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Improving Fodder Yields and Nutritive Value of Some Forage Grasses as Animal Feeds through Intercropping with Egyptian Clover (*Trifolium alexandrinum* L.)

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Abstract: The present study aimed to evaluate the potential of improving the feeding value of Egyptian clover (EC), ryegrass (R), triticale (T), barley (B), and oats (O) monoculture, or Egyptian clover mixed with ryegrass (EC+R), oats (EC+O), barely (EC+B), and triticale (EC+T) at 75:25% seeding rate, respectively, during two successive winter seasons of 2018/19 and 2019/20. Harvesting of plots was carried out at 5 cm stubble height after 60, 100, and 140 days from sowing. The in vitro nutritive value and ruminal fermentation of the monoculture and intercropping containing EC were evaluated. Green forage yield of EC was higher than other plants with about 160% of fresh forage compared with T, O, or EC+T intercropping. The highest crude protein (CP) concentration was noted in EC, while the lowest ($p < 0.001$) concentration was observed in T, which had the highest fiber fractions content. Ryegrass had the highest net in vitro gas production (GP), while EC+R had the lowest GP ($p < 0.05$). The EC increased dry matter and organic matter degradability. EC and R reduced protozoal count, while total volatile fatty acids (VFA), acetate, and propionate were increased with B and EC+T intercropping ($p < 0.05$). Overall, intercropping of EC with grass of triticale or ryegrass at mixing rates of 75:25% resulted in improving fresh and dry forage yields. The legume–grass intercropping improved the protozoa count partitioning factor as an index of microbial protein synthesis and total VFA concentration.

Keywords: Egyptian clover; feeds; grasses; intercropping; nutritive value

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1. Introduction

In Egypt, the competition between humans and animals for the use of the limited arable land area reflects the core of animal feed shortages. This forces animal nutritionists to explore unconventional strategies to meet animals' needs from the same land by improving nutritive value of already available feed. Attempts for this purpose and successful applications began in the early 1970s [1].

According to the Central Agency for Public Mobilization and Statistics (CAPMAS), Egyptian clover is the principal forage crop in Egypt, occupying the second largest area in the winter season, following wheat [2]. Egyptian clover forage at early cuttings is characterized by high moisture content, which induces bloat and diarrhea in ruminants [3]. Moreover, Egyptian clover in all cuts is rich in crude protein (CP) and poor in fibers (structural carbohydrates) [4]. With advancing cuts, the CP content becomes lower while the fiber content becomes higher causing a loss of valuable protein in Egyptian clover [5,6].

In Egypt, researchers proposed planting Egyptian clover intercropping with compatible grass species. This practice might increase forage yield and nutritive value [7,8]. The forage barley (*Hordeum vulgare*), forage oat (*Avena sativa*, L.), or triticale (*Triticosecale wittmack*) are proposed winter cereals to complement the productivity and quality of Egyptian clover forage [8–10]. Barley forages were reported to have a high digestible dry matter (DM) content, a low acid detergent fiber (ADF), and higher CP than oat forage [11]. Egyptian clover and barley intercropping enjoyed higher total seasonal CP in comparison with Egyptian clover and oat intercropping or Egyptian clover and triticale intercropping [12]. Additionally, interseeding grass species with Egyptian clover resulted in better growth [13]. Moreover, intercropping Egyptian clover and barley produced higher forage yield than intercropping Egyptian clover and oats [14].

The nutritive value of feed/plants can be reflected in many angles including concentrations of nutrients (e.g., organic matter (OM), CP, and essential micronutrients), nutrient degradability, and animal's acceptability [15]. High CP (with low ruminal degradability) and NSC support animal requirements for maintenance, growth, and milk production. The nutritive value of feed/diets depends mainly on the chemical composition and nutrient digestibility [16]. Mixing more than one plant (grass and/or legume) varies the nutritive value of the mixture compared with the individual plants due to changes in the chemical composition. Mixing legumes with grass can increase animal performance with environmental benefits (decreases methane and increases the symbiotic nitrogen fixation) with possible associative effects between them [17]. Mixing grasses with legumes decreases ruminal protein degradation and urinary excretion of N by ruminants resulting in an increase in the flux of dietary protein for absorption in the intestine due to the presence of secondary metabolites in some species, providing a positive impact on ruminal fermentation [18]. Some studies focused on mixing grasses with legumes evaluated the associative effects, which showed improved ruminal fermentation [17,19]. Gas production (GP) appeared to be related to the chemical composition of the feed, and in particular to the fiber content [20]. Increasing/decreasing cell wall content and CP results in decreasing/increasing microbial activities and ruminal fermentation [21]. The hypothesis of the current study is based on the theory that intercropping grasses with legumes will improve forage production, nutritive value, ruminal degradation, and fermentation. This study is novel in the field of agronomy and animal feeding since in the Middle East no scientific reports are available on this topic and today sustainable farming systems need to find alternatives that boost yields rationally and those field practices include intercropping. Therefore, the objective of this study was to assess the potential of improving ryegrass, triticale, barley, or oats forage yields and feeding value due to intercropping legumes and grasses with Egyptian clover.

2. Materials and Methods

The study was carried out at the experimental farm of the Crop Science Department, Faculty of Agriculture, Alexandria University (31°200 N, 30° E), during two successive winter seasons of 2018/19 and 2019/20. The in vitro assays were performed at the advanced laboratory of animal nutrition of the Animal and Fish Production Department, Faculty of Agriculture, Alexandria University, Egypt. The soil at the experimental location was a sandy loam, moderately alkaline (pH 8.4), with 1.5% OM content and electrical conductivity of 1.30 dSm^{-1}.

2.1. Agronomic Cultivation and Evaluation

Nine different treatments represented the proposed cultivations of EC, and four types of grasses are listed in Table 1. Multicuts Egyptian clover seeds (Giza 10), and triticale and oat seeds were obtained from the Forage Research Section, Agricultural Research Center, Giza, Egypt. Barley (Giza 123) was obtained from the Barley Research Section, Field Crops Institute, Agricultural Research Center. Seeding rates for Egyptian clover, ryegrass, triticale, barley, and oat were 10.5 Kg, 10.08 Kg, 16.8 Kg, 16.8 Kg, and 54.6 Kg per ha, respectively. The Egyptian clover monoculture was considered as the control treatment.

