Asian Journal of Crop Science

ISSN 1994-7879

OPEN ACCESS Asian Journal of Crop Science

ISSN 1994-7879
DOI: 10.3923/ajcs.2016.96.102

Research Article
Variations of the Cell Wall Components of Multi-cut Forage Legumes, Grasses and Legume-grass Binary Mixtures Grown in Egypt

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Abstract
Background: The cell wall components are considered among the most important determinants of forage quality and its effect on the animal's performance. It is therefore, important to investigate the response of the different forage grasses, legumes and their mixtures at successive cuttings in terms of their cell wall components under the different agricultural systems. **Materials and Methods:** The present study was carried out in the summer seasons of 2012 and 2013 in the experimental station of the Faculty of Agriculture, Alexandria University, Egypt. The main aim was to analyze the response of cell wall components, namely Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL), cellulose and hemicellulose of legume, grass and legume-grass forage groups to three successive cuttings. **Results:** Results revealed that the amount of the tested cell wall components of the three forage groups increased with the successive cuttings following a linear trend, however, this increase was more pronounced in case of the ADF and cellulose contents. The difference between the 1st and 3rd cuts amounted to 3.9, 3.3, 0.3 and 3.0% for NDF, ADF, ADL and cellulose contents, respectively. Grasses, in general were superior to the other two forage groups in NDF, ADF and cellulose contents with a content that amounted to 643.05, 374.91 and 345.86 g kg^{-1} for the three respective components. On the other hand, forage legumes produced the highest amount of lignin (39.73 g kg^{-1}). **Conclusion:** The amounts of the different cell wall components and the rates by which they increased among the three successive cuttings were less in case of forage mixtures than forage grasses, indicating better nutritional value for livestock in Egypt in the summer season.

Key words: Cell wall, forages, NDF, ADF, ADL, cellulose, hemicellulose, legumes, grasses, legume-grass mixtures

Received: June 23, 2016 Accepted: August 04, 2016 Published: September 15, 2016

Citation: Heba Sabry Attia Salama and Ali Issa Nawar, 2016. Variations of the cell wall components of multi-cut forage legumes, grasses and legume-grass binary mixtures grown in Egypt. Asian J. Crop Sci., 8: 96-102.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The animal production sector in Egypt is experiencing marked fluctuations. High feed prices are impacting the sector as are reduced demand for high priced local beef during this period of low economic growth[1]. In Egypt, as well as other developing countries, animals are likely to have a lower genetic potential for production and to partition proportionally more nutrients into maintenance and survival strategies than those found in industrialized countries. Also, dual and triple purpose animals may have different efficiencies for any one of the major uses of energy than animals that have been highly selected for a single production goal, such as high producing dairy cows[2]. Energy substrates, largely fiber make up a greater proportion of common forages, fodders and crop residues. Total dietary fiber is composed of soluble and insoluble fractions and the proportions of these fractions may be important in determining animal responses to forages[3]. Many chemical assays have been proposed to estimate herbage quality and its relationships to animal performance[4]. The majority of the respondents indicated that the Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) analyses were the chemical assays of c hoice to estimate *in vivo* dry matter digestibility and dry matter intake, respectively[5,6]. The ADF concentration refers to the cell wall portions of the forage. These portions consist of cellulose and lignin. The ADF values are important because they describe the ability of an animal to digest the forage. As the ADF increases, the digestibility of the forage usually decreases. The NDF value refers to the total cell wall composed of the ADF fraction plus hemicellulose. Neutral detergent fiber values are important in ration formulation because they reflect the amount of forage that the animal can consume[7]. Van Soest[8] classified the chemical fractions of forages and feedstuffs according to their nutritive availability to animals into two categories, category A contained proteins, soluble carbohydrates and other constituents of the plant cell that are generally available to ruminants and non-ruminants alike. However, components in category B included the cellulose, hemicellulose and lignin which are either of limited availability or not available at all. Enzymes that hydrolyze cellulose and hemicellulose are not secreted by higher animals and a considerable difference exists between ruminants and non-ruminants in their ability to utilize these structural carbohydrates. Utilization is possible only through microbial fermentation, which gives ruminants a considerable advantage. The high correlation of the lignin content with decreased forage digestibility and more specifically with decreased digestibility of particular cell wall polysaccharides has been reported[9-11]. Thus, cell wall components such as NDF, ADF, cellulose, hemicellulose and lignin are very important limiting factors to the feeding processes and to the ability of the animal to utilize the consumed forage. Forage grasses are known to have higher fiber content than forage legumes, thus expected to have relatively lower digestibility. Meanwhile, there are some disadvantages associated with feeding only legumes to ruminants such as bloat[12] and infertility in cattle and sheep[13]. While legume-grass mixtures, where the legume content is at maximum 50% are bloat safe[12] and the occurrence of infertility has decreased with higher proportion of grass in the feed. Therefore, growing mixtures of grasses and legumes is a proposed strategy to improve forage quality[14,15]. Main aim of the present study was to analyze the response of cell wall components, namely; NDF, ADF, cellulose, hemicellulose and lignin of legume, grass and legume-grass forage groups to three successive cuttings.

