Performance and chemical composition of three *Echinochloa* grasses over short term experiment

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Abstract

Three weedy grasses, *Echinochloa crus-galli* (L.) P. Beauv. *Echinochloa colona* (L.) Link. and *Echinochloa stagnina* (Retz.) P. Beauv. were grown for four months in the greenhouse of Botany Department, Faculty of Science at New Damietta to examine the effect of age on plant biomass, ash, proteins, total carbohydrates, lipids and fiber content. Plants were collected from the field and replanted in greenhouse, with the same age and size. Biomass and ash content increased by increasing the age in *E. colona* and *E. stagnina* but increased in *E. crus-galli* till the fourth harvest then decreased. Protein, crude lipid and crude fiber content were decreased gradually by the increase of the age, in the three species throughout the progressive harvests. By the increase of the age total carbohydrates increased in *E. crus-galli* and *E. colona* but remained largely unchanged in *E. stagnina*. The crude fiber was a relatively high in *E. crus-galli* and *E. colona* but this was not good for forage use of the grass. The amount of PEPC and Rubisco protein was higher in *E. stagnina* than in *E. crus-galli* and *E. colona*. It is recommended using *E. stagnina* as fodder additives due to its high content of protein and biomass and low fiber content.

The obtained results will be useful for the optimum managing strategy of these three grasses as invasive weeds in field crop as well as use them as a source of natural forage in particular in summer.

Keywords: Ash, Biomass, Carbohydrates, Echinochloa, Fibers, Forage, Lipids PEPC, Rubisco

Introduction

Echinochloa is a cosmopolitan genus belonging to Subfamily Panicoideae of the family Poaceae comprised of 20 to 25 species [1]. Although considered a weed in many agricultural systems, it is also valued by wildlife managers as a food source for waterfowl [2]. The genus *Echinochloa* includes serious weeds in agriculture being the third and fourth most important weeds in the world [3,4]. *Echinochloa crus-galli* (L.) Beauv. is a C4 weed that grows in paddy fields and distributed worldwide. It has adapted to temperate regions and anaerobic conditions such

as rice fields and wetlands, but it is also used for reclamation of saline soil especially in Egypt [5]. The broad ecological adaptation, such as the ability to mimic rice, quick germination, rapid growth and abundant seed production, make it a successful weed [6]. E. crus-galli includes many sub-species and varieties. Echinochloa colona, is a vigorous C4 annual species, is one of the world's most serious grass weeds in rice [7,4]. More than 60 countries have reported it as a weed problem in 35 crops, including rice, maize (Zea mays L.), sorghum (Sorghum bicolor L.), cotton (Gossypium hirsutum L.), and peanuts (Arachis hypogea L.) [7]. E. colona is an example of crop mimicry because it closely resembles rice at the seedling stage and is sometimes unintentionally transplanted into fields together with the crop. By the time the weed can be easily recognized by farmers and removed, crop yield losses may already be unavoidable [7]. E. colona had been reported to reduce yield of direct-seeded rice by as much as 76% [8]. Echinochloa stagnina, is a C4 plant, a perennial grass, it presents as weeds in deepwater paddies and river channels [9,10].

Nutritive value of forage refers to its chemical composition, digestibility and the nature of digested products [11,12]. The amount of forage consumed by the animal is very important, as it affects total nutrient intake and therefore the animal's response. The efficiency of ruminant production systems based on forages as the main source of protein and energy is strongly dependent on forage maturity, which is considered a primary factor decreasing its nutritional quality [13]. These modifications are well evident in forages at different stages of development (e.g. vegetative vs. reproductive or mature). Age and the prevailing environmental conditions may also affect the growth and nutritive value of the used grass. Variation in growth patterns, dry matter accumulation and tiller production [14].

The highest known productivity in natural vegetation is for a C4 perennial grass which achieves about of 100 t (dry matter) ha⁻¹ year⁻¹ [15,16]. Some of the world's most productive crops and pasture, such as maize (Zea mays), sugarcane (Saccharum officinarum), sorghum (Sorghum bicolor), Bermuda grass (Cynodon dactylon), and rhodes grass (Chlorisgayana) are C4 plants. In addition, the most troublesome weeds like nutgrass, crabgrass and barnyard, are also C4 species. Although C4 plants represent only a small portion of the world's plant species,

accounting for only 3 % of the vascular plants, they contribute about 20% to the global primary productivity because of the highly productive C4- grass-lands [17].

