADF, ADL, hemicellulose and cellulose). Increasing level of spraying significantly (P<0.05) increased CP content, but it significantly (P<0.05) decreased NFE content compared to control (Zero mg/L). Spraying BGF by high level (100 mg/L) recorded the highest values of energetic values (GE, DE, ME and NE) and nutritive values (TDN and DCP percentages). Cell wall constituents includes (NDF, ADF, ADL, cellulose) significantly (P<0.05) decreased with increasing the level of supplementation.

In addition to the data of Tables (5 & 6) mentioned that the main effect of cut stages had significantly affecting (P<0.05) on all parameters of chemical analysis, energetic and nutritive values and cell wall constituents that includes (NDF, ADF, ADL, hemicellulose and cellulose). With increasing the cut stage the DM and CF contents were significantly (P<0.05) increased. The highest values of CF, NDF, ADF, ADL and cellulose were recorded at fourth cut stage. The highest values of CP, EE, ash, GE, DE, ME, NE, TDN and DCP contents were notices with the third cut stages. The highest value of hemicellulose content was recorded with the second cut stages. The highest values of both moisture and OM was observed with the first cut sages.

Moreover, data presented in Tables (6 & 7) showed that, there were significantly (P<0.05) interactions between source and level of supplementation (S x L); source and cut stages (S x C); level of supplementation and cut stages (L x C) and source, level of supplementation and cut stages (S x L x C) on all parameters of chemical analysis; energetic & nutritive values and cell wall constituents. The present results within the range of the results obtained by Zeweil [43]; Gupta *et al*. [44]; Sarhan [45]; Stanton and LeVally [46]; Omer *et al.* [47]; Hassan *et al.* [48]; El-Garhy *et al.* [49]; Omer *et al.* [50]; Ibrahim *et al.* [51]; Omer *et al.* [52]; Omer *et al.* [53]; Omer and Badr [54]; Abdel-Magid *et al.* [55]; Bakhoum *et al.* [42] and Omer *et al.* [56] who noticed that clover hay on dry matter basis (in average) contained 92.00, 87.17, 13.40, 26.03, 4.03, 43.71, 43.20, 30.06, 5.54 %, 4153 and 2661 kca/ kg DM of DM, OM, CP, CF, EE, NFE, ash, NDF, ADF and ADL, gross energy (GE) and digestible energy (DE), respectively.

Main Effects of Spraying Berseem Green Fodder (BGF) By Tryptophan or Pyridoxine at Different Levels and Different Cut Stages on Their Changes in Photosynthetic Pigments: As presented in Tables (8 and 9) it clear that the main effects of supplementation source had significant effect on total carbohydrates, total soluble sugars, chlorophyll a, phenolic compounds and flavonoids. But the other parameters of photosynthetic pigments was not affected (P>0.05) by the source of the supplementation. Spraying BGF by pyridoxine solution

Table 5: Main effects of dietary treatments on chemical analysis; energetic & nutritive values and cell wall constituents of tested berseem green fodder samples