Table 1. Monocultures and intercropping of Egyptian clover/grass.

Serial	Treatment	Designation	Mixing Ratio	
			Egyptian Clover %	Grass %
1	Egyptian clover monoculture (Control)	EC	100%	-
2	Ryegrass monoculture	R	-	100%
3	Triticale monoculture	T	-	100%
4	Barley monoculture	B	-	100%
5	Oat monoculture	O	-	100%
6	Egyptian clover + ryegrass intercropping	EC+R	75%	25%
7	Egyptian clover + triticale intercropping	EC+T	75%	25%
8	Egyptian clover + barley intercropping	EC+B	75%	25%
9	Egyptian clover + bats intercropping	EC+O	75%	25%

The evaluated treatments represented five monocultures and four Egyptian clover-grass intercropping. Egyptian clover was represented by 75% of its monoculture seeding rate, whereas grass was represented by 25% of its seeding rate in monoculture. Harvesting of plots was carried out from the end of November to the end of March each year. Plots were manually harvested with a garden shear to a 5 cm stubble height and the fresh herbage per plot was weighed in the field. The plot area was 42 m^2. The recommended crop management practices were applied uniformly for all experimental plots concerning irrigation schemes and weed management. Irrigation was scheduled on a 10-day interval and applied equally to all plots to avoid induced water stress. Based on the soil nutrient's profile, and following the recommendations in the area, phosphorous was added once with seed bed preparation at a rate of 200 kg P_2O_5/ha, in the form of calcium mono phosphate (15.5% P_2O_5). Nitrogen, in the form of ammonium nitrate (33.5% N), was applied at a rate of 120 kg N/ha and split into three equal doses that were applied to the experimental plots with sowing, directly after cutting, and 1 month after cutting [22]. The seed mixtures were mixed before seeding in rows 20 cm apart. Three cuttings were taken after 60, 100, and 140 days from sowing. Data were recorded for fresh forage yield and dry forage yield depending on samples for dry matter determination at each cutting date.

2.2. Laboratory Analysis

A sub-sample of approximately 300 g of fresh matter per plot was dried at 60 °C until constant weight was achieved to determine the DM content. The dried sub-samples were uniformly ground to a particle size of 1 mm. The concentrations of neutral detergent fiber (NDF), ADF, and acid detergent lignin (ADL) were determined sequentially using the semiautomatic ANKOM220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) as described by Van Soest et al. [23]. 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 AOAC [24]. Prior to total N analysis, the dried samples were ground again to a particle size of 10 µm. The N content was analyzed by the Kjeldahl procedure AOAC [24], and CP content was calculated (CP = N × 6.25). Total carbohydrate content (TC) was determined using the phenol–sulfuric acid method as described by DuBois et al. [25].

2.3. In Vitro Assay

The in vitro study was carried out at the Advanced Laboratory of Animal Nutrition at the Animal and Fish Production Department, Faculty of Agriculture, Alexandria University (Egypt) using a semi-automatic system of GP equipped with a pressure transducer and a data logger (Pressure Press Data GN200, Sao Paulo, Brazil) as described by Bueno et al. [26].

Rumen contents were freshly collected from three adult fasted, slaughtered Egyptian buffalo steers at the slaughterhouse of the Faculty of Agriculture, Alexandria University. Rumen contents were collected and kept separately in pre-warmed thermos containers (39 °C) under anaerobic conditions. To prepare the rumen inoculate, the rumen contents

of each animal were blended for 10 s, squeezed through three layers of cheesecloth, and maintained in a water bath (39 °C) under CO_2 until inoculation.

For each treatment (i.e., feed/feed mixture), six replicates (bottles) were used; three for the fermentation parameters and protozoal count, and the other three were for the determination of rumen degradability (truly degraded DM (TDDM) and truly degraded OM (TDOM)). Three blank (containing rumen fluid and buffer solution) and internal standard bottles (containing rumen inoculum, buffer solution, and clover hay) were prepared to correct for the sensitivity variations induced by the inoculum. Ground samples (0.5 g) of feeds under evaluation were put into numbered bottles and were incubated with 45 mL of diluted rumen fluid (15 mL mixed rumen fluid + 30 mL of Menkes buffered medium) in 120 mL serum incubation bottles for 24 h.

Once filled, bottles were sealed immediately with 20 mm butyl septum stoppers (Bellco Glass Inc., Vineland, NJ, USA), manually mixed, and incubated in a forced-air oven (FLAC STF-N 52 Lt, Treviglio, Italy) at 39 °C for 24 h. The bottles were shaken manually after the recording of the gas headspace pressure at 3 h, 6 h, 12 h, and 24 h of incubation. The amount of the GP in all bottles at each measuring time was estimated according to the regression equation predicated between gas volume versus pressure relationship $V = 4.974 \times p + 0.171$ (n = 500; R^2 = 0.98); where: V is a gas volume (mL) and p is the measured pressure (psi).

2.4. Rumen Fermentation and Degradability

After 24 h, all incubation bottles were placed in cold water (4 °C) to stop the microbial fermentation process. Determination of TDDM and TDOM was carried out according to Blümmel et al. [27] by immediate addition of neutral detergent solution (70 mL) without heat stable α-amylase and incubated in a forced-air oven (FLAC STF-N 52 Lt, Treviglio, Italy) at 105 °C for 3 h. The residue was filtered in pre-weighed crucibles, washed with hot water and acetone, and oven-dried at 105 °C for 16 h, then ashed at 550 °C for 4 h with correction for the corresponding blank. The TDDM and TDOM values were calculated from the difference between the amounts of the incubated DM and OM and those remaining non-degraded. The PF was estimated as the ratio of TDOM (mg) and gas volume (mL) [27].