The objective of the present study was to examine the effect of age on plant biomass, ash, proteins, total carbohydrates, lipids and fiber content of the three weedy grasses in the coastal area of Damietta.

Materials and methods

Studied species

Echinochloa crus-galli is an erect to decumbent, often branched at base, tufted, stout annual, graminoid; up to 3 ft. (100 cm) tall or more, fibrous roots, culms often branching at base, and branching culms ascending from decumbent branches; sometimes rooting at lower culms nodes, culms stout, up to 60 in. (50-150 cm) high, glabrous, nodes slightly swollen [18-22].

Echinochloa colona is a tufted, erect to spreading and semi-prostrate, annual graminoid; up to 16 in. (60 cm) tall or more, fibrous roots, culms freely branching at base and lower nodes, culms 8-16 in. (20-60 cm) or more high, glabrous or nodes pubescent [18-23].

Echinochloa stagnina is an annual or perennial, coarse, often succulent grass which floats in deep water or creeps with rhizomes and stolons, to 2 m long, readily and sprouts at nodes, stems up to about 1 m long under non-flooded conditions and elongating up to 2 m in flooded areas, under flooded conditions, the stem trails on the surface of the water but the leaves and inflorescence are held in an upright position above the water [18,24,25].

Experimental design

The study was conducted using a randomized complete block design with 3 replicates, 9 blocks represent all treatments, the area of each block approximately 0.5 m^2 . The abovementioned grasses were collected from the natural habitat and replanted in green house in the third week of May 2011, the age of the plant was one month before beginning the experiment in both species E. crus-galli and E. colona but in E. stagnina similar cuts of 3 internodes are planted. Nitrogen fertilizer was applied a sure a in the third week of experiment. The first harvest began after one month for two species E. crus-galli and E. colona

and after two months for *E. stagnina*. The other harvest was taken monthly till the end of experiment.

Chemical Analysis

E. crus-galli, *E. colona* and *E. stagnina* shoots were harvested and oven-dried at 65 C° for 72 hr. Biomass was calculated as dry weight per plant. Dried shoots were used for chemical analysis.

Dried shoot were ground to pass a 1 mm sieve and stored in airtight containers prior to chemical analysis. Total ash was determined by igniting at 550°C for 2 h. Protein content of dry powdered plant material was determined according to the method of Bradford [26]; while, carbohydrates were determined according to the method of Schortemeyer et al. [27]. Crude lipids and crude fibers were estimated according to the method of Nesamvuni et al. [28].

Measurement of PEPC and Rubisco

The protein content of fresh plant material was determined according to the method of Bradford [26].

SDS-PAGE of protein for PEPC and Rubisco quantification

Proteins were resolved as described by Laemmlli [29] using the BioRad Mini Protean 3 (BioRad laboratories, Hercules, CA, USA). The resolving gel contained 11% acrylamide and the stacking gel contained 5% acrylamide. Proteins from 3 mg leaf fresh weight were loaded onto each lane. The proteins were resolved at 100 V for 90 min. The gels were stained with brilliant blue R-250 (BioRad) and then destained with 20% methanol, scanned and used for PEPC and Rubisco quantification.

Statistical analysis

One-way ANOVA-LSD was performed by entering the data into two computer programs SPSS 18.0 and CoStat 6.311.

Results

Dry weight of *E. colona* and *E. stagnina* increased but in *E. crus-galli* it increased until the fourth harvest then decreased in the last one, the highest dry weight was found in the fourth

harvest of *E. stagnina* (115.16 g plant⁻¹) and the lowest one was found in the first harvest of *E. crus-galli* (6.34g plant⁻¹) (Fig. 1).



Fig. 1. Changes in dry biomass over monthly harvests of *E. crus-galli*, *E. colona* and *E. stagnina*.h1 (55 days), h2 (80 days), h3 (105 days), h4 (130 days), h5 (156 days), (ND) not detected. Data is mean \pm SE. Bars labeled with different letters are significantly different at *p*<0.05.

Ash content was increased by increasing the age of both *E. colona* and *E. stagnina* but in *E. crus-galli* it increased in the third harvest then decreased in the fourth and the fifth harvest, the highest ash content was found in the fourth harvest of *E. stagnina* (19.67%), the lowest value was observed in the first harvest of *E. stagnina* (11%) (Fig. 2).