MATERIALS AND METHODS

Experimental site, design and treatments: Field experiments were conducted during the summer seasons of the years, 2012 and 2013 at the experimental farm of the Faculty of Agriculture, Alexandria University in Alexandria, Egypt. Forage treatments comprised three tested forage groups, namely; legumes, grasses and legume-grass mixtures. The first group contained two summer forage legumes, namely; forage cowpea (*Vigna unguiculata* L.) and guar (*Cyamopsis tetragonoloba* L., Taub.), while the second group consisted of two summer grasses, which were sudan grass (*Sorghum sudanense*) and pearl millet (*Pennisetum glaucum* L.). The third group contained the legume-grass mixtures; forage cow pea-sudan grass and forage cow pea-pearl millet with the ratio 50-50%. For all the investigated parameters, data were aggregated over forage treatments within each forage group, resulting design is a randomized complete block design with three replications arranged as a 3$\times$3 factorial split plot with forage group as the main-plot factor with three levels and cut sequence over time was the sub-plot factor. All the forage treatments were drilled with the recommended seeding rates by the Egyptian Ministry of Agriculture and land reclamation, amounting to 50 kg ha^{-1} for all the tested species.

Management and sampling: The experimental plots were sown on late May and early June in 2012 and 2013, respectively. The plot size was 14.4 m^2. All plots were treated similarly, i.e., fertilized and harvested 3 times at the same interval in each growing season. Fertilizer applications were split into 3 equal applications, applied before the first, second and third harvests. Broadleaf and grass weeds were hand-removed from plots and no serious incidence of insects or diseases was observed. Each plot was cut 3 times over the growing season. First cut was taken at 55 Days After Sowing (DAS) with 45 days interval between each two successive cuts. Plots were manually harvested with a sickle to a 5 cm stubble height.

Laboratory analyses: A representative sub-sample of approximately 500 g fresh matter per plot was dried at 60°C until constant weight, then the dried sub-samples were uniformly ground to a particle size of 1 mm. The concentrations of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were determined sequentially using the semiautomatic ANKOM^{220} Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) as described by Van Soest *et al.*[16]. The NDF and ADF were analyzed without a heat stable amylase and expressed inclusive of residual ash, while ADL content was corrected after the residual ash content. Ash was determined by combusting the sub-sample in a muffle oven at 550°C for 3 h[17]. Hemicellulose was then calculated by subtracting the ADF from the NDF and cellulose was calculated by subtracting the lignin from the ADF.

Statistical analyses: Data was analyzed with SAS® software, version 9.4[18]. Overall significance level a was set up to 0.05 (a = 0.05). A mixed model was fitted for each dependent variable using the mixed SAS procedure. Fixed effects were forage group, cut sequence and their interaction. Random effects were blocks (REP) and interaction between forage group and block. *Post-hoc* analysis of relevant significant effects was conducted through pairwise least square (predicted) means comparisons, using Tukey's studentized range test at a 5% significance level to control type I error. When interaction effect was significant, simple effect analyses were carried out using the SLICE option in the LSMEANS statement. One-degree of freedom contrasts were used to analyze the linear and deviation from linear effects for cut sequence and their interaction with forage group. Interactions not discussed in results and discussion were not significant at 0.05. Analysis for forage group effect on observed response (parameters) trend over time was conducted with PROCs REG and mixed. A linear regression was fitted within each plot, with cut-interval as explanatory variable and an observed response (parameter) as dependent variable. A linear model was fit to each set of regression coefficients (intercept and slope) with forage group as a fixed effect factor and REP as random effect. Pairwise predicted regression coefficient (least square means) comparisons were carried out using Tukey's studentized range test at 5% significance level.