	Green berseem fodder cut stages					Levels of supplementations					Sources of supplementations		
Item	First cut	Second cut	Third cut	Fourth cut	SEM	Zero mg/L	50 mg/L	75 mg/L	100 mg/L	SEM	Tryptophan	Pyridoxine	SEM
1-Chemical analyses:													
Moisture	10.24^{a}	9.91^{b}	9.13^{c}	9.01^{d}	0.14	9.39^{c}	9.61^{b}	9.69^{a}	9.60^{b}	0.14	9.45^{b}	9.69^{a}	0.14
Dry matter (DM)	89.76^{d}	90.09^{c}	90.87^{b}	90.99^{a}	0.14	90.61^{a}	90.39^{b}	90.31^{c}	90.40^{b}	0.14	90.55^{a}	90.31^{b}	0.14
Chemical analysis on DM basis:													
Organic matter (OM)	88.31^{a}	87.71^{b}	87.33^{d}	87.46^{c}	0.12	88.01^{a}	87.10^{c}	87.68^{b}	88.00^{a}	0.12	87.81^{a}	87.59^{b}	0.12
Crude protein (CP)	17.07^{d}	17.85^{b}	19.05^{a}	17.63^{c}	0.10	17.09^{c}	17.76^{b}	18.38^{a}	18.38^{a}	0.10	17.78^{b}	18.02^{a}	0.10
Crude fiber (CF)	20.29^{b}	18.84^{d}	19.86^{c}	23.46^{a}	0.31	21.04^{a}	20.75^{c}	19.75^{d}	20.91^{b}	0.31	20.12^{b}	21.10^{a}	0.31
Ether extract (EE)	2.91^{b}	2.46^{d}	3.52^{a}	2.76^{c}	0.10	3.03^{a}	2.88^{c}	2.78^{d}	2.96^{b}	0.10	2.91	2.91	0.10
Nitrogen-free extract (NFE)	48.04^{b}	48.56^{a}	44.90^{c}	43.61^{d}	0.35	46.85^{a}	45.71^{c}	46.77^{b}	45.75^{c}	0.35	47.00^{a}	45.56^{b}	0.35
Ash	11.69^{d}	12.29^{c}	12.67^{a}	12.54^{b}	0.12	11.99^{c}	12.90^{a}	12.32^{b}	12.00^{c}	0.12	12.19^{b}	12.41^{a}	0.12
2-Energetic values (kilo calories / Kg DM)													
Gross energy (GE)	4074^{b}	4037^{c}	4095^{a}	4039^{c}	6.54	4068^{b}	4032^{d}	4060^{c}	4083^{a}	6.54	4064^{a}	4058^{b}	6.54
Digestible energy (DE)	3096^{b}	3068^{c}	3112^{a}	3069^{c}	4.97	3092^{b}	3065^{d}	3086^{c}	3103^{a}	4.97	3088^{a}	3084^{b}	4.97
Metabolizable energy (ME)	2539^{b}	2516^{c}	2552^{a}	2517^{c}	4.06	2535^{b}	2513^{d}	2530^{c}	2545^{a}	4.06	2533^{a}	2529^{b}	4.06
Net energy (NE)	1422^{b}	1409^{c}	1429^{a}	1410^{c}	2.29	1420^{b}	1407^{d}	1417^{c}	1425^{a}	2.29	1418^{a}	1416^{b}	2.29
3-Nutritive values (%)													
Total digestible nutrients (TDN)	69.89^{b}	69.26^{c}	70.25^{a}	69.28^{c}	0.11	69.79^{b}	69.18^{d}	69.66^{c}	70.57^{a}	0.11	69.72^{a}	69.62^{b}	0.11
Digestible crude protein (DCP)	12.01^{d}	12.68^{b}	13.69^{a}	12.49^{c}	0.09	12.03^{c}	12.59^{b}	13.12^{a}	13.12^{a}	0.09	12.61^{b}	12.82^{a}	0.09
4-Cell wall constituents(%)													
Neutral detergent fiber (NDF)	42.25^{b}	41.30^{d}	41.97^{c}	44.33^{a}	0.21	42.75^{a}	42.56^{c}	41.90^{d}	42.66^{b}	0.21	42.14^{b}	42.79^{a}	0.21
Acid detergent fiber (ADF)	27.93^{b}	26.62^{d}	27.54^{c}	30.83^{a}	0.29	28.62^{a}	28.36^{c}	27.44^{d}	28.50^{b}	0.29	27.78^{b}	28.68^{a}	0.29
Acid detergent lignin (ADL)	4.96^{b}	4.72^{d}	4.89^{c}	5.50^{a}	0.05	5.09^{a}	5.04^{c}	4.87^{d}	5.07^{b}	0.05	4.93^{b}	5.10^{a}	0.05
Hemicellulose*	14.32^{c}	14.68^{a}	14.43^{b}	13.50^{d}	0.08	14.13^{d}	14.20^{b}	14.46^{a}	14.16^{c}	0.08	14.36^{a}	14.11^{b}	0.08
Cellulose**	22.97^{b}	21.90^{d}	22.65^{c}	25.33^{a}	0.23	23.53^{a}	23.32^{c}	22.57^{d}	23.43^{b}	0.23	22.85^{b}	23.58^{a}	0.23

a,b,c and d: Means in the same row within each treatments having different superscripts differ significantly ($P<0.05$).

SEM: standard error of the mean.

*Hemicellulose = NDF – ADF.

** Cellulose = ADF – ADL.

Table 6: Results of ANOVA for chemical analysis, energetic & nutritive values and cell wall constituents of different tested berseem green fodder samples