Fig. 2.Changes in ash content over monthly harvests of *E. crus-galli, E. colona* and *E. stagnina.* h1 (55 days), h2 (80 days), h3 (105 days), h4 (130 days), h5 (156 days), (ND) not detected. Data is mean \pm SE. Bars labeled with different letters are significantly different at *p*<0.05.

Monthly harvests indicated different changes in the chemical composition of *E. crus-galli*, *E. colona* and *E. stagnina*. Protein content decreased by increasing the age in the three plants gradually and significantly along the progressive harvests, except that in *E. colona*, it similar in the third harvest to the first one, the highest protein content was observed in the first harvest of *E. crus-galli* (266.43 mg g⁻¹) and the lowest one was found in the fourth harvest of *E. stagnina* (83.23 mg g⁻¹) (Fig. 3).



Fig. 3. Changes in protein content over monthly harvests of *E. crus-galli, E. colona* and *E. stagnina*. h1 (55 days), h2 (80 days), h3 (105 days), h4 (130 days), h5 (156 days), (ND) not detected. Data is mean \pm SE. Bars labeled with different letters are significantly different at *p*<0.05.

By increasing the age, total carbohydrates of *E. crus-galli* and *E. colona* increased but remained largely unchanged in *E. stagnina*. The highest total carbohydrate content (Fig. 4) was found in the third harvest of *E. stagnina* (2.2 mg g⁻¹) and the lowest one was observed in the first harvest of *E. crus-galli* (0.76 mg g⁻¹). The crude lipids content decreased by increasing the age of *E. colona*, but in *E. crus-galli* and *E. stagnina* where it decreased by increasing the age then increased in the last harvest (Fig. 5). The highest crude lipid content was found in the fourth harvest of *E. stagnina* (3.23%) and the lowest one was observed in the fourth harvest of *E. crus-galli* (0.78 %).

Crude fibers decreased by increasing the age of plants in *E. crus-galli*, but not in *E. colona* and *E. stagnina* where it decreased in the second harvest then increased in the last harvest than the first. Highest crude fibers was found in the third harvest (Fig. 6) of *E. colona* (81.3%) and the

lowest one was found in the second harvest of *E. stagnina* (36.9%). The amount of PEPC protein in *E. stagnina* was higher than that in *E. crusgalli* and *E. colona* (3-fold and 95.4%, respectively). Rubisco protein content in *E. stagnina* was slightly higher than those of *E. crus-galli* and *E. colona*. The ratio of Rubisco/PEPC in *E. crus-galli* was higher than in *E. colona* and *E. stagnina* where increased by 38% and 2.58-fold, respectively (Fig.7 A&B).



Fig. 4. Changes in carbohydrates content over monthly harvests of *E. crus-galli, E. colona* and *E. stagnina*.h1 (55 days), h2 (80 days), h3 (105 days), h4 (130 days), h5 (156 days), (ND) not detected. Data is mean \pm SE. Bars labeled with different letters are significantly different at *p*<0.05.



Fig. 5. Changes in crude lipid content over monthly harvests of *E. crus-galli, E. colona* and *E. stagnina*. h1 (55 days), h2 (80 days), h3 (105 days), h4 (130 days), h5 (156 days), (ND) not detected. Data is mean \pm SE. Bars labeled with different letters are significantly different at *p*<0.05.



Fig. 6. Changes in fiber content over monthly harvests of *E. crus-galli, E. colona* and *E. stagnina*. h1 (55 days), h2 (80 days), h3 (105 days), h4 (130 days), h5 (156 days), (ND) not detected. Data is mean±SE. Bars labeled with different letters are significantly different at p<0.05.

Discussion

The biomass (dry weight) increased by increasing the age. In *E. crus-galli* it increased from 6.3 g plant⁻¹ in 55-d old to 81 g plant⁻¹ in 130-d old. In *E. colona* it increased from 6.5 g plant⁻¹ in 55-d old to 22 g plant⁻¹ in 105-d old. In *E. stagnina* it increased from 75 g plant⁻¹ in 55-d old to 115 g plant⁻¹ 130-d old. The present results of dry weight in line with wheat grass (*Thinopyrum paniticum*) 94-780 g plant⁻¹ [30].

