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Insights into forage quality of grass-legume mixtures and monocultures as affected by nitrogen fertilization

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Introduction

Nitrogen (N) is a major plant nutrient and plays an important role in the plant growth and development. N fertilization is a very important input in the Egyptian agricultural system. The quality of the produced forage in monocultures or binary mixtures is to a large extent affected by the rate of applied N. In grass-legume mixtures, the requirements of plants for N often cannot be fully satisfied through nitrogen fixation, therefore, this deficiency has to be compensated by mineral fertilizers. Main aim of the current study, therefore, was to analyze the variations in dry matter content and some forage quality parameters, namely; crude protein, carbohydrates, fiber fractions, cellulose and hemicellulose, under different levels of N fertilizer applications. Data set comprised two winter annual forage crops, namely; Egyptian clover (*Trifolium alexandrinum* L.) and annual ryegrass (*Lolium multiflorum* Lam.) grown in monocultures and binary mixtures with different mixing rates. In this paper results of the linear and quadratic regression analysis for the tested N levels and quality parameters, as well as simple correlation analysis for the investigated parameters, for the first cut, will be presented and discussed.

Materials and Methods

Field experiments were conducted during the winter seasons of two successive years (2012-2013) at the experimental farm of the Faculty of Agriculture, Alexandria University in Alexandria, Egypt. Data set comprised two winter annual forage crops, namely; Egyptian clover (*Trifolium alexandrinum* L.) and annual ryegrass (*Lolium multiflorum* Lam.) grown in monocultures and binary mixtures with three different mixing rates, and tested under three levels of N fertilizer applications (72, 107 and 143 kg N ha^{-1}) in three replicates. A split plot experimental design was used, where the main plots were assigned to the three N levels, while the forage monocultures and binary mixtures were tested in the sub plots. After determination of the dry matter (DM) content, forage quality was investigated in terms of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) which were determined sequentially using the semiautomatic ANKOM apparatus (VAN SOEST *et al.,* 1991). The nitrogen content was traditionally analyzed by the Kjeldahl procedure (AOAC, 1990), and crude protein (CP) content was calculated from the N content (CP = N x 6.25). Carbohydrate content was determined using the phenol-sulfuric acid method as described by DUBOIS *et al*. (1956). Hemicellulose was then calculated by subtracting the ADF from the NDF, and cellulose was calculated by subtracting the lignin from the ADF. Linear and quadratic regression analyses for the tested N levels and quality parameters were investigated and curves were fitted using the CurveExpert 1.4, while multiple correlation analysis for the investigated parameters was done using the SPSS 18.

Results and Discussion

Table 1 revealed highly significant correlation among all the investigated parameters. Positive correlation was observed in case of correlations between DM content, carbohydrates, NDF, ADF, cellulose and hemicellulose contents. On the contrary negative correlations were recorded between the previously mentioned parameters and CP and ADL contents. Levels of structural and non structural carbohydrates increase with increasing plant maturity, this is usually associated with in-

crease in the DM content of the forage material. This could explain the positive correlation between the DM content and the carbohydrate parameters. On the other hand, protein content reached its highest levels in the young forage plants & decreases as the plants matures. Furthermore, the regression analysis presented in Figure 1, reveals that N levels did not affect, or slightly increased, DM, ADF, ADL, cellulose and hemicellulose contents of forage plants, either in pure stands or in mixtures. However crude protein was linearly increased with increasing N level, with minor effect of quadratic response. Both carbohydrate content and NDF content were significantly influenced by increasing N level with a decreasing rate of increase at higher levels of N application.

Conclusion

Significant correlation, either in the positive or negative directions, was observed among all the investigated parameters. The applied N applications exerted a significant positive effect on the protein content of the tested forages. On the other hand, no or slight effect of the N levels was observed in case of the carbohydrate and fiber components.

References

AOAC. (1990): Official Methods of Analysis (15th Ed.) *Association of Official Analytical Chemists*. Arlington, VA.

DUBOIS, M., GILLES, K., HAMILTON, J., REBERS, P. & SMITH, F. (1956): Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28, 350-356.

STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES. SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.