The rumen pH was measured directly within 2–3 min of sampling using a portable pH meter (GLP 21 model: CRISON, Barcelona, Spain) in the fermentation bottles. Two ml of rumen fluid sample was mixed with 2 mL of methylgreen-formalin-saline solution and stored in a glass bottle at room temperature for microscopic determination of protozoal count and differentiation by Digital Zoom Video microscope (LCD 3D, GiPPON; Japan) following the procedure described by Dehority [28]. Individual volatile fatty acids (VFA) concentrations were determined according to Palmquist and Conrad [29] using gas chromatography (GC). Briefly, all fermentation samples were centrifuged at 10,000× *g* for 15 min and an aliquot of 1.6 mL of the filtrate was mixed with 0.4 mL of 25% metaphosphoric acid (4:1 ratio) and centrifuged at 15,000 rpm for 20 min and 4 °C (K1015 Micro Prime; Centurion Scientific Ltd., Stoughton, Chichester, UK). The supernatant was used to determine VFA concentrations with a gas chromatograph (Thermo Fisher Scientific, Inc., TRACE1300, Rodano, Milan, Italy) fitted with an AS3800 autosampler and equipped with a capillary column HP-FFAP (19091F-112; 0.320 mm o.d., 0.50 µm i.d., and 25 m length; J&W Agilent Technologies Inc., Palo Alto, CA, USA). Hydrogen at 1.35 mL/min was used as a carrier gas. Air, hydrogen, and nitrogen fluxes (make up gas) were kept at 450 mL/min, 40 mL/min, and 35 mL/min, respectively. A 0.1 µL aliquot was injected in the splitless mode for the entire run with 31.35 mL/min of H2 flux (63.432 Pa). Injector and flame ionization detector (FID) temperatures were held isothermally at 250 °C. The oven heating slope was 80 °C (1 min), 120 °C (20 °C/min for 3 min), and 205 °C (10 °C/min for 2 min), with 9 min of overall analytical time. A mixture of known concentrations of VFA was used as an external standard (Sigma Chemie GmbH, Steinheim, Germany) to calibrate the integrator. Ruminal NH_3-N concentration was measured calorimetrically by spectrophotometer (Alpha-1101 model; Labnics Equipment, Fremont, CA, USA).

2.5. Statistical Analysis

Data of forage yields were analyzed in a randomized complete block design with four blocks. Data were combined over cuttings and over years and cuttings according to Steel and Torrie [30].

Data of in vitro ruminal GP and fermentation parameters were analyzed as a randomized design using the PROC MIXED procedure of SAS (SAS Institute, Cary, NC, USA). Mean values of each individual run (3 runs) were used as the experimental unit. The statistical model was:

$$Y_{ij} = \mu + D_i + \varepsilon_{ij}; \quad (1)$$

where: Y_{ij} represents every observation of the *i*th plant/mixture and ε_{ij} is the experimental error. Duncan's test was used for the multiple comparisons among mean values.

3. Results

3.1. Field Evaluation

Mean squares of fresh and dry forage yields for the studied forages combined over cuttings and years are presented in Table 2. The obtained forage yields (fresh and dry) were different ($p \leq 0.01$) among the study years. Moreover, the successive cuttings gave variable yields ($p \leq 0.01$). The magnitude of dry forage and/or the rank of obtained forage from any of the studied forages was different ($p \leq 0.01$) among cuttings. Fresh and dry yields obtained from the different forages were different ($p \leq 0.01$) over cuttings and years and among years. Meanwhile, only fresh forage yield was affected ($p \leq 0.01$) by the time of cutting. The second-order interaction among year × cutting × forage was not significant for any of the fresh or dry forage yields.

Table 2. Mean squares of fresh and dry forage yields for the studied forages combined over cuttings and years. * $p < 0.05$, ** $p < 0.01$, n.s = not significant.

Source of Variance	Degree of Freedom	Mean Squares	
		Fresh Forage Yield	Dry Forage Yield
Years	1	49.56 **	0.74 $^{n.s}$
Replications × years interaction	2	2.33	1.33
Cutting	2	47.28 **	8.56 **
Years × cutting interaction	2	0.43 $^{n.s}$	3.34 **
Error	4	1.63	0.78
Forages	8	160.78 **	8.03 **
Years × forages interaction	8	15.69 **	1.19 **
Cutting × forages interaction	16	4.54 **	0.47 $^{n.s}$
Years × cutting × forages interaction	16	2.11 $^{n.s}$	0.88 *
Error	48	1.71	0.31

Figure 1 shows the means of fresh forage yields that resulted from the different studied forages in each season of the study. As an average of the two study seasons, EC monoculture significantly gave the highest fresh forage yield of 18.92 ton/ha, while the second fresh forage yield was obtained with B monoculture (12.91 ton/ha). All of R, EC+R, and EC+B gave similar fresh forage (10.32 ton/ha, 10.26 ton/ha, and 11.62 ton/ha, respectively). The lowest significant fresh yields were observed in T monoculture, O monoculture, and (EC+T) intercropping representing 7.9 ton/ha, 8.29 ton/ha, and 8.32 ton/ha, respectively. The lowest fresh forage yield was observed with EC+O intercropping (6.91 ton/ha). The green forage yield of EC amounted to about 125% of the respective yield produced by any of R, EC+R intercropping, or EC+B intercropping. Moreover, EC yield amounted to about 160% of fresh forage produced by any of T, O, or EC+T intercropping. In the meantime, the fresh

yield represented about 180% of the EC+O intercropping. The EC fresh yield suppression due to grass mixing was minimal with R or B inclusion, while it reached its maximum with O.