RESULTS AND DISCUSSION

The regression analysis revealed that the amount of the tested cell wall components of the three forage groups increased with the successive cuttings following a linear trend (Fig. 1). Slopes and intercepts of the different forage groups were analysed for significance for all the tested parameters and results presented in Table 1 reveal that, the slopes were highly significant only in case of ADF and cellulose. However, the intercepts were highly significant for all the parameters. The predicted slopes of the three forage groups for all the parameters are presented in Table 2. Data of the ADF and cellulose reveal that the slopes of the grass and legume-grass forage groups were significantly higher than the slope of the legume forage group. This result shows that the increase in the ADF and cellulose components from one cut to the following was more pronounced in case of the grass and legume-grass forage groups than in case of the legume forage group. The similar response of the ADF and cellulose to the treatments was obvious as the cellulose constitute the largest portion of the cell wall ADF.

Table 1: Mean squares and levels of significance of the slopes and intercepts of the forage group factor

Dependent variable	Source of variation	Mean squares				
		NDF	ADF	ADL	Cellulose	Hemicellulose
Slope	Forage group	30.30^{ns}	241.82**	0.04^{ns}	236.72***	111.06^{ns}
Intercept		43982***	26087***	89.45**	29230***	3036.10**

**Significant at 0.01 level of probability, ***Significant at 0.001 level of probability and ns: Non-significant

Table 2: Predicted slopes of the three tested forage groups for the investigated parameters (g kg^{-1})

Forage groups	NDF	ADF	ADL	Cellulose	Hemicellulose
L	16.08^{a}*	6.03^{b}	1.29^{a}	4.73^{b}	10.05^{a}
G	22.35^{a}	21.71^{a}	1.53^{a}	20.18^{a}	0.64^{a}
LG	20.10^{a}	21.44^{a}	1.39^{a}	20.05^{a}	-1.34^{a}

*Means followed by different small letter(s) within the same column for each parameter are significantly different according to the LSD test at 0.05 level of probability