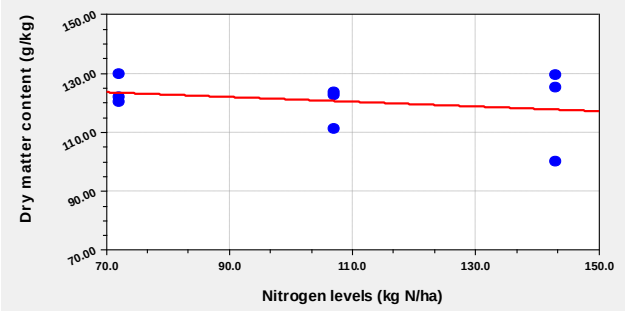
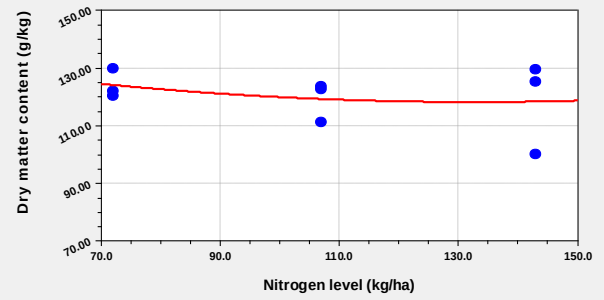
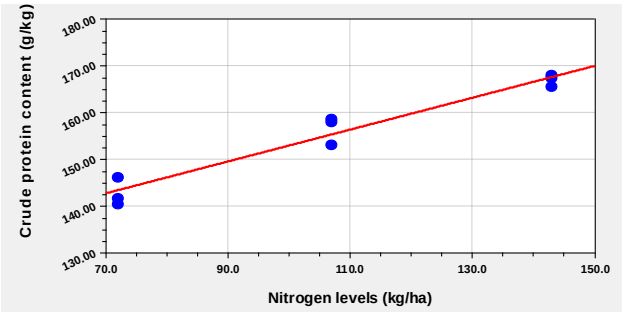
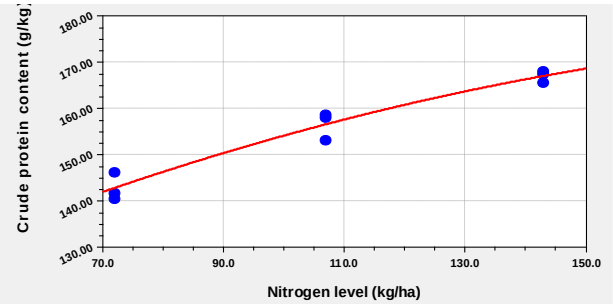
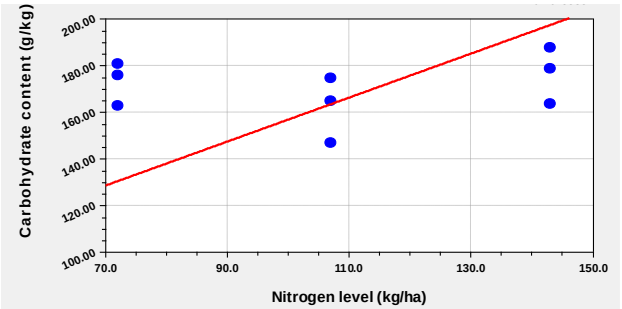
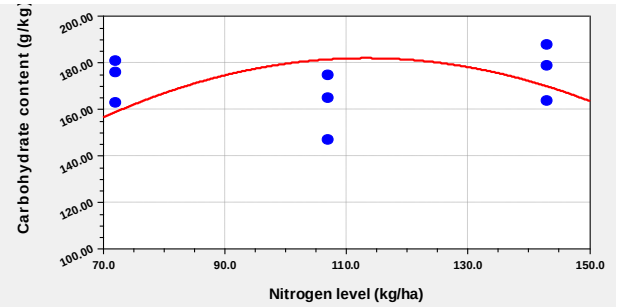
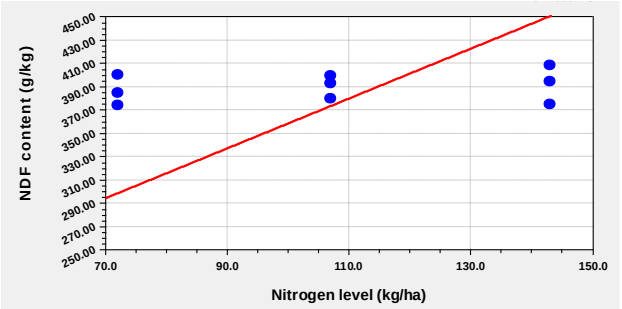
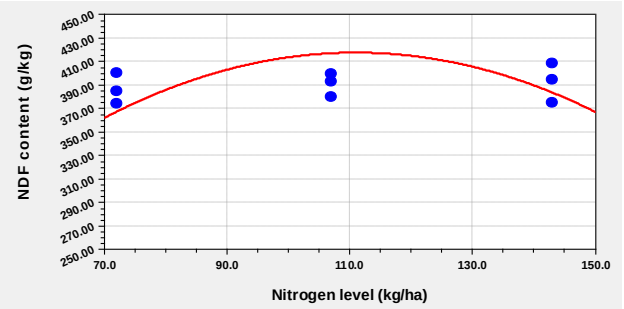
VAN SOEST, P.J., ROBERTSON, J.B. & LEWIS, B.A. (1991): Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583-3597.