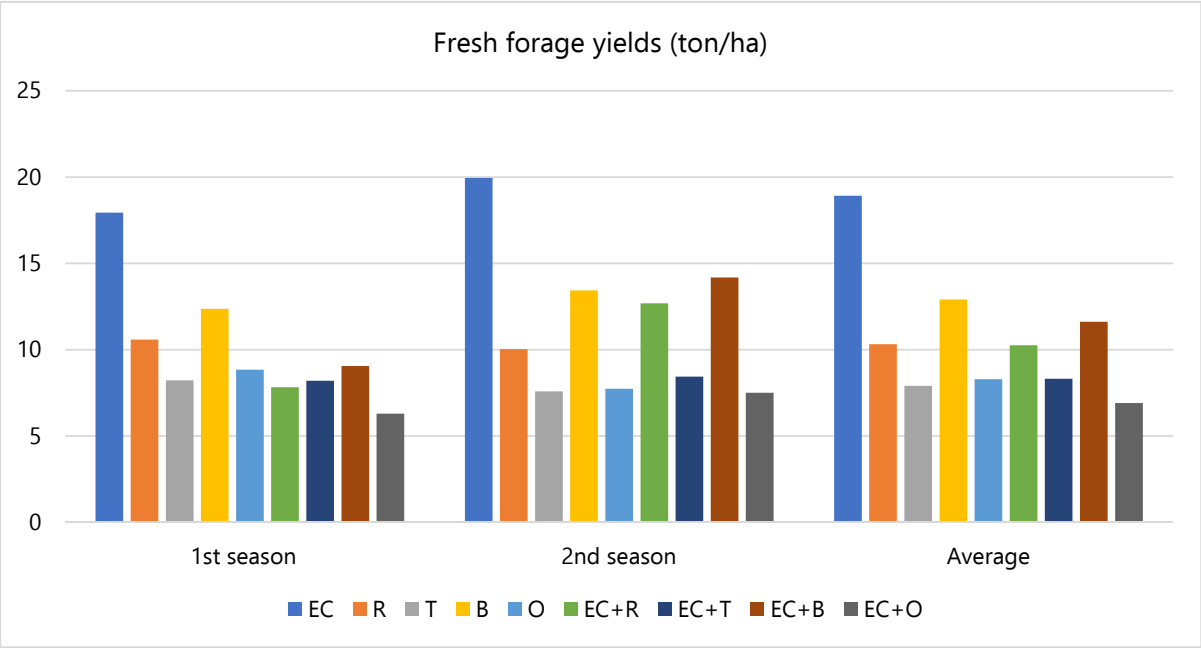


Figure 1. Means of fresh forage yields (ton/ha) from the different studied forages in each season of the study. EC = Egyptian clover monoculture, R = ryegrass monoculture, T = triticale monoculture, B = barley monoculture, O = oat monoculture, EC+R = Egyptian clover + ryegrass intercropping, EC+O = Egyptian clover + oat intercropping, EC+B = Egyptian clover + barley intercropping, and EC+T = Egyptian clover + triticale intercropping ($L.S.D._{season}$ = 2.91, $L.S.D._{forages}$ = 1.43, $L.S.D._{forage \times season}$ = 2.02).

The obtained fresh forage yields from the studied forages varied from one season to the other, where EC+R intercropping and EC+B intercropping significantly responded differently to seasonal conditions (7.83 ton/ha vs. 12.69 ton/ha for EC+R, and 9.06 ton/ha vs. 14.18 ton/ha, for EC+B at the first and the second seasons, respectively). The second season of the study significantly yielded higher fresh forage (11.28 ton/ha vs. 9.93 ton/ha for the two successive seasons, respectively).

Figure 2 shows the means of fresh forage yields obtained at different cuttings of the study. The cutting that had the highest significant green forage yield was 12.48 ton/ha. The second significant rank was presented by the third cutting in the second season (11.28 ton/ha). The lowest significant green forage was in the first cut in the first season (8.74 ton/ha). Although, the trend matched with obtained forage yield from T monoculture, O monoculture, EC+O or EC+B intercropping, the differences among cuttings of fresh forage were not of significant. For each studied forage, the second and the third cuttings significantly produced a fresh yield of higher magnitude.

Figure 3 shows the means of dry forage yields that resulted from the different forages in each season of the study. The highest significant dry forage yield was presented by EC monoculture over the two seasons (4.37 ton/ha). The yield was not significantly different from the yield of the B monoculture (3.52 ton/ha). The other cultivated forages gave significantly similar dry forage yield which ranged between 1.79 to 2.79 ton/ha. Moreover, dry forage yields were significantly similar among seasons. Dry forage yields that were obtained at the second season were only significantly different from the corresponding dry yield of the first season only, for EC monoculture (4.85 vs. 3.88 ton/ha) and EC+B intercropping (3.39 vs. 2.18 ton/ha).