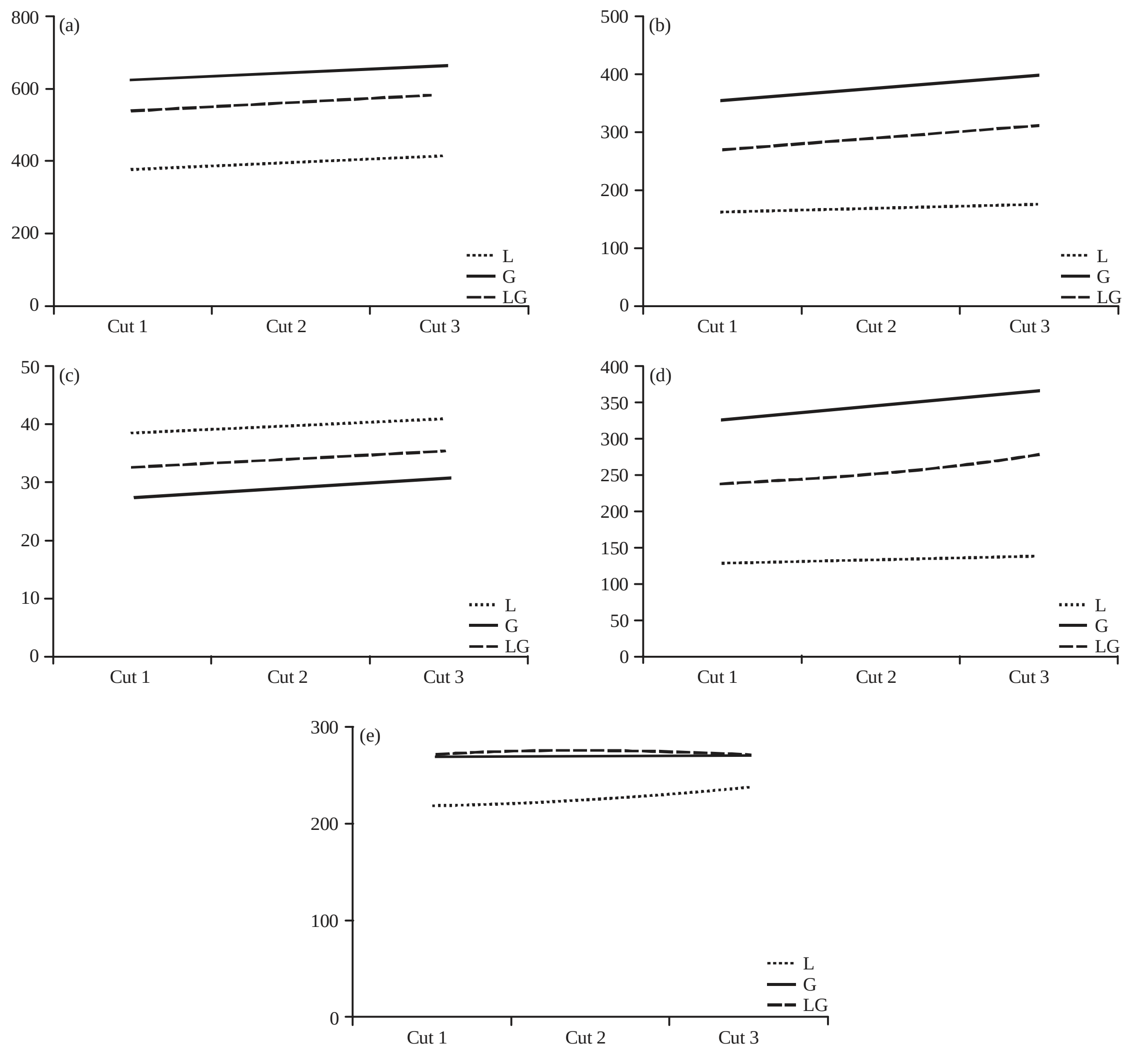

Fig. 1(a-e): Linear regression analysis of the three cuts of the tested forage groups for the investigated fiber fractions (g kg^{-1}), (a) Neutral detergant fiber, (b) Acid detergant fiber, (c) Acid detergant lignin, (d) Cellulose and (e) Hemicellulose

Table 3: Predicted intercepts of the three tested forage groups for the investigated parameters (g kg^{-1})

Forage groups	NDF	ADF	ADL	Cellulose	Hemicellulose
L	383.37^{c*}	166.98^{c}	38.43^{a}	128.55^{c}	216.38^{b}
G	620.70^{a}	353.20^{a}	27.52^{b}	325.68^{a}	267.50^{a}
LG	543.69^{b}	268.94^{b}	32.63^{ab}	236.31^{b}	274.75^{a}

*Means followed by different small letter(s) within the same column for each parameter are significantly different according to the LSD test at 0.05 level of probability

Table 3 demonstrates the predicted intercepts of the three forage groups for all the parameters. When comparing the intercepts of NDF, ADF, cellulose and hemicellulose produced by the grass forage group with those produced by the legumes and mixed forage groups at any certain cut, it becomes clear that the grass forages had significantly higher intercepts of the four parameters than the legume and mixed forages. The NDF, ADF, cellulose and hemicellulose intercepts of the grass forage group amounted to 620.70, 353.20, 325.68 and 267.50 g kg^{-1}, respectively. Only in case of lignin (ADL) did the legume and mixed forage groups have significantly higher intercepts than the grasses with 38.43 and 32.63 g kg^{-1}, respectively.