	Main effects of			Interactions			
Item	Sources (S)	Levels (L)	Cuts (C)	(S x L)	(S x C)	(L x C)	(S x L x C)
1-Chemical analyses:							
Moisture	*	*	*	*	*	*	*
Dry matter (DM)	*	*	*	*	*	*	*
Chemical analysis on DM basis:							
Organic matter (OM)	*	*	*	*	*	*	*
Crude protein (CP)	*	*	*	*	*	*	*
Crude fiber (CF)	*	*	*	*	*	*	*
Ether extract (EE)	NS	*	*	*	*	*	*
Nitrogen-free extract (NFE)	*	*	*	*	*	*	*
Ash	*	*	*	*	*	*	*
2-Energetic values (kilo calories / Kg DM)							
Gross energy (GE)	*	*	*	*	*	*	*
Digestible energy (DE)	*	*	*	*	*	*	*
Metabolizable energy (ME)	*	*	*	*	*	*	*
Net energy (NE)	*	*	*	*	*	*	*
3-Nutritive values (%)							
Total digestible nutrients (TDN)	*	*	*	*	*	*	*
Digestible crude protein (DCP)	*	*	*	*	*	*	*
4-Cell wall constituents (%)							
Neutral detergent fiber (NDF)	*	*	*	*	*	*	*
Acid detergent fiber (ADF)	*	*	*	*	*	*	*
Acid detergent lignin (ADL)	*	*	*	*	*	*	*
Hemicellulose*	*	*	*	*	*	*	*
Cellulose**	*	*	*	*	*	*	*

*: Significant (P<05).

NS: not significant.

Table 7: Effect interactions between source & level of supplementation and green berseem fodder cut stages (S x L x C) on chemical analysis; energetic & nutritive values and cell wall constituents