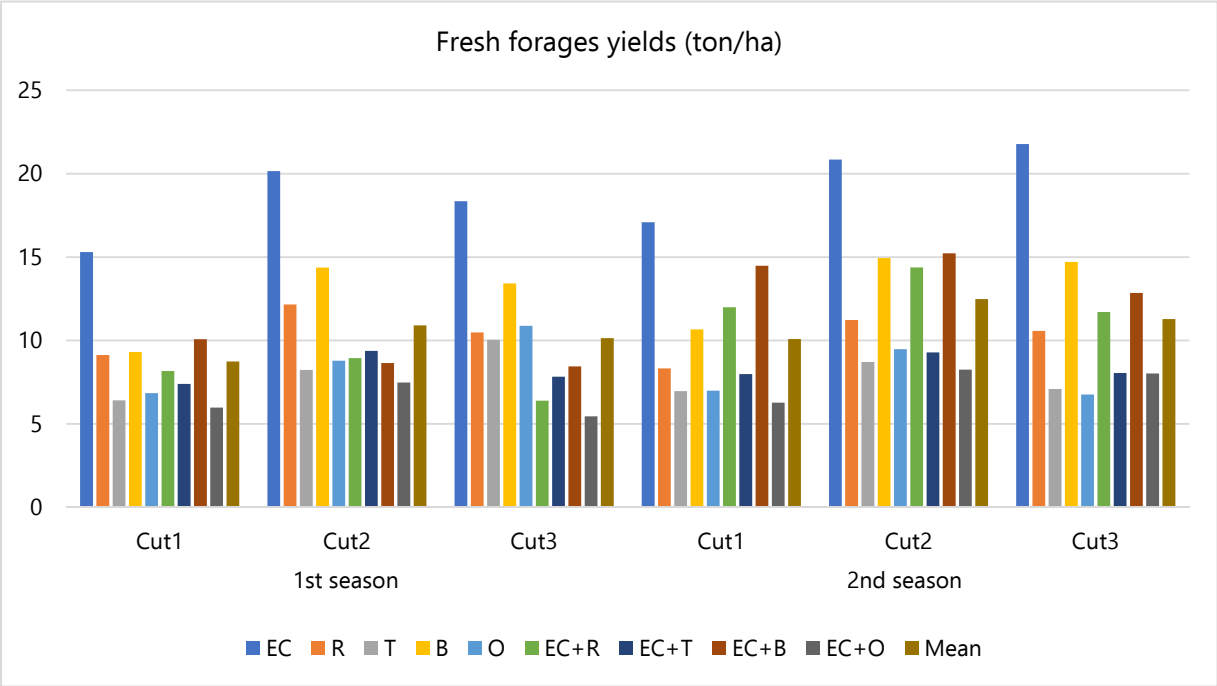


Figure 2. Means of fresh forage yields (ton/ha) obtained at different cuttings of the study. EC = Egyptian clover monoculture, R = ryegrass monoculture, T = triticale monoculture, B = barley monoculture, O = oat monoculture, EC+R = Egyptian clover + ryegrass intercropping, EC+O = Egyptian clover + oat intercropping, EC+B = Egyptian clover + barley intercropping, and EC+T = Egyptian clover + triticale intercropping ($L.S.D._{forage \times cutting}$).

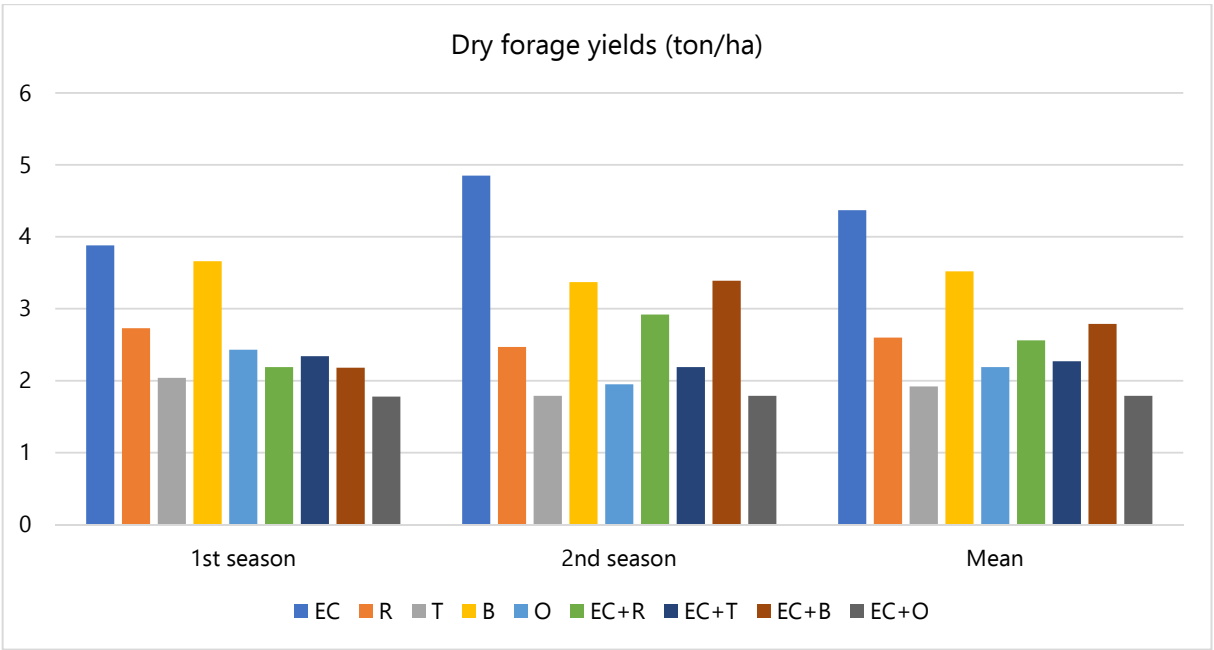


Figure 3. Means of dry forage yields (ton/ha) produced from the different studied forages in each season of the study. EC = Egyptian clover monoculture, R = ryegrass monoculture, T = triticale monoculture, B = barley monoculture, O = oat monoculture, EC+R = Egyptian clover + ryegrass intercropping, EC+O = Egyptian clover + oat intercropping, EC+B = Egyptian clover + barley intercropping, and EC+T = Egyptian clover + triticale intercropping ($L.S.D._{season}$ = 0.47, $L.S.D._{forages}$ = 1.72, and $L.S.D._{forage \times season}$ = 0.860).

Figure 4 shows the means of dry forage yields that were obtained at different cuttings of the study. The EC monoculture produced significantly variable dry forage yield from the same cutting with a variable season (1.97 ton/ha vs. 4.96 ton/ha, 5.94 ton/ha vs. 5.22 ton/ha, and 3.73 ton/ha vs. 4.36 ton/ha for the 1st, t2nd, and 3rd cuttings, respectively). The Triticale monoculture presented the best dry forage yields by the first cutting irrespective of the study season (1.21 ton/ha and 1.62 ton/ha at the first and the second seasons, respectively). The EC monoculture at the second cutting of each season gave the highest production of dry forage (5.44 ton/ha and 5.22 ton/ha) for the first and the second seasons, respectively.