The previous regression analysis results were confirmed by the analysis of variance of the variations within the cell wall components caused by the tested treatments and their interaction. The analysis of variance demonstrated highly significant variations among the three tested forage groups and the three cuts in case of NDF, ADF, ADL and cellulose, while the hemicellulose was significantly variable only among

Table 4: Mean squares and levels of significance of the investigated parameters (g kg^{-1}) as affected by the three forage groups among the three cuts

Effect	DF	NDF	ADF	ADL	Cellulose	Hemicellulose
Forage group	2	3425.03**	92531**	256.93**	102526**	5961.48**
Cut	2	33.26**	2423.84**	17.91*	2025.98**	91.48ns
Forage group×cut	4	120.25^{ns}	261.49*	0.11^{ns}	258.05*	138.67^{ns}

*Significant at 0.05 level of probability, **Significant at 0.01 level of probability and ns: Non-significant

Table 5: Predicted least square means of the investigated parameters (g kg^{-1}) for the three forage groups

Forage groups	NDF	ADF	ADL	Cellulose	Hemicellulose
L	399.44^{c*}	173.01^{c}	39.73^{a}	133.28^{c}	226.43^{b}
G	643.05^{a}	374.91^{a}	29.05^{c}	345.86^{a}	268.14^{a}
LG	563.79^{b}	290.38^{b}	34.01^{b}	256.37^{b}	273.41^{a}

*Means followed by different small letter(s) within the same column for each parameter are significantly different according to the LSD test at 0.05 level of probability

Table 6: Predicted least square means of the investigated parameters (g kg^{-1}) for three cuts

Cuts	NDF	ADF	ADL	Cellulose	Hemicellulose
1	515.99^{c*}	263.50^{c}	32.95^{b}	230.55^{c}	252.49^{a}
2	535.30^{b}	278.52^{b}	34.08^{ab}	244.44^{b}	256.78^{a}
3	555.00^{a}	296.28^{a}	35.76^{a}	260.53^{a}	258.72^{a}

*Means followed by different small letter(s) within the same column for each parameter are significantly different according to the LSD test at 0.05 level of probability

the three forage groups. Moreover, the two way interaction between the forage group and the cutting sequence exerted a significant influence on the ADF and cellulose contents (Table 4). Means of the tested parameters as affected by the three forage groups are presented in Table 5. It is clear that the grasses were superior to the other two forage groups in almost all the cell wall components except for lignin, where the legumes were superior. The NDF and ADF amounts produced by the forage grasses were 643.05 and 374.91 g kg^{-1}, respectively. These amounts were around 24 and 8% NDF and 20 and 8% ADF higher than those amounts produced by the forage legumes and legume-grass mixtures, respectively. Similarly, the grass forage group produced significantly higher cellulose (345.86 g kg^{-1}) content than the other two forage groups, while both the grass and the legume-grass forage groups produced significantly higher hemicellulose contents than the forage legumes. Forage legumes are known to have lower fiber contents than grasses. Comparing legumes to grasses in the dairy studies is usually confounded by the NDF differences between the two species. Grasses generally contain more NDF and therefore, when diets are formulated to contain an equal amount of forage DM, the total dietary NDF concentration will be higher for diets containing grasses compared to legumes. Sanderson[19] and Erla[20] reported that the proportion of legumes in grass-legume mixtures was negatively correlated with NDF, while the proportion of grass was positively correlated with NDF. Similar results were reported by Lithourgidis *et al.*[21], Albayrak *et al.*[7] and Albayrak and Turk[22] for different types of legume-grass mixtures. On the other hand, the forage legumes were superior to the two other forage groups in its lignin content, amounting to 39.73 g kg^{-1}. Caballero *et al.*[23] and Laidlaw and Teuber[24] reported that forage legume monocultures are generally higher in lignin content than forage grass monocultures. This trend in the lignin content is most probably because the cell wall of grasses contains less lignin than the cell wall of dicots, thus, legumes has more lignin associated with the fiber[25,26].