Item					Chemical analysis on DM basis						Energetic values				Nutritive values		Cell wall constituents				
Berseem Cuts	Source s	Levels mg/L	Moisture	DM	OM	CP	CF	EE	NFE	Ash	GE	DE	ME	NE	TDN	DCP	NDF	ADF	ADL	Hemi cellulose	Cell ulose
First cut (1st cut)	Trypto phan	0	9.71^{g}	90.29^{l}	86.18^{t}	16.00^{r}	18.10^{q}	3.96^{a}	28.12^{g}	13.82^{c}	4024^{l}	3058^{l}	2508^{kl}	1404^{i}	69.03^{l}	11.10^{r}	40.82^{r}	25.94^{q}	4.59^{o}	14.88^{g}	21.35^{r}
		50	10.59^{d}	89.41^{o}	88.26^{g}	16.47^{q}	19.30^{m}	3.75^{c}	48.74^{f}	11.74^{p}	4107^{cd}	3121^{cd}	2559^{cd}	1433^{bcd}	70.45^{cd}	11.50^{q}	41.60^{n}	27.03^{m}	4.79^{l}	14.57^{k}	22.24^{n}
		75	9.26^{k}	90.74^{h}	89.53^{c}	17.38^{m}	17.48^{t}	1.06^{op}	53.61^{a}	10.47^{t}	4032^{k}	3064^{k}	2512^{k}	1407^{i}	69.16^{k}	12.27^{mn}	40.41^{u}	25.37^{t}	4.48^{r}	15.04^{d}	20.89^{u}
		100	9.60^{h}	90.40^{k}	89.90^{b}	17.09^{o}	20.33^{l}	1.23^{n}	51.25^{b}	10.10^{u}	4052^{j}	3080^{j}	2526^{j}	1415^{h}	69.53^{j}	12.03^{o}	42.28^{m}	27.97^{l}	4.97^{k}	14.31^{l}	23.00^{m}
	Pyrido xine	0	9.71^{g}	90.29^{l}	86.18^{t}	16.00^{r}	18.10^{q}	3.96^{a}	48.12^{g}	13.82^{c}	4024^{l}	3058^{l}	2508^{kl}	1404^{i}	69.03^{l}	11.10^{r}	40.82^{r}	25.94^{q}	4.59^{o}	14.88^{g}	21.35^{r}
		50	7.85^{p}	92.15^{c}	88.02^{h}	16.60^{p}	22.67^{g}	1.82^{m}	46.93^{i}	11.98^{o}	3997^{m}	3038^{m}	2491^{m}	1395^{j}	68.58^{m}	11.61^{p}	43.82^{g}	30.11^{g}	5.36^{g}	13.71^{q}	24.75^{g}
		75	10.73^{c}	89.27^{P}	87.57^{k}	18.20^{i}	22.58^{gh}	3.65^{de}	43.14^{q}	12.43^{l}	4099^{ef}	3115^{ef}	2554^{ef}	1430de	70.32^{ef}	12.97^{i}	43.76^{hi}	30.02^{h}	5.35^{g}	13.74^{p}	24.67^{hi}
		100	14.51^{a}	85.49^{r}	90.82^{a}	18.81^{f}	23.73^{d}	3.86^{b}	44.42^{n}	9.18^{v}	4254^{a}	3233^{a}	2651^{a}	1485^{a}	72.98^{a}	13.49^{f}	44.51^{d}	31.07^{d}	5.54^{d}	13.44^{t}	25.53^{d}
Second cut (2nd cut)	Trypto phan	0	10.52^{de}	89.48^{no}	88.73^{e}	17.42^{m}	17.15^{u}	3.28^{hi}	50.88^{c}	11.27^{r}	4116^{b}	3128^{b}	2565^{b}	1436^{b}	70.61^{b}	12.31^{m}	40.19^{v}	25.07^{u}	4.43^{s}	15.12^{c}	20.64^{v}
		50	9.74^{g}	90.26^{l}	87.92^{i}	17.57^{l}	21.46^{i}	3.60^{ef}	45.29^{l}	12.08^{n}	4101de	3117de	2556de	1431^{cde}	70.36^{de}	12.43^{l}	43.02^{j}	29.00^{i}	5.16^{h}	14.02^{o}	23.84^{j}
		75	10.17^{f}	89.83^{m}	87.41^{mn}	18.20^{i}	23.57^{e}	3.11^{k}	42.53^{s}	12.59^{ij}	4064^{hi}	3089^{hi}	2533^{hi}	1418^{gh}	69.73^{hi}	12.97^{i}	44.41^{e}	30.93^{e}	5.52^{e}	13.48^{s}	25.41^{e}
		100	9.31^{jk}	90.69^{hi}	87.23^{o}	18.22^{i}	18.29^{p}	1.06^{op}	49.66^{e}	12.77^{h}	3949^{o}	3001^{o}	2461^{o}	1378^{l}	67.74^{o}	12.99^{i}	40.94^{q}	26.11^{p}	4.62^{n}	14.83^{h}	21.49^{q}
	Pyrido xine	0	10.52^{de}	89.48^{no}	88.73^{e}	17.42^{m}	17.15^{u}	3.28^{hi}	50.88^{c}	11.27^{r}	4116^{b}	3128^{b}	2565^{b}	1436^{b}	70.61^{b}	12.31^{m}	40.19^{v}	25.07^{u}	4.43^{s}	15.12^{c}	20.64^{v}
		50	8.71^{m}	91.29^{f}	86.34^{s}	17.29^{n}	16.71^{v}	1.03^{p}	51.31^{b}	13.66^{d}	3897^{p}	2962^{p}	2429^{p}	1360^{m}	66.86^{p}	12.20^{n}	39.90^{w}	24.67^{v}	4.35^{t}	15.23^{b}	20.32^{w}
		75	9.56^{hi}	90.44^{jk}	88.40^{f}	17.91^{k}	18.68^{n}	1.10^{o}	50.71^{c}	11.60^{q}	3995^{m}	3036^{m}	2490^{m}	1394^{j}	68.53^{m}	12.72^{k}	41.20^{o}	26.47^{n}	4.68^{m}	14.73^{j}	21.79^{o}
		100	10.72^{c}	89.28^{p}	86.92^{q}	18.79^{f}	17.74^{s}	3.24^{ij}	47.15^{h}	13.08^{f}	4059^{ij}	3085^{ij}	2530^{ij}	1417^{gh}	69.64^{hij}	13.47^{f}	40.58^{t}	25.61^{s}	4.53^{q}	14.97^{e}	21.08^{t}