Ash content was increased by increasing the age of *E. crus-galli*, *E. colona* and *E. stagnina*. It increased in *E. crus-galli*, and *E. stagnina* from 12.9 and 11% in 55-d old, respectively to 15.6 and 19.6 % in 130-d old, respectively. And in *E. colona* it increased from 13% in 55-d old to 18.3 % in 105-d old. Ash content of three species of *Echinochloa* were similar to that of *Atriplex ummularia* 18.1 % [31], lower than that of *Atriplex halimus* 26.9 % [32], but higher than those reported for *Bromus erectus*, *Horedum marinum and Dactylis glomerata* [33].

Total carbohydrates content increased by increasing the age of *E. crus-galli*, *E. colona* and *E. stagnina*. It increased in *E. crus-galli* and *E. stagnina* from 0.7 and 1.9 mg g⁻¹ in 55-d old to about 2, 2.2 mg g⁻¹ in 130-d old. And in *E.*

colona it increased from 0.8 mg g⁻¹ in 55-d old to 1.26 mg g⁻¹ in 105-d old. Results of our study are in agree with Donaghy and Fulkerson [34] who reported that carbohydrates and its composition in grass had been correlated with its growth.



Fig. 7. (A): SDS-page shows the protein bands of PEPC and Rubisco in (1) *E. cruss-galli*, (2) *E. colona* and (3) *E. stagnina*. (B) Level of PEPC, Rubisco and Rubisco/PEPC in *E. crus-galli*, *E. colona* and *E. stagnina*. Data is mean±SE.

Also, the obtained results are in agreement with those of cocksfoot (*Dactylis glomerata*) where total carbohydrate increased from 101 mg g^{-1} in September to 214 mg g^{-1} in November [35]. Carbohydrate content of *Echinochloa* sp. was lowest than that of other grasses crested wheatgrass (*Agropyron cristatum*) 39 g g^{-1} and wide leaf orchard grass (*Dactylis glomerata*) 23 g g^{-1} [36]. The nonstructural carbohydrates and structural polysaccharides or dietary fiber which comprises the principal components of cell walls [37] are considered to be the primary energy source for ruminant animals.

However, the fiber content of ryegrass can vary from being deficient in a vegetative state (between May and August) [38], to excess [39], after seed set or where moisture is deficient in summer. Non-structural carbohydrates are important in that they are a readily fermentable source of energy in the rumen and may also be important in synchronizing with rumen ammonia from the high protein intakes and common in cows grazing pasture [40].

Protein content was high in young plants in E. crus-galli and E. stagnina which began with 266 mg g^{-1} , 207 mg g^{-1} in 55-d old, respectively then decreased to 113 mg g⁻¹, 83mg g⁻¹ in 130-d old, respectively. It increased by the increase of the age in *E. colona* where began with 219 mg g^{-1} in 55-d old and reached to 262 mg g^{-1} in 105-d old. Tuna et al. [41] reported that the protein content of some grasses was between 385 and 780 mg g ¹, these values are not in agreement with the present study where protein content of *Echinochloa* sp. is between 83 and 266 mg g^{-1} . The results of E. colona are in line with Minson [42] who showed that the crude protein content was lowest in mid-summer (170 g kg⁻¹) and highest in autumn (230 g kg⁻¹), due to the increased proportion of leaf in the forage. Results of E. crus-galli and E. stagnina agree with Lima et al. [43] which reported that hay components decreased linearly with increased growth age of Echinochloa sp. (rice grass). Also, the results agree with those in soybean HLW-18 where it began with 228 mg g⁻¹ in July and decreased to 177 mg g⁻¹ in October [44]. Protein content of Echinochloa sp was similar to those of Medicago sativa and Trifolium pretense (206, 242 g kg⁻¹), respectively [45], but higher than that of *Panicum maximum* 177 g kg⁻¹ [46] and *Atriplex nummularia* (92-131 mg g^{-1}) [31] and lowest than soybean (310-340 g kg⁻¹) [44].

Lipid content decreased by the increase of the

age in *E. crus-galli* and *E. colona* but the contrast happen in *E. stagnina*. In *E. crus-galli* began with 3.15% in 55-d old and decreased to 1.6% in 156-d old. In *E. colona* it began with 3.16% in 55-d old and decreased to 1.3% in 105-d old. In *E. stagnina* began with 1.8% in 55-d old and reached to 3.2% in 130-d old. Lipid content of *Echinochloa* sp highest than other in *Medicago sativa*, *Trifolium pretense* 1.3, 1.8%, respectively [38] and *Atriplexhalimus*1.8 % [32].

