Legume-grass mixtures improve forage productivity and quality under irrigated conditions of the Mediterranean basin

H. S. A. Salama
Crop Science Department, Faculty of Agriculture, Alexandria University, Aflaton Street, El-Shatby 21545 Alexandria, Egypt.
heba.salama@alexu.edu.eg

Introduction

Mixture cropping of annual forage legumes and grasses is a common strategy to support sustainable forage supply in low input agricultural systems, especially in the Mediterranean region (Lithourgidis et al. 2006). In an arid region like Egypt, with a negligible amount of precipitation, resulting in agriculture mainly dependent on irrigation, mixture cropping would increase water use efficiency over sole cropping. Berseem clover (*Trifolium alexandrinum* L.) (BC) is the most important annual forage legume in Egypt and the region. Despite that the yield and protein content of BC are high, its cultivation in Egypt is currently facing several challenges, among them is its relatively high water requirements, low dry matter content especially in the first cut (Salama 2015), and high sensitivity to climate change, especially rising temperatures associated with global warming (Bakheit et al., 2012; Dost et al. 2014). On the other hand, grass monocultures provide a level of quality that is insufficient for many categories of livestock (Lithourgidis et al. 2006). Thus, mixture cropping of BC with forage grasses is a proposed low input technology that might solve these problems (Muhammad et al. 2014). Triticale (X *Triticosecale Wittmack*) and oat (*Avena sativa* L.) are among the promising cereals currently occupying a considerable agricultural area in Egypt, however, mostly for grain production, whereas their full potential as annual winter forage crops is not yet exploited.
The specific objective of the current study was to investigate the productivity and nutritive value of three cuts of berseem clover, triticale and oat when grown as monocultures, as well as binary mixtures with varying mixing rates.

Materials and Methods

A 2-year field trial (2017/2018 and 2018/2019) was conducted at the experimental station of the Faculty of Agriculture, Alexandria University, Alexandria, Egypt. A split plot experimental design with three replicates was used to evaluate three successive cuts of two forage grass species, triticale (X Triticosecale Wittmack) and oat (Avena sativa L.), sown in monocultures and binary mixtures with berseem clover (Trifolium alexandrinum L.), cultivar “Helaly”, with the following mixing rates: 1. 0% Grass (G) + 100% Berseem clover (BC), 2. 25% G + 75% BC, 3. 50% G + 50% BC, 4. 75% G + 25% BC, and 5. 100% G + 0% BC. Cuts were allocated to the main plots, while the combinations between grass species and mixing rates were tested in the subplots. All plots received equal amounts of fertilization and irrigation. Selective herbicides were sprayed against broadleaf weeds.
At cutting, plants were manually clipped at a 7 cm stubble height and fresh yield (FY) was directly weighed for each plot. A 1 kg sample was taken from each plot, dried at 60℃ until constant weight was reached, and dry matter (DM) content of the herbage was determined, followed by grounding the dried samples to 1-mm particle size. Crude protein (CP = N × 6.25), crude ash (CA) and crude fat (CF) were determined as described in the AOAC (2012). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were sequentially determined as described by Van Soest et al. (1991). Non-fiber carbohydrates (NFC) were then calculated as follows:

$$NFC = 1000 - (CP + CF + NDF + CA)$$

Digestibility was determined using the in vitro cellulase technique developed by De Boever et al. (1988). The percentage of digestible organic matter (DOM) was then calculated by applying the following equation of Weissbach et al. (1999):

$$DOM\ (\%) = 100 * \frac{(940 - CA - 0.62 \times EULOS - 0.000221 \times EULOS^2)}{1000 - CA}$$

EULOS = enzyme insoluble organic matter; CA and EULOS were expressed as g kg^{-1} DM.
Analysis of variance (ANOVA) was conducted using PROC Glimmix of SAS 9.4 (SAS 2012). Data were presented in a combined analysis for the two growing seasons upon homogeneity of variance’s error (Winer 1971). Means comparison was done using the Fisher’s Protected Least Significant Difference (L.S.D.) procedure at $p \leq 0.05$.

Results and Discussion

Tab. 1: Means of fresh yield (t ha^{-1}), dry matter (DM), crude protein (CP), non-fiber carbohydrates (NFC), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents (g kg^{-1} DM), as affected by the grass species × mixing rate interaction, combined over the two growing seasons.