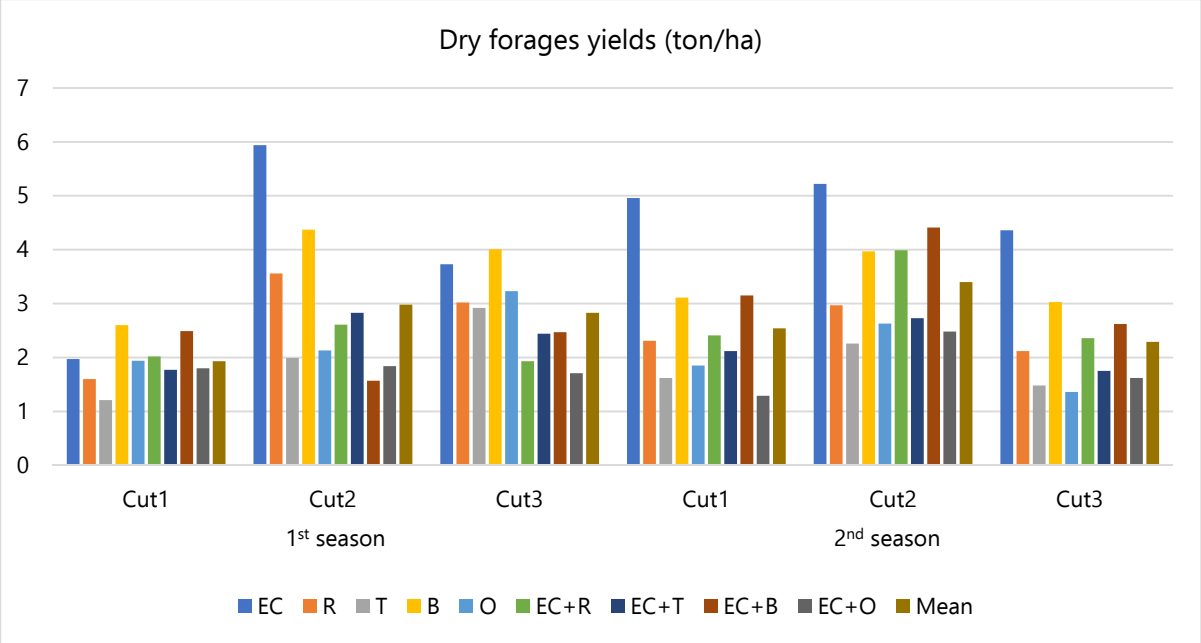


Figure 4. Means of dry forage yields (ton/ha) obtained at different cuttings of the study. EC = Egyptian clover monoculture, R = ryegrass monoculture, T = triticale monoculture, B = barley monoculture, O = oat monoculture, EC+R = Egyptian clover + ryegrass intercropping, EC+O = Egyptian clover + oat intercropping, EC+B = Egyptian clover + barley intercropping, and EC+T = Egyptian clover + triticale intercropping (L.S.D.$_{\text{forage}\times\text{cutting}}$ =1.054).

3.2. Chemical Composition and Fermentation

The chemical composition of EC grasses is shown in Table 3. The highest OM concentration was observed with oats (p = 0.030), while the lowest concentration was observed with EC. For CP concentration, the highest concentration was noted in EC while the lowest concentration was observed in T ($p < 0.001$). T and O had the highest NDF and ADF while EC and R had the lowest NDF and ADF concentrations. Moreover, EC+T and EC+R intercropping had the highest hemicellulose while R, B, O, EC+O, and EC+B intercropping had the lowest concentrations; R had the highest lignin while T had the lowest concentration.

Gas production differed between the incubated substrates as shown in Table 4. The highest net GP was observed with ryegrass, while the lowest one was observed in EC+R intercropping. EC showed the highest TDDM and TDOM, while T had the lowest TDDM, and O had the lowest TDOM. EC showed the highest NH_3-N concentration, while EC+R intercropping had the lowest NH_3-N concentration. In vitro incubation of EC showed the highest protozoal count in the incubation medium (p = 0.009), while fermentation of EC+O had the lowest protozoal count. The highest PF was observed with EC+B and EC+T intercropping, while T showed the lowest PF.

Table 3. Chemical composition (g/kg DM) of intercropping of Egyptian clover/grass.

	OM	CP	EE	NSC	NDF	ADF	Hemicellulose	Cellulose	Lignin
EC	862.5 ^{c}	155.3 ^{a}	17.1 ^{cd}	136.9 ^{cd}	553.2 ^{d}	490.1 ^{e}	63.1 ^{bc}	335.0 ^{d}	155.1 ^{b}
R	872.7 ^{bc}	114.1 ^{cd}	13.4 ^{f}	214.0 ^{a}	531.3 ^{e}	472.0 ^{f}	59.3 ^{c}	290.5 ^{e}	181.5 ^{a}
T	897.8 ^{ab}	50.7 ^{f}	23.0 ^{b}	139.0 ^{cd}	685.0 ^{a}	621.3 ^{b}	63.7 ^{bc}	520.1 ^{a}	101.2 ^{d}
B	901.8 ^{ab}	99.4 ^{de}	16.0 ^{de}	196.2 ^{ab}	590.2 ^{bc}	541.5 ^{c}	48.7 ^{c}	410.5 ^{b}	131.0 ^{bc}
O	910.9 ^{a}	83.0 ^{e}	25.8 ^{a}	110.6 ^{e}	691.6 ^{a}	646.7 ^{a}	44.8 ^{c}	533.9 ^{a}	112.9 ^{cd}
EC+R	875.7 ^{bc}	134.5 ^{ab}	12.7 ^{f}	118.0 ^{d}	610.4 ^{b}	530.1 ^{cd}	80.3 ^{ab}	397.8 ^{bc}	132.3 ^{bc}
EC+T	889.8 ^{abc}	145.0 ^{ab}	18.8^{8}	154.7 ^{cd}	571.2 ^{cd}	520.5 ^{d}	50.7 ^{c}	389.7 ^{bc}	130.8 ^{bc}
EC+B	876.2 ^{bc}	125.1 ^{bc}	14.5 ^{ef}	166.4 ^{bc}	570.1 ^{cd}	522.0 ^{d}	48.1 ^{c}	381.8 ^{c}	140.2 ^{b}
EC+O	893.6 ^{ab}	143.8 ^{ab}	23.9 ^{b}	113.8 ^{d}	612.0 ^{b}	525.0 ^{d}	87.1 ^{a}	384.5 ^{c}	140.5 ^{b}
SEM	9.35	6.67	0.64	12.56	7.31	4.92	5.79	6.86	7.41
p value	0.030	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	<0.001	<0.001

Means in the same row with different superscripts differ, $p < 0.05$. ADF, acid detergent fibers; CP, crude protein; EE, ether extract; NDF, neutral detergent fibers; NSC, nonstructural carbohydrates; OM, organic matter; EC = Egyptian clover monoculture; R = ryegrass monoculture; T = triticale monoculture; B = barley monoculture; O = oat monoculture; EC+R = Egyptian clover + ryegrass intercropping; EC+O = Egyptian clover + oat intercropping; EC+B = Egyptian clover + barley intercropping; and EC+T = Egyptian clover + triticale intercropping.