Third cut (3rd cut)	Trypto phan	0	9.79^{g}	90.21^{l}	87.73^{j}	18.53^{g}	21.34^{j}	3.62^{de}	44.24^{no}	44.24^{m}	4109^{bc}	3123^{bc}	2561^{bc}	1434^{bc}	70.50^{bc}	13.25^{g}	42.94^{k}	28.89^{j}	5.14^{i}	14.05^{n}	23.75^{k}
		50	9.71^{g}	90.29^{l}	87.35^{n}	19.13^{d}	20.92^{k}	3.87^{b}	43.43^{p}	43.43^{i}	4115^{b}	3127^{b}	2564^{b}	1436^{b}	70.59^{b}	13.76^{d}	42.67^{l}	28.51^{k}	5.07^{j}	14.16^{m}	23.44^{l}
		75	7.96^{o}	92.04^{d}	87.38^{mn}	19.32^{c}	17.70^{s}	3.36^{g}	47.00^{hi}	47.00^{ij}	4092^{f}	3110^{f}	2550^{f}	1428^{e}	70.20^{f}	13.92^{c}	40.55^{t}	25.57^{s}	4.52^{q}	14.98^{e}	21.05^{t}
		100	8.19^{n}	91.81^{e}	87.33^{n}	19.00^{e}	17.86^{r}	3.88^{b}	46.59^{j}	46.59^{i}	4113^{bc}	3126^{bc}	2563^{bc}	1435^{b}	70.56^{bc}	13.65^{e}	40.66^{s}	25.72^{r}	4.55^{p}	14.94^{f}	21.17^{s}
	Pyrido xine	0	9.79^{g}	90.21^{l}	87.73^{j}	18.53^{g}	21.34^{j}	3.62^{de}	44.24^{no}	44.24^{m}	4109^{bc}	3123^{bc}	2561^{bc}	1434^{bc}	70.50^{bc}	13.25^{g}	42.94^{k}	28.89^{j}	5.14^{i}	14.05^{n}	23.75^{k}
		50	10.45^{e}	89.55^{n}	87.06^{p}	19.54^{b}	18.26^{p}	3.31^{gh}	45.95^{k}	45.95^{g}	4080^{g}	3101^{g}	2543^{g}	1424^{f}	70.00^{g}	14.11^{b}	40.92^{q}	26.09^{p}	4.62^{n}	14.83^{h}	21.47^{q}
		75	11.00^{b}	89.00^{q}	86.58^{r}	19.75^{a}	18.56^{o}	3.27^{hi}	45.00^{m}	45.00^{e}	4061^{hi}	3086^{hi}	2531^{hi}	1417^{gh}	69.66^{hij}	14.29^{a}	41.12^{p}	26.36^{o}	4.67^{m}	14.76^{i}	21.69^{p}
		100	6.16^{r}	93.84^{a}	87.47^{lm}	18.61^{g}	22.87^{f}	3.19^{j}	42.80^{r}	42.80^{jk}	4077^{g}	3099^{g}	2541^{g}	1423^{f}	69.95^{g}	13.32^{g}	43.95^{f}	30.29^{f}	5.40^{f}	13.66^{r}	24.89^{f}
Fourth cut (4th cut)	Trypto phan	0	7.54^{q}	92.46^{b}	89.41^{d}	16.41^{q}	27.56^{a}	1.25^{n}	44.19^{o}	44.19^{s}	4022^{l}	3057^{l}	2507^{l}	1404^{i}	69.01^{l}	11.45^{q}	47.03^{a}	34.57^{a}	6.19^{a}	12.46^{w}	28.38^{a}
		50	10.49^{e}	89.51^{n}	86.07^{u}	17.55^{l}	22.53^{h}	2.57^{l}	43.42^{p}	43.42^{b}	3970^{n}	3017^{n}	2474^{n}	1385^{k}	68.10^{n}	12.42^{l}	43.73^{i}	29.98^{h}	5.34^{g}	13.75^{p}	24.64^{i}
		75	9.50^{i}	90.50^{j}	87.06^{p}	18.06^{j}	15.69^{w}	3.31^{gh}	50.00^{d}	50.00^{g}	4058^{ij}	3084^{ij}	2529^{ij}	1416^{h}	69.62^{ij}	12.85^{j}	39.23^{x}	23.74^{w}	4.18^{u}	15.49^{a}	19.56^{x}
		100	9.15^{l}	90.85^{g}	87.46^{lm}	18.15^{i}	22.62^{gh}	3.67^{d}	43.02^{q}	43.02^{jk}	4095^{ef}	3112^{ef}	2552^{ef}	1429^{e}	70.25^{ef}	12.93^{i}	43.79^{gh}	30.06^{gh}	5.35^{g}	13.73^{pq}	24.71^{gh}
	Pyrido xine	0	7.54^{q}	92.46^{b}	89.41^{d}	16.41^{q}	27.56^{a}	1.25^{n}	44.19^{o}	44.19^{s}	4022^{l}	3057^{l}	2507^{l}	1404^{i}	69.01^{l}	11.45^{q}	47.03^{a}	34.57^{a}	6.19^{a}	12.46^{w}	28.38^{a}
		50	9.37^{j}	90.63^{i}	85.81^{v}	17.89^{k}	24.16^{b}	3.09^{k}	40.67^{v}	40.67^{a}	3992^{m}	3034^{m}	2488^{m}	1393^{j}	68.49^{m}	12.71^{k}	44.80^{b}	31.47^{b}	5.62^{b}	13.33^{v}	25.85^{b}
		75	9.33^{jk}	90.67^{hi}	87.53^{kl}	18.24^{i}	23.70^{d}	3.36^{g}	42.23^{t}	42.23^{kl}	4082^{g}	3102^{g}	2544^{g}	1425^{f}	70.02^{g}	13.00^{i}	44.49^{d}	31.05^{d}	5.54^{d}	13.44^{t}	25.51^{d}
		100	9.16^{l}	90.84^{g}	86.89^{q}	18.34^{h}	23.82^{c}	3.54^{f}	41.19^{u}	41.19^{f}	4067^{h}	3091^{h}	2535^{h}	1420^{g}	69.77^{h}	13.09^{h}	44.57^{c}	31.16^{c}	5.56^{c}	13.41^{u}	25.60^{c}
SEM			0.14	0.14	0.12	0.10	0.31	0.10	0.35	0.12	6.54	4.97	4.06	2.29	0.11	0.09	0.21	0.29	0.05	0.08	0.23