Table 6 presents the variations among the three cuts for all the investigated cell wall components. It was clear that the NDF, ADF, ADL and cellulose contents increased with the successive cuts, where the difference between the 1st and 3rd cuts amounted to 3.9, 3.3, 0.3 and 3.0%. The hemicellulose amounts produced from the three cuts were insignificantly variable. A steady increase in the different cell wall components with successive cutting might be attributed to the continuous increase in temperature in Egypt during the summer growing season. It is well documented that temperature can alter the carbohydrate status of metabolic sinks by speeding/slowing individual metabolic reactions, by changing rates of active transport across membranes by changing the concentration of different enzymes (through modification of gene expression) and by changing enzyme activity[27]. Deinum and Dirven[28,29] associated the increases in temperature from 24 to between 28 and 33°C with greater stem weight and increased crude fiber of both C3 and C4 forage grasses. As for forage legumes, Wilson and Minson[30] reported lower digestibility of the tropical legume siratro (*Macroptillium atropurpureum* (DC.) Urb.) with increasing growth temperature. This decrease was associated with an increase in cell wall and lignin concentration, mainly in stem fraction. It has been suggested that temperature effects on the nutritive value of legumes are often not as great as those on grasses where lignin accumulation in the cell wall was

Table 7: Predicted least square means of the ADF and cellulose contents (g kg^{-1}) as affected by the interaction between the three forage groups and the three cuts

	ADF			Cellulose		
	Cuts			Cuts		
Forage groups	1	2	3	1	2	3
L	165.95^{cA*}	175.08^{cA}	178.00^{cA}	127.40^{cA}	135.58^{cA}	136.87^{cA}
G	353.68^{aB}	373.95^{aB}	397.11^{aA}	326.00^{aB}	345.22^{aAB}	366.37^{aA}
LG	270.86^{bB}	286.54^{bB}	313.74^{bA}	238.24^{bB}	252.51^{bB}	278.35^{bA}

*Means followed by different small letter(s) within the same column or different capital letter(s) within the same row for each parameter are significantly different according to the LSD test at 0.05 level of probability

observed throughout the plant[31]. Moreover, reduced leaf-to-stem ratio is a major cause of the decline in forage quality with maturity[20,32]. It is known that leaves are higher in quality than stems and since the reproductive growth lowers leaf-to-stem ratio and hence forage quality, therefore, the higher proportion of leaves in forage in the 1st cut compared to the successive cuts is usually accompanied by higher quality in terms of higher digestibility and lower amounts of fiber fractions[33,34]. For the same reason, the fiber fractions usually increase with the successive cuts.

The effect of the two way interaction between the forage group and the cut on the ADF and cellulose contents is presented in Table 7. The ADF and cellulose contents peaked with the 3rd cut for the grass and mixed forage groups. However, no significant variation among the three cuts was detected in case of the forage legumes. Concerning the three forage groups, a significant variation was detected in favor of the forage grasses. As expected, the forage legumes produced the lowest ADF and cellulose contents followed by the legume-grass mixtures, whereas the forage grasses recorded the highest values for both components. Noticeably, the differences between the highest and lowest forage groups for both parameters were greater and more distinguished in case of the 1st cut than the 3rd cut.

CONCLUSION

Results of the present study demonstrated that the cell wall components of forage grasses and legumes and their mixtures varied with the successive cuttings and generally, increased in amount from the first to the last cut. This would result in reducing the quality of the produced herbage through negatively affecting its digestibility. In this regard, the legume-grass mixtures showed better cell wall profile than the sole grasses, i.e., the amounts of the different cell wall components and the rates by which they increase among the successive cuttings were less in case of forage mixtures than forage grasses. Thus, growing legume-grass forage mixtures in Egypt in the summer season might help provide the livestock with feedstuff of better cell wall profile along the growing season.

SIGNIFICANCE STATEMENTS

The animal production sector in Egypt is experiencing marked fluctuations. Where animals have very low efficiency in utilizing the consumed forage to support their maintenance and production requirements. The cell wall components are considered among the most important determinants of forage quality and its effect on the animal's performance. It is therefore, important to investigate the response of the different forage grasses, legumes and their mixtures at successive cuttings in terms of their cell wall components under the Egyptian agricultural system. As a result of the present study, it is recommended to grow legume-grass forage mixtures in Egypt in the summer season, which might help provide the livestock with feedstuff of better cell wall profile along the growing season.

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