Table 4. Gas production, degradability, protozoal count, partitioning factor, and fermentation pH of intercropping of Egyptian clover/grass.

	Net GP	TDDM	TDOM	pH	NH_3-N	Protozoa	PF
EC	168.0 ^{b}	759.4 ^{a}	733.9 ^{a}	6.31	20.8 ^{a}	3.00 ^{d}	3.70 ^{bc}
R	180.2 ^{a}	710.6 ^{b}	683.5 ^{b}	6.12	15.4 ^{bc}	4.80 ^{bc}	3.25 ^{cd}
T	165.4 ^{b}	547.5 ^{f}	532.5 ^{fg}	6.14	16.6 ^{bc}	6.50 ^{ab}	2.95 ^{d}
B	172.5 ^{b}	637.2 ^{d}	620.1 ^{d}	6.10	15.3 ^{bc}	3.55 ^{cd}	3.27 ^{cd}
O	137.2 ^{cd}	533.1 ^{f}	508.2 ^{g}	6.09	16.9 ^{bc}	5.85 ^{ab}	3.68 ^{bc}
EC+R	123.5 ^{e}	576.1 ^{e}	544.0 ^{f}	6.20	14.7 ^{c}	6.50 ^{ab}	3.93 ^{ab}
EC+T	132.6 ^{d}	599.4 ^{e}	574.1 ^{e}	6.19	16.0 ^{bc}	6.90 ^{a}	4.16 ^{ab}
EC+B	141.4 ^{c}	686.1 ^{bc}	657.2 ^{bc}	6.16	16.9 ^{bc}	5.05 ^{abc}	4.36 ^{a}
EC+O	138.5 ^{cd}	659.6 ^{cd}	630.4 ^{cd}	6.12	18.8 ^{ab}	6.40 ^{ab}	4.30 ^{a}
SEM	2.52	9.77	10.30	0.051	1.17	0.577	0.163
p value	<0.001	<0.001	<0.001	0.095	0.007	0.009	<0.001

Means in the same row with different superscripts differ, $p < 0.05$. Net GP = net gas production (mL/g DM); NH_3-N = Ammonia-N (mg/dL); PF, partitioning factor (mg OMD/mL gas); TDDM = total digestible DM; TDOM = total digestible OM; EC = Egyptian clover monoculture; R = ryegrass monoculture; T = triticale monoculture; B = barley monoculture; O = oat monoculture; EC+R = Egyptian clover + ryegrass intercropping; EC+O = Egyptian clover + oat intercropping; EC+B = Egyptian clover + barley intercropping; and EC+T = Egyptian clover + triticale intercropping.

Fermentation of B and EC+O intercropping produced greater total VFA ($p = 0.003$), acetate ($p = 0.030$), and propionate ($p = 0.014$), while T, O, EC+R, and EC+B intercropping produced the lowest concentrations as shown in Table 5. Both of B and EC+O intercropping had higher OM concentrations, and B had the highest NSC concentration.

Table 5. Volatile fatty acids production (mmol/L) of intercropping of Egyptian clover/grass.

	Total	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate
EC	44.1 ^{c}	28.4 ^{d}	7.66 ^{d}	0.93	3.74 ^{cd}	2.56	0.80 ^{d}
R	49.9 ^{b}	31.8 ^{b}	8.95 ^{ab}	1.00	4.51 ^{b}	2.76	0.89 ^{c}
T	43.4 ^{c}	27.4 ^{d}	7.52 ^{d}	0.92	4.35 ^{b}	2.60	0.65 ^{ef}
B	52.4 ^{a}	33.0 ^{a}	9.52 ^{a}	0.94	5.17 ^{a}	2.71	1.03 ^{a}
O	43.9 ^{c}	27.9 ^{d}	7.81 ^{d}	0.86	4.21 ^{bc}	2.37	0.83 ^{d}
EC+R	44.6 ^{c}	28.3 ^{d}	7.48 ^{d}	0.95	4.53 ^{b}	2.66	0.71 ^{e}
EC+T	51.3 ^{a}	32.8 ^{a}	8.62 ^{b}	1.02	5.06 ^{a}	2.84	0.93 ^{b}
EC+B	45.5 ^{c}	28.8 ^{d}	8.17 ^{c}	0.90	4.27 ^{c}	2.53	0.88 ^{c}
EC+O	47.2 ^{bc}	30.5 ^{c}	8.24 ^{c}	0.92	4.06 ^{d}	2.58	0.92 ^{b}
SEM	2.34	1.22	0.314	0.196	0.230	0.659	0.090
p value	0.004	0.030	0.014	0.088	0.044	0.921	0.023

Means in the same row with different superscripts differ, $p < 0.05$. EC = Egyptian clover monoculture; R = ryegrass monoculture; T = triticale monoculture; B = barley monoculture; O = oat monoculture; EC+R = Egyptian clover + ryegrass intercropping; EC+O = Egyptian clover + oat intercropping; EC+B = Egyptian clover + barley intercropping; and EC+T = Egyptian clover + triticale intercropping.

4. Discussion

4.1. Field Evaluation

Increasing variability in cultivated forage species might increase fresh and dry forage yields [31]. Obtaining high forage yield might not be the choice of the forage grower, since stability of produced quantity along with good quality represents the major factors of importance [32].

Grass–legume intercropping, particularly those from Egyptian clover and Italian ryegrass (*Lolium multiflorum* L.) or with barely, are very effective for improving fresh and dry forage quantity, quality, and nutritive value, which agrees with Salem et al. [9]. Intercropping Italian ryegrass with Egyptian clover has been recommended previously [9,33]. It has been reported that intercropping ryegrass and Egyptian clover produces higher yields and nutritive quality than cropping them individually [34]. Thus, Egyptian clover and Italian ryegrass can be used as winter annual intercropping under Egyptian conditions because both possess compatible maturity and harvesting schemes, complement each other in growth distribution and ecological niche, and do not, severely, compete for growth requirements [35,36].