Mixing rate		Fresh yield		DM	
Grass %	**Berseem clover %**	**Triticale**	**Oat**	**Triticale**	**Oat**
0	100	38.1aA	36.8aA	108.0aE	106.7aD
25	75	29.6bB	40.3aA	117.0aD	114.1bC
50	50	24.6bC	36.3aA	125.4aC	119.7bB
75	25	18.6bD	31.8aB	130.4aB	122.4bB
100	0	16.0bD	25.4aC	137.9aA	128.6bA
Grass %	**Berseem clover %**	**CP**		**NFC**	
		Triticale	**Oat**	**Triticale**	**Oat**
0	100	149.1aA	147.8aA	235.2aC	233.9aD
25	75	146.6aA	137.2bB	255.1bB	276.9aAB
50	50	138.4aB	127.6bC	267.1aAB	266.0aBC
75	25	133.3aB	123.5bC	279.8aA	281.8aA
100	0	121.2aC	97.2bD	193.2bD	256.0aC
Grass %	**Berseem clover %**	**NDF**		**ADF**	
		Triticale	**Oat**	**Triticale**	**Oat**
0	100	455.8aC	454.5aB	287.7aC	286.4aB
25	75	468.4aBC	436.0bC	282.0aC	260.6bD
50	50	474.6aB	466.5bB	277.3aD	267.3bC
75	25	477.0aB	464.7bB	290.9aB	268.4bC
100	0	589.7aA	533.8bA	353.0aA	316.6bA

Means followed by different small letter(s) within the same row, and different capital letter(s) within the same column, for each studied parameter, are significantly different according to the L.S.D. test at 0.05 level of probability.

Mixtures of BC with oat resulted in significantly higher FY than mixtures with triticale, across all tested mixing rates (Tab. 1). In a similar study, Lithourgidis et al. (2006) concluded that mixing common vetch with oat resulted in 18% more forage yield than mixtures of common vetch with triticale. It is evident that oat plants are leafier than triticale plants, especially after cutting/grazing, oat regrowth will be characterized with higher green leaf yields than triticale. Pure BC stands produced the highest significant FY, thus, increasing the BC percentage in the mixture uplifted the FY, which could be mainly attributed to the higher branching ability and denser canopy structure of BC in comparison to forage grasses (El Karamany et al. 2012). On the contrary to FY, DM content of mixtures with triticale was significantly highest than mixtures with oat. This was in line with the findings of McCartney and Vaage (1994), who compared the DM content of the two crops at different growth stages. In addition, BC was characterized by significantly lowest DM content and, thus, its increased percentage in the mixture contributed to decreasing DM of the whole mixture.

Similar to the DM content, pure triticale had significantly higher CP content than pure oat, therefore, mixtures with triticale were significantly higher in CP content than mixtures with oat, while, increasing the BC percentage in mixtures with both grass crops significantly increased CP content. Similar results were reported by Gill and Omokanye (2018) for mixtures of peas with oat and triticale. Pure oat stands were richer in NFC content than pure triticale stands. Keles et al. (2016) reported that oat had higher NFC content than triticale when compared at the tillering and stem elongation growth stages. This was obviously reflected on a higher NFC content of BC mixtures with oat, than with triticale in the current study. Opposite to NFC, triticale, either as pure stand or in the mixtures with BC was significantly higher in the fiber components than oat. Similar findings were reported for mixtures of common vetch with oat and triticale by Lithourgidis et al. (2006). Nonetheless, pure grass was richer in NDF and ADF than pure BC stands, therefore, the fiber content was decreased as a result of mixing BC with forage grasses (Salama and Zeid 2016).

Tab. 2: Means of neutral detergent lignin (ADL), and digestible organic matter (DOM) contents (g kg^{-1} DM), as affected by the cut × grass species × mixing rate interaction, combined over the two growing seasons.

Mixing rate		**ADL**					
Grass %	**Berseem clover %**	**Cut 1**		**Cut 2**		**Cut 3**	
		Triticale	**Oat**	**Triticale**	**Oat**	**Triticale**	**Oat**
0	100	48.5	47.2	42.7	41.5	55.2	54.0
25	75	56.6	43.7	50.0	46.6	55.9	59.3
50	50	47.5	42.6	42.2	42.6	49.8	55.3
75	25	43.8	39.0	39.5	42.2	48.3	53.0
100	0	28.5	29.7	23.0	29.7	25.5	29.0
L.S.D.$_{0.05}$		5.57					
		DOM					
Grass %	**Berseem clover %**	**Cut 1**		**Cut 2**		**Cut 3**	
		Triticale	**Oat**	**Triticale**	**Oat**	**Triticale**	**Oat**
0	100	671.0	669.7	656.6	655.3	669.4	668.1
25	75	659.1	640.7	640.1	639.9	649.9	647.8
50	50	649.8	625.0	625.4	604.5	640.1	614.9
75	25	610.4	591.5	584.2	576.3	609.8	590.3
100	0	591.8	551.3	589.5	540.1	601.3	557.9
L.S.D.$_{0.05}$		52.1					