Fiber content was decreased by increasing the age of *E. crus-galli* it began with 61% in 5-d old and decreased to 40% in 156-d old. But increased in both *E. colona* and *E. stagnina* from 68.6, 47% in 55-d old, respectively to 81, 80% in 130-d old, respectively. Fiber content of *Echinochloa* sp was higher than other in *A. halimus*12.8% [32], *Atriplex numularia* 2.15 % [31] and soybean (HLW-18) 58.0 % [44] and *Panicum maxima* 71.3 % [46].

Protein and carbohydrates content changes in relation to season [47,48], stage of growth [49,50], time of day [51], soil fertility or fertilizer application rate (particularly nitrogen (N)) [52] and probably soil moisture status.

Photosynthetic efficiency depends on the activity of ribulose-1,5-bisphoshate carboxylase/oxygenase (Rubisco, EC 4.1.1.39). When the atmospheric CO_2 decreased and O_2 increased, CO_2 concentration mechanisms evolved to reduce photorespiration (oxygenase activity of Rubisco) [53].

The primary CO_2 fixation to PEP is catalyzed by phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) in mesophyll cells. C4 acids are transported to bundle sheath cells, where they provide CO_2 to Rubisco [53,54]. Plants with C4 photosynthesis have advantages in extreme growth conditions such as high temperature, low water availability, high irradiation or saline soils [53].

The amount of PEPC and Rubisco protein was higher in *E. stagnina* than in *E. crus-galli* and *E. colona* and this lead to increase in plant biomass of *E. stagnina* than *E. crus-galli* and *E. colona*. The present results are in line with Piedade et al. [15] and Long [16] who reported that C4 plants exhibit higher photosynthetic and growth rates due to gains in the water, carbon and nitrogen efficiency uses, The highest known productivity in natural vegetation is for a C4 perennial grass in the central Amazon, which achieves a net production of 100 t (dry matter) ha⁻¹ year⁻¹.

Conclusion

The present study indicated that E. crus-galli, E. colona and E. stagnina had a relatively high proteins in young plants, but because the low carbohydrate content were low in these plants, therefore, it can be considered as a source of protein as well as additives to the fodder. Crude fibers were relatively high in E. crus-galli and E. colona and this was not good for forage use. It is recommended to use E. stagnina as a summer fodder in marshlands where these grasses are dominant. It is a cheap source of natural forage. Furthermore, the obtained results will be useful for managing these invasive weedy grasses in field crops.

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الملخص العريبي

الأداء والتركيب الكيميائي لثلاثة أعشاب في تجربة على المدى القصير

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تم عمل دراسة حقلية على نباتات الدنيبة وأبو ركبة والأمشوط حيث تم تجميع هذه النباتات من الحقل وتم إعادة زراعتها في الصوبة الحقلية و كانت النباتات لها نفس العمر و نفس الحجم . تهدف الدراسة إلى إيضاح تأثير عمر النبات على محتواه الكيمائي لكل من الكتلة الحية- الرماد- البروتين- الكربوهيدرات -الدهون - الألياف. وقد وجد ان الكتلة الحية و الرماد يزداد بزيادة عمر النبات في نباتي أبو ركبة و الأمشوط لكن في نبات الدنيبه يزداد حتى عمر 130 يوم ثم ينخفض. البروتينات و الدهون و الالياف تقل تدريجيا بزيادة عمر النبات في الثلاثة انواع بينما الكربو هيدرات تزداد بزيادة عمر النبات في نباتي الدنيبة وأبو ركبة و تظل ثابته في نبات الأمشوط. وجد ان نسبة كل من (PEPC and Rubisco) اعلى في نبات الأمشوط عن نبات الدنيبه وأبو ركبة لذلك لوحظ زيادة الكتلة الحيه في الامشوط عن باقي النباتات. نظرا لتواجد الألياف بنسبه اكبر في نباتي الدنيبه وأبو ركبة و ذلك يعتبر غير جيد لتغذية الحيوانات , لذلك تبين أنه يمكن استخدام نبات الأمشوط كمدعم للأعلاف نظرا لاحتوائه على نسبه اقل من الألياف ونسبه كبيرة من البروتين و الكتلة الحية.