The two old forages, Egyptian clover (i.e., *Trifolium alexandrinum* L.). and Italian ryegrass (i.e., *Lolium multiflorium* L.), proved to have high forage yield potentiality and wide adaptability to variable conditions within a season (among cuttings) and among seasons [37,38]. Adaptability and sustainable productivity were also provided by the old-world crop barley (*Hordeum vulgare*) [39,40]. Intercropping of Egyptian clover and ryegrass or barley enjoyed a reasonably good fresh and dry forage yield [41]. Oats and triticale wither in monoculture or in intercropping with Egyptian clover and were proved to have low compatibility with the study conditions [42,43].

4.2. Chemical Composition and Fermentation

Oats had the highest OM compared with BC which had the lowest OM and fiber and highest CP concentrations indicating a higher nutritive value of BC compared with other grasses. Intercropping EC with T and R increased hemicellulose while intercropping EC with B lowered its concentrations. Feeding high-fiber diets to animals does not encourage microbial growth and ruminal fermentation enough, causing decreased ration digestibility [16]. The genotype of the plants, differences in production environments (e.g., climate, soil, and agronomic practice), and interaction between environment and genotypes are the main

reasons for different chemical compositions [44]. Environmental conditions greatly affect a plant's chemical composition, especially crude protein, fiber, and secondary metabolites. Plants absorb many elements from soil that have a biological function, however, some of them are known as toxic even in low amounts. If environmental conditions change, plants respond by changing some of their metabolic activities and thus, some components will change [45]. Different chemical composition means different nutritive value for ruminants. As previously noted, the nutritive value depends on the concentrations of certain nutrients (e.g., CP, OM, NSC, fiber, lipids, and secondary metabolites) [46]. High CP (with low ruminal degradability) and NSC support animal requirements for growth and milk production.

One of the simple, powerful, and sensitive techniques to evaluate ingredients as feeds for ruminants is the GP technique. Gas production differed between the incubated substrates because of different nutrient concentrations. Gas production depends mainly on nutrient availability for ruminal microbes. The highest net GP was observed with R, while the lowest one was observed in EC+R intercropping indicating lowered nutritive value of R when mixed with EC. The insignificant differences between R and EC+R intercropping for OM, EE, and cellulose concentrations show their minimal effects on GP, while the low CP and hemicellulose concentrations in ryegrass compared with EC+R intercropping show the role of fiber and protein in GP from feeds [20,47]. Not only do nutrient concentrations affect the fermentation and GP but also nutrient digestibility [48].

The highest TDDM and TDOM with EC and the lowest TDDM with T and TDOM with O partially explain the results of fermentation measurements (e.g., GP, total and individual VFA) and confirm the fact that ruminal fermentation does not depend only on nutrient concentration but also nutrient digestibility [49].

The lowest NH_3-N concentration in the incubation medium when EC is mixed with R indicates that intercropping R with EC has a high advantage to decrease the high degradability of protein in EC [4]. Low ruminal CP degradability indicates higher amounts of protein escaping from the rumen, resulting in an increased absorption at the small intestine [50]. The result of ruminal NH_3-N confirms the role of ruminal protozoa on protein and amino acid degradation as is explained later [51]. Decreasing ruminal protein degradation and NH_3-N production in the rumen is recommended to decrease the overall N excretion and NH_3-N emissions by ruminants.

EC showed the highest protozoal count ($p = 0.009$), while it had the lowest protozoal count. The suppression of ruminal ciliate protozoa with EC+O is very important in animal feeding due to its role in methanogenesis [52]. Ruminal protozoa are one of the main ruminal methane producers in ruminants [52]. These results are converse to the results of TDDM and TDOM, which confirm the observations of Kholif et al. [53] who observed that decreasing protozoal count increases nutrient digestibility. Ivan et al. [51] stated that protozoa engulf and digest ruminal bacteria, therefore, decreasing them allows a higher number and activity of ruminal bacteria.

The highest PF when EC was intercropped with B and T reflects the conversion of degraded substrate into microbial biomass [54]. A decrease in ammonia-N concentration with decreases in total VFA concentration is evidence of improved synchronization between dietary energy and protein, which is expected to increase microbial-N production within the rumen and can explain the improved TDDM and TDOM [55].

Volatile fatty acids are produced because of dietary nutrients, especially carbohydrates (structural and nonstructural), to acetate, propionate, butyrate, and isoacids [56]. The high concentration of OM and NSC in B and EC+O intercropping can explain the results of produced total and individual VFA. Ruminal fermentation of OM and NSC produces more VFA and propionate, while fermentation of fiber fractions produces more acetate [57]. Therefore, the high OM and NSC may explain the results of total and individual VFA, especially propionate. Moreover, the high fiber concentrations in T and O were expected to increase acetate production, but this was not observed. Similarly, the high OM concentration in O was not the main reason for the high content of detected VFA. These results confirm the fact that VFA (total and individual) productions did not depend only on the concentration

of nutrients but mainly on their ruminal/total tract digestibility. High fiber digestibility favors the production of ruminal acetate [58], while high NSC and OM digestibility favor the production of ruminal propionate [59].

As it has been shown in the manuscript, EC is already of nutritional value; however, intercropping with other grasses may boost both of their nutritive value (i.e., EC and intercropped grass) to increase NDF, and CP, with better agronomic characteristics and synergy with other nutrients from grasses.

5. Conclusions

The results from the current study confirmed that intercropping Egyptian clover with grass of triticale or ryegrass at mixing rates of 75:25% resulted in improved fresh and dry forage yield and nutritive value. However, the intercropping of Egyptian clover with barely or oat suppressed the fresh and dry forage yield and enhanced the nutritive value. The legume–grass intercropping improved the chemical composition (increased CP and decreased fiber fractions) of the individual grass monoculture, protozoa count, and partitioning factor as an index of microbial protein synthesis and total VFA concentration, in particular with triticale monoculture but declined in the net GP and degradation of dry and OM. Therefore, grass–legume intercropping is an effective technique to improve the green and dry forage yield and nutritive value of some, but not all, grasses.

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