Tab. 1: Correlation matrix for the investigated parameters (g kg^{-1}).

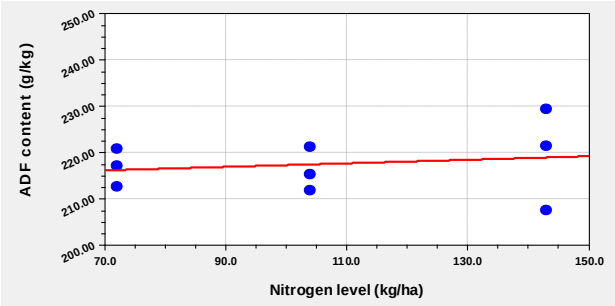
Parameter	DM	CP	Carbohydrates	NDF	ADF	ADL	Cellulose	Hemicellulose
DM	1	-0.759**	0.769**	0.744**	0.794**	-0.764**	0.596**	0.821**
CP	-0.759**	1	-0.510**	-0.641**	-0.549**	0.588**	-0.585**	-0.576**
Carbohydrates	0.769**	-0.510**	1	0.861**	0.792**	-0.699**	0.757**	0.812**
NDF	0.744**	-0.641**	0.861**	1	0.791**	-0.614**	0.947**	0.801**
ADF	0.794**	-0.549**	0.792**	0.791**	1	-0.629**	0.551**	0.996**
ADL	-0.764**	0.588**	-0.699**	-0.614**	-0.629**	1	-0.505**	-0.698**
Cellulose	0.596**	-0.585**	0.757**	0.947**	0.551**	-0.505**	1	0.568**
Hemicellulose	0.821**	-0.576**	0.812**	0.801**	0.996**	-0.698**	0.568**	1

** Correlation is highly significant at 0.01 level of probablility

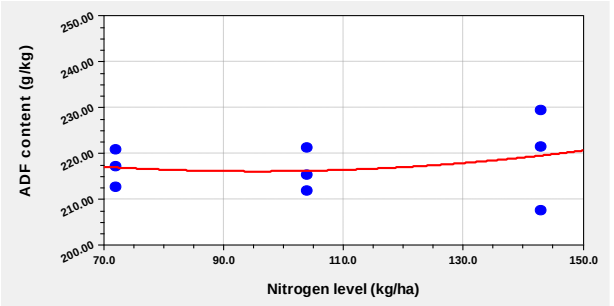
Fig. 1: Linear and quadratic regression analyses for the tested N levels and quality parameters.