Means presented in Tab. 2 showed that BC and its contribution in the forage mixture with 75 % were characterized with the significantly highest lignin content, which decreased with decreasing the BC percentage in the forage mixture, and reached its minimal values with 100% G. Noticeably, pure BC stands produced up to two-fold lignin content than that of grasses among all cuts. This is mainly because of the low lignin proportion in the cell wall of monocots compared to the cell wall of dicots (Buchanan et al. 2015; Carpita and McCann 2015). In general, the lignin content of the 3rd cut was significantly higher than that of the 1st and 2nd cuts. It was observed that pure oat had higher lignin content than pure triticale among the three cuts.
The significantly highest DOM was a character of the pure BC stands, and mixtures with 75% BC. Meanwhile, DOM significantly decreased with increasing grass percentage in the mixture. Despite its higher NDF and ADF contents, triticale was more digestible than oat, probably because of its higher CP and lower lignin contents. This was in line with the findings of Sullivan et al. (1982) who reported higher nutritive value of triticale than oat, in terms of higher intake and digestibility that was reflected on the better performance of cattle fed triticale. They, thus, recommended triticale as an alternative forage crop for fattening cattle.
Under similar Mediterranean conditions, mixing BC with triticale at 75 %:25 % mixing rate would produce high fresh forage yield with balanced nutritional options.

References

AOAC (2012): International Official Methods of Analysis (19th Ed.) Association of Official Analytical Chemists. Gaithersburg, MD, USA.

Bakheit, B.R., Ali, M.A., Helmy, A.A. (2012): The influence of temperature, genotype and genotype x temperature interaction on seed yield of berseem clover (Trifolium alexandrinum L.). Asian J. Crop Sci, 4, 63-71.

Buchanan, B.B., Gruissem, W., Jones, R.L. (Eds.) (2015): Biochemistry and molecular biology of plants. John Wiley & Sons.

Carpita, N., McCann, M. (2015): The cell wall. In: Buchanan, B.B., Gruissem, W., Jones, R.L. (Eds.), Biochemistry and Molecular Biology of Plants. American Society of Plant Biologists, Maryland, USA, pp. 52–108.

De Boever, J.L., Cottyn, B.L., Andries, J.I., Buysse, F.X., Vanacker, J.M. (1988): The use of cellulose technique to predict digestibility, metabolizable and net energy of forages. Anim. Feed Sci. Technol., 19, 247-260.

Dost, M., Misri, B., El-Nahrawy, M., Khan, S. and Serkan, A. (2014): Egyptian Clover (Trifolium alexandrinum); King of Forage Crops. Regional Office for the Near East and North Africa, Food and Agriculture Organization of the United Nations, Cairo.

El Kramany, M.F., Elewa, T.A., Bakry, A.B. (2012): Effect of mixture rates on forage mixture of Egyptian clover (Trifolium alexandrinum L.) with triticale (× Triticosecale Wittmack) under newly reclaimed sandy soil. Aust. J. Basic Appl. Sci., 6(5), 40-44.

Gill, K.S., Omokanye, A.T. (2018): Potential of spring barley, oat and triticale intercrops with field peas for forage production, nutrition quality and beef cattle diet. J. Agric. Sci., 10, 1-17.

Keles, G., Ates, S., Coskun, B., Alatas, M.S., Isik, S. (2016): Forage yields and feeding value of small grain winter cereals for lambs. J. Sci. Food Agric., 96(12), 4168-4177.

Lithourgidis, A.S., Vasilakoglou, I.B., Dhima, K.V., Dordas, C.A., Yiakoulaki, M.D. (2006): Forage yield and quality of common vetch mixtures with oat and triticale in two seeding ratios. Field Crops Res., 99(2-3), 106-113.

McCartney, D.H., Vaage, A.S. (1994): Comparative yield and feeding value of barley, oat and triticale silages. Can. J. Anim. Sci., 74(1), 91-96.

Muhammad, D., Misri, B., EL-Nahrawy, M., Khan, S., Serkan, A. (2014): Egyptian Clover (Trifolium alexandrinum) King of Forage Crops. FAO, Regional Office for the Near East and North Africa, Cairo, Egypt.

Salama, H.S.A., 2015. Interactive effect of forage mixing rates and organic fertilizers on the yield and nutritive value of berseem clover (Trifolium alexandrinum L.) and annual ryegrass (Lolium multiflorum Lam.). Agric. Sci., 6(04), 415.

Salama, H.S.A., Zeid, M.M.K. (2016): Hay quality evaluation of summer grass and legume forage monocultures and mixtures grown under irrigated conditions. Aust. J. Crop Sci., 11(11), 1543.

Statistical Analysis System (2012): SAS PC Windows Version 9.4 SAS Institute Inc. Cary, NC, USA.

Sullivan, M.T., Hales, J.W., Norton, B.W. (1982): A comparison of triticale and oats as forages for fattening cattle in south-east Queensland. In Proceedings of the Australian Society of Animal Production, 14, 289-92.

Van Soest, P. V., 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(10): 3583-3597.

Weißbach, F., Kuhla, S., Schmidt, L., Henkels, A. (1999): Estimation of the digestibility and the metabolizable energy of grass and grass products. In Proceedings of the Society of Nutrition Physiology (Germany).

Winer, B. J. (1971): Statistical principles in experimental design. 2nd Edition. McGraw-Hill Kogakusha, LTD.