a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s, t, u, v, w and x: Means in the same colum having different superscripts differ significantly ($P<0.05$).
SEM: standard error of the mean. *Hemicellulose = NDF – ADF. ** Cellulose = ADF – ADL

significantly ($P<0.05$) increased the total carbohydrates, total soluble sugars, chlorophyll a phenolic compounds and flavonoids in comparison with the spraying BGF by tryptophan. Meanwhile, the other determined components of photosynthetic pigments that includes (polysaccharides, chlorophyll b, carotenoids and total pigments) were in the near value among the two sources of supplementation (tryptophan and pyridoxine).

On the other hand, Tables (8 and 9) showed that levels of supplementation had significant effect ($P<0.05$) on all determined nutrients of photosynthetic pigments of BGF. With increasing the level of supplementation the values of total carbohydrates, total soluble sugars, polysaccharides, chlorophyll b, carotenoids and total pigments comparing to control one (zero ml/ L). The best values were recorded when BGF sprayed by 75 or 100 ml/ L for most component determined.

Also, Tables (8 and 9) showed that berseem cut stages had significant effect ($P<0.05$) on all determined nutrients of photosynthetic pigments of BGF. Third 3rd and fourth 4th followed by the second 2nd berseem cut stages recorded the higher values of photosynthetic pigments of BGF comparing to the first 1st berseem cut stage.

Results of Tables (9 & 10) mentioned that their were significant ($P<0.05$) interactions among (S x L), (S x C), (L x C) for all parameters determined of photosynthetic pigments of BGF. Meanwhile interaction among (S x L x C) were significant ($P<0.05$) for all parameters determined of photosynthetic pigments of BGF except for plysaccharides contents.

The promotive effect of pyridoxine on the green berseem fodder leaves chlorophyll a, chlorophyll b, carotenoids and total pigments could be resulted from increased activities of different enzymes responsible for biosynthesis of these pigments or preservation of chromo proteins [57]. In addition, pyridoxine increased biosynthesis of chloroplast via its role in indole acetic acid formation [58]. Similar results were obtained earlier by Dawood and Sadak [10] on canola; Bakry *et al.* [16] on quinoa and El-Awadi *et al.* [32] on chickpea plant.

Table 8: Main effects of dietary treatments on photosynthetic pigments of berseem green fodder

	Green berseem fodder cut stages					Levels of supplementations					Sources of supplementations		
Item	First cut	Second cut	Third cut	Fourth cut	SEM	Zero mg/L	50 mg/L	75 Mg/L	100 mg/L	SEM	Tryptophan	Pyridoxine	SEM
Total carbohydrates %	15.31^{c}	16.08^{b}	17.31^{a}	16.04^{b}	0.09	15.48^{c}	16.05^{b}	16.60^{a}	16.61^{a}	0.09	16.10^{b}	16.27^{a}	0.09
Total soluble sugars%	3.90^{d}	5.02^{a}	4.57^{b}	4.16^{c}	0.06	3.89^{d}	4.27^{c}	4.81^{a}	4.69^{b}	0.06	4.36^{b}	4.47^{a}	0.06
Polysaccharides %	11.41^{c}	11.06^{d}	12.74^{a}	11.88^{b}	0.07	11.59^{c}	11.78^{b}	11.79^{b}	11.92^{a}	0.07	11.74	11.80	0.07
Chlorophyll a	962^{d}	1231^{b}	1291^{a}	1099^{c}	14.05	1077^{d}	1151^{c}	1184^{a}	1173^{b}	14.05	1142^{b}	1150^{a}	14.05
Chlorophyll b	638^{c}	655^{b}	660^{a}	619^{d}	2.19	625^{d}	638^{c}	652^{b}	657^{a}	2.19	644	642	2.19
Carotenoids	311^{c}	320^{b}	322^{a}	302^{d}	1.08	304^{d}	312^{c}	318^{b}	320^{a}	1.08	314	313	1.08
Total pigments	1911^{d}	2206^{b}	2273^{a}	2020^{c}	16.33	2006^{c}	2101^{b}	2154^{a}	2150^{a}	16.33	2100	2105	16.33
Phenolic compounds	64.29^{d}	68.57^{c}	81.56^{b}	93.16^{a}	1.56	77.39^{b}	76.78^{c}	78.35^{a}	75.05^{d}	1.56	68.83^{b}	84.95^{a}	1.56
Flavonoids	28.11^{d}	29.99^{c}	35.67^{b}	40.74^{a}	0.68	33.85^{b}	33.58^{c}	34.27^{a}	32.82^{d}	0.68	30.10^{b}	37.15^{a}	0.68

a,b,c and d: Means in the same row within each treatments having different superscripts differ significantly (P<0.05)
SEM: standard error of the mean.

