J. Agric. Sci. Mansoura Univ., 30 (1): 205 - 214, 2005

EFFECT OF FEEDING LETTUCE AND GARDEN ROCKET ON SOME BIOCHEMICAL PARAMETERS IN: 2- DIABETIC RATS

Hegazi ,A. M.E.* and S.S. Ragab**

*** Dept. of Food Science, Agric. College, Cairo Univ.**

**** Dept. of Nutr. and Food Science, Faculty of Home Economics, Minufiya Uinv.**

ABSTRACT

The present study showed the effect of feeding lettuce and garden rocket to diabetic rats on some biochemical indices.

Diabetic rats fed experimental diets for 28 days including lettuce, garden rocket or their mixture presented a gradual increase of relative liver, kidney and spleen weight. Serum level of calcium, phosphorus, magnesium, zinc, iron, ferritin and blood hemoglobin and hematocrit, were also increased.

Rats fed experimental diets showed a gradual decrease of blood and liver cholesterol, triglycerides, phospholipids, free fatty acids, AST, ALT, ALP, creatinine, urea and blood glucose, while HDL-cholesterol increased.

INTRODUCTION

Vegetables such as lettuce (lactuca sativa) and garden rocket (Eruca sativa) are good sources of vitamins, minerals as well as fibers. Lettuce is used in several forms, it is used mainly as an ingredient in most salads. Dark green leaves are good sources of vitamins A and C, iron, folic acid and calcium. Lettuce content of fibers is 1.0%. Insoluble fiber was greater than soluble fiber (Filisetti and Lajolo, 1991).

Garden rocket is a green vegetable which have a favorable flavor and a good nutritional value. (Haag and Minami, 1988) stated that garden rocket is consumed in Brazil as a salad crop and is rich in iron and vitamin C and its fiber content is 1.0%.

Diabetes is now the fourth or the fifth leading cause of death in the world, with the total economic burden of type 2 diabetes estimated to be in the order of 8% of the total health care expenditure of the United States (Songer, 1992. O'Brien *et al.* 1998). Dietary therapy in type 2 diabetes remains a cornerstone of treatment and management. The key feature of diabetic nutritional therapy has been in the minimization of plasma glucose concentrations accompanied by increased insulin action.

The potential protective role of dietary fiber promoted the investigation of the action of dietary fiber as a component of a high carbohydrate diet on glucose metabolism in people who already had diabetes.

MATERIALS AND METHODS

Materials:

*** Vegetable samples:**

Lettuce and garden rocket leaves were bought from different sites of the local market in Cairo. They were washed and dried over night at 63°C using fan oven and powdered.

*** Animals:**

A total of 60 male adult albino rats 120-130g. (Sprague Dawley strain), were used in the study. (Desia and Bhide, 1985). Rats were divided into 10 groups, 6 rats in each group and fed the experimental diets for 28 days as follows.

Group 1: Fed control diet.
Group 2: Fed control diet + 1 % lettuce powder.
Group 3: Fed control diet + 2.5 % lettuce powder.
Group 4: Fed control diet + 5 % lettuce powder.
Group 5: Fed control diet + 1 % garden rocket powder.
Group 6: Fed control diet + 2.5% garden rocket powder.
Group 7: Fed control diet + 5% garden rocket powder.
Group 8: Fed control diet + 1% of mixture powder.
Group 9: Fed control diet + 2.5% of mixture powder.
Group 10: Fed control diet + 5% of mixture powder.

- Control diet consists of 10% protein (casein), corn oil 10%, salt mixture 4%, vitamin mixture 1%, choline chloride 0.2%, corn starch to complete 100%.
- Salt mixture of (Hegested *et al*. 1941) and vitamin mixture of (Bunce and Bloomer; 1972), were used.
- Mixture powder consists of equal parts of powdered lettuce and garden rocket leaves.
- Blood sampling: At the end of the experiment rats were fasted overnight and anesthetized with chloroform. Blood samples were collected for hemoglobin and hematocrit by heparinized micro tubes, the rest of blood was collected in a clean dry tube and serum was separated for biochemical analysis. Rats organs (liver, kidney, spleen) were taken, washed with saline and dried, then kept frozen till analysis.
- Extraction of liver lipids: was done according to (Bligh and Dyer 1959).