Linear Regression	Quadratic Regression
$Y = 1.29 - 8.05X$ $r^2 = 0.068$	$Y = 1.46-4.26X+1.61X^2$ $r^2 = 0.08$
	
$Y = 1.19 + 3.41X$ $r^2 = 0.94$	$Y = 1.03+6.54X-1.46X^2$ $r^2 = 0.96$
	
$Y = 6.27 + 9.43X$ $r^2 = 0.58$	$Y = 7.05+3.09X-1.37X^2$ $r^2 = 0.90$
	
$Y = 1.45 + 2.14X$ $r^2 = 0.60$	$Y = 9.47+7036X-3.32X^2$ $r^2 = 0.96$
	

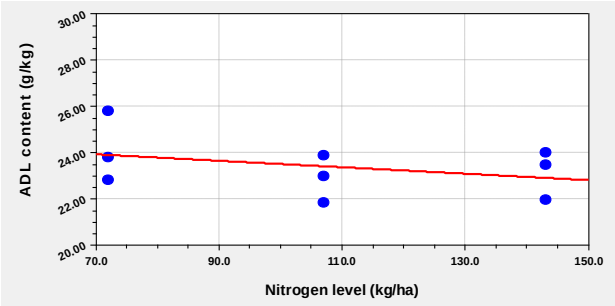
Y = 2.14 + 3.82 X r^2 = 0.03



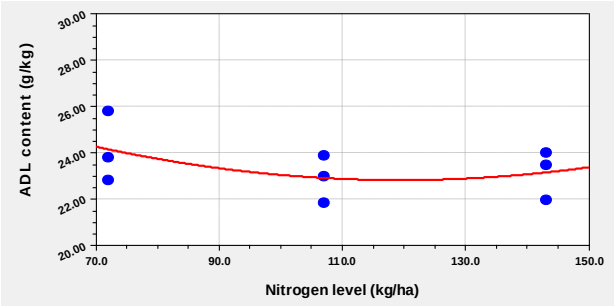
Y = 2.30-2.93X+1.53X^2 r^2 = 0.05



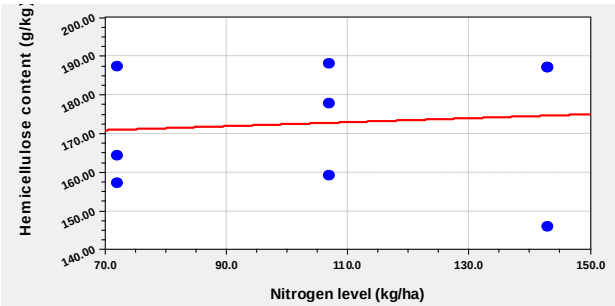
Y = 2.49 - 1.39X r^2 = 0.13



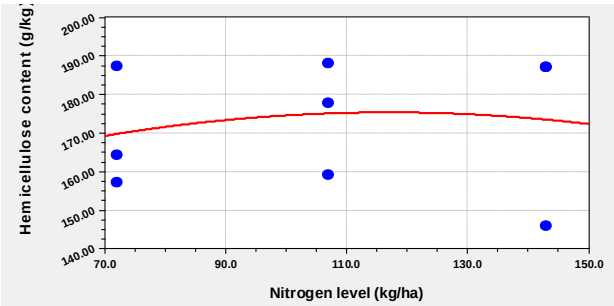
Y = 3.13-1.41X+5.92X^2 r^2 = 0.22



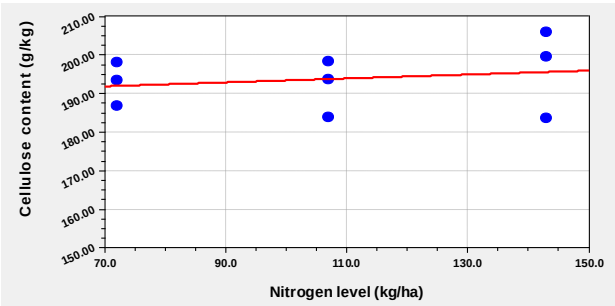
Y = 1.67 + 5.16X r^2 = 0.01



Y = 1.38+6.47X-2.77X^2 r^2 = 0.02



Y = 1.88 + 5.08X r^2 = 0.04



Y = 2.11-3.95X+2.07X^2 r^2 = 0.07