Table 9: Results of ANOVA for photosynthetic pigments of berseem green fodder

	Main effects of			Interactions			
Item	Sources of supplementation (S)	Levels of supplementation (L)	Berseem cut stages (C)	(S x L)	(S x C)	(L x C)	(S x L x C)
Total carbohydrates %	*	*	*	*	*	*	*
Total soluble sugars%	*	*	*	*	*	*	*
Polysaccharides %	NS	*	*	*	*	*	NS
Chlorophyll a	*	*	*	*	*	*	*
Chlorophyll b	NS	*	*	*	*	*	*
Carotenoids	NS	*	*	*	*	*	*
Total pigments	NS	*	*	*	*	*	*
Phenolic compounds	*	*	*	*	*	*	*
Flavonoids	*	*	*	*	*	*	*

*: Significant (P<05). NS: not significant

Table 10: Interactions between source & level of supplementation and green berseem fodder cut stages (S x L x C) on photosynthetic pigments

Item											
Berseem cuts stage	Source	Levels (mg/L)	Total carbohydrates %	Total soluble sugars %	Polysacch arides %	Chlorophyll a	Chlorophyll b	Carotenoids	Total pigments	Phenolic compounds	Flavonoids
First cut (1st cut)	Tryptophan	0	14.45^{q}	3.45^{k}	11.00^{ijk}	927^{l}	612^{p}	299^{o}	1838^{n}	63.15^{p}	27.62^{p}
		50	14.73^{p}	3.68^{j}	11.05^{ijk}	961^{k}	635^{jk}	310^{i}	1906^{l}	59.62^{r}	26.07^{r}
		75	15.77^{kl}	4.09^{h}	11.68^{fg}	981^{jk}	654^{g}	319^{f}	1954^{k}	55.02^{s}	24.06^{s}
		100	14.45^{mn}	4.26^{gh}	11.19^{ij}	984^{jk}	661^{ef}	323^{de}	1968^{k}	50.91^{t}	22.26^{t}
	Pyridoxine	0	14.45^{q}	3.45^{k}	11.00^{ijk}	927^{l}	612^{p}	299^{o}	1838^{n}	63.15^{p}	27.62^{p}
		50	15.30^{no}	3.52^{jk}	11.78^{efg}	955^{kl}	624^{m}	$304l^{m}$	$1883l^{m}$	70.39^{n}	30.78^{n}
		75	16.25^{ghi}	4.60^{f}	11.65^{fgh}	993^{j}	637^{jk}	311^{hi}	1941^{k}	78.77^{j}	34.45^{j}
		100	16.08^{ij}	4.16^{gh}	11.92^{ef}	972^{jk}	665^{bcd}	324^{cde}	1961^{k}	73.27^{m}	32.04^{m}
Second cut (2nd cut)	Tryptophan	0	15.59^{lm}	4.27^{gh}	11.32^{hi}	1150^{gh}	638^{j}	311^{hi}	2099^{gh}	69.08^{o}	30.21^{o}
		50	15.86^{jk}	5.02^{d}	10.84^{k}	1200^{f}	648^{h}	316^{g}	2164^{f}	61.06^{q}	26.70^{q}
		75	16.35^{fgh}	5.27^{c}	11.08^{ijk}	1239^{e}	664^{cde}	324^{cde}	2227^{de}	59.76^{r}	26.13^{r}
		100	16.52^{def}	5.47^{b}	11.05^{ijk}	1268^{cde}	674^{a}	329^{a}	2271^{c}	55.75^{s}	24.38^{s}
	Pyridoxine	0	15.59^{lm}	4.27^{gh}	11.32^{hi}	1150^{gh}	638^{j}	311^{hi}	2099^{gh}	69.08^{o}	30.21^{o}
		50	15.78^{kl}	4.83^{e}	10.95^{jk}	1273^{cd}	651^{gh}	317^{fg}	2241^{cde}	73.90^{m}	32.32^{m}
		75	16.20^{hi}	5.78^{a}	10.42^{l}	1308^{b}	668^{b}	326^{bc}	2302^{b}	83.29^{g}	36.48^{g}
		100	16.78^{d}	5.24^{c}	11.54^{gh}	1263^{cde}	659^{f}	322^{e}	2244^{cd}	76.65^{k}	33.52^{k}
Third cut (3rd cut)	Tryptophan	0	16.72^{de}	4.20^{gh}	12.52^{cd}	1256^{de}	643^{i}	313^{h}	2212^{e}	83.20^{g}	36.38^{g}
		50	17.27^{c}	4.23^{gh}	13.04^{ab}	1293^{bc}	653^{g}	319^{f}	2265^{c}	75.39^{l}	32.97^{l}
		75	17.78^{a}	4.60^{f}	13.18^{a}	1311^{b}	665^{bcd}	325^{cd}	2307^{b}	68.05^{o}	29.76^{o}
		100	17.44^{bc}	4.83^{e}	12.61^{cd}	1343^{a}	675^{a}	329^{a}	2347^{a}	70.33^{n}	30.76^{n}
	Pyridoxine	0	16.72^{de}	4.20^{gh}	12.52^{cd}	1256^{de}	643^{i}	313^{h}	2212^{e}	83.20^{g}	36.38^{g}
		50	17.50^{bc}	4.68^{ef}	12.82^{bc}	1275^{cd}	663^{de}	324^{cde}	2262^{c}	90.39^{f}	39.53^{f}
		75	17.58^{ab}	5.19^{cd}	12.39^{d}	1317^{ab}	672^{a}	328^{ab}	2317^{b}	98.47^{d}	43.06^{d}
		100	17.46^{bc}	4.65^{ef}	12.81^{bc}	1277^{cd}	667^{bc}	325^{cd}	2269^{c}	83.44^{g}	36.49^{g}
Fourth cut (4th cut)	Tryptophan	0	15.17^{o}	3.63^{jk}	11.54^{gh}	974^{jk}	606^{p}	295^{p}	1875^{m}	94.14^{e}	41.17^{e}
		50	15.71^{kl}	3.87^{i}	11.84^{efg}	1095^{i}	614^{o}	300^{no}	2009^{j}	81.00^{i}	35.42^{i}
		75	16.34^{fgh}	4.28^{gh}	12.06^{e}	1147^{gh}	624^{l}	305^{kl}	2076^{hi}	72.85^{m}	31.86^{m}
		100	16.49^{efg}	4.59^{f}	11.90^{ef}	1141^{h}	634^{kl}	309^{ij}	2084^{ghi}	82.04^{h}	35.88^{h}
	Pyridoxine	0	15.17^{o}	3.63^{jk}	11.54^{gh}	974^{jk}	606^{p}	295^{p}	1875^{m}	94.14^{e}	41.17^{e}
		50	16.21^{hi}	4.32^{g}	11.89^{ef}	1155^{gh}	619^{n}	302^{mn}	2076^{hi}	102.48^{c}	44.82^{c}
		75	16.54^{def}	4.65^{ef}	11.89^{ef}	1173^{g}	629^{kl}	307^{jk}	2109^{g}	110.62^{a}	48.38^{a}
		100	16.66^{de}	4.29^{2}	12.37^{d}	1134^{h}	620^{n}	302^{mn}	2056^{i}	107.98^{b}	47.22^{b}
	SEM		0.09	0.06	0.07	14.05	2.19	1.08	16.00	1.56	0.68

a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s and t: Means in the same colum having different superscripts differ significantly (P<0.05). SEM: standard error of the mean.

These increasing in carbohydrate constituents of green berseem fodder in response to pyridoxine treatment could be attributed to its important role on chlorophyll biosynthesis which reflected on the biosynthesis of carbohydrates as well as Inodole acetic acid (IAA) and its precursor pyridoxine enhance translocation of sugars during its biosynthesis [59]. The present results are in harmony with those noted by Abdel-Monem *et al.* [60] who found that carbohydrates contents were increased in sunflower plant by tryptophan treatment and El-Awadi *et al.* [32] who noted that tryptophan increased carbohydrates contents of chickpea plant.

External treatment of pyridoxine as foliar application with different concentrations (50, 75 and 100 mg/l) on green berseem fodder grown under sandy soil enhance and significantly increased both total phenol and flavonoids contents.

These increases in phenolic and flavonoids are gradually in the four cuts. Phenolic and flavonoids contents increased in second, third and fourth cuts comparing to the control n agreement with those noticed by Dawood and Sadak [10]; Sadak *et al.* [9]; Bakry *et al.* [16] and El-Awadi *et al.* [32]. In addition, these increases in total phenolic and flavonoids compounds might decrease or inhibit activity of IAA oxidase enzyme thus leads to increased levels of IAA which lead to improved growth and yield of green berseem fodder [10].

CONCLUSION

From the present results it can be mentioned that under conditions as this available during carrying out of this work, it can be concluded that, tryptophan or pyridoxine can be used safety without realized any adverse effect on plant grown and it occurred an increasing in their forage yield and improving their nutritional values and photosynthetic pigments of berseem green fodder.

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