Biochemical Analysis:

1- Blood and liver lipids: were determined according to (Richmond 1973) for total cholesterol, (Young and Pestaner 1975) for triglycerides, (Richard *et al*. 1974) for phospholipids and free fatty acids, (Richmond 1973) for HDL-cholesterol.

2-Liver function tests: AST and ALT were determined by the method of (Reitman and Frankel 1957), ALP by Haussemet (1977) method.

3-Kidney function test: creatinine was estimated by the method of (Henry 1974), urea by the method of (Patton and Crouch 1977).

4-Iron indices: Serum ferritin was determined b the method of Cook *et al.* (1974), hemoglobin by the method of (Bernard 1965), hematocrit by the method of (Mc-Inory 1957).

5-Blood glucose: was determined by the method of (Trinder 1969).

RESULTS AND DISCUSSION

The effect of feeding, different levels of lettuce (L), garden rocket (GR) or their mixture (M), on relative weight of organs in alloxan-induced diabetic rats is shown in table (1). Data obtained showed that relative liver weight value elevated gradually in diabetic rats fed (L), (GR) or (M) which increase the feeding supplement increased. El-Adawi (1997), reported that the lack of insulin causes dissolution of the fat stores in adipose tissue. Stored triglycerides in the liver, lead to a very fatty liver. The liver can store 30% or more of its weight in the form of fat. A gradual increase in relative kidney weight of diabetic rats fed (L), (GR) or (M), as the feeding supplement increased. The increase in kidney weight was probably due to the fatty infiltration as reported by Akia and Matsumoto, (1978). A gradual elevation of relative spleen weight level in diabetic rats was shown in the table, with the increase of feeding supplement (L), (GR) or (M). This result agrees with that of El-Sayed (2001), who found an increase in the relative spleen weight of rats fed diet containing 5% p. oleracea leaves.

Table (2) shows the effect of feeding different levels of (L), (GR) or (M) on blood lipids profile in alloxan induced diabetic rats. A gradual decrease in the level of blood lipids, total cholesterol, triglycerides, phospholipids and free fatty acids, while HDL-cholesterol gradual increase with the increase of feeding supplement. The results are in a good agreement with El-Adawi (1997), who recorded highly significant reduction in serum total cholesterol with dietary fiber source. Fried *et al*. (1989) suggested that leaf of (L) diet led to a decrease in average lipid content in the plasma of B. glabrata snails. The results are also supported by El-Adawi (1997), he investigated that the fibers of broad bean, tomatoes, luprine and (L) highly significant decreased serum triglycerides.

The presented data agree with those of El-Adawi (1997), he found that the fibers of (L) highly significantly decreased the serum phospholipids. The result is coinciding with that obtained by Iman (1988). The results are also coinciding with that obtained by Sousa *et al.* (1990), who found that diets of (L) leaf reduced plasma free fatty acids. The results are in accordance with the hypothesis reported by El-Sayed (2001), that HDL-cholesterol was inversely related to total cholesterol.

The effect of feeding different levels of (L), (GR) or (M), on liver lipids profile in alloxan-induced diabetic rats, is presented in table (3). Liver lipids profile followed the same pattern as plasma lipids profile. Total cholesterol, triglycerides, phospholipids, free fatty acids were gradually decreased as the feeding supplement increased while HDL-cholesterol gradually increased.

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Fiber source containing a mixture of both soluble and insoluble fibers, have intermediate effect on serum and liver cholesterol values, the cholesterol lowering effect is due to increasing fecal bile acid excretion by soluble fibers which increase short chain fatty acids, reported by Anderson *et al*. (1994). Table (4) showed the effect of feeding different levels of (L), (GR), or (M) on liver function is alloxan-induced diabetic rats. The data of the present work indicated that there were a gradual decrease of the AST, ALT and ALP. The results are supported by the hypothesis of El-Missiry and El-Gindy (2000) that Eruca sativa seeds oil (ESS) may have favorable effects on liver damage produced by the generation of free radicals.

The effect of feeding different levels of (L), (GR) or (M) on kidney functions in alloxan-induced diabetic rats is presented in table (5). In the present study, a gradual decrease of blood creatinine and urea of diabetic rats, as the feeding level of (L), (GR) or (M) increased. The results are in agreement with those investigated by El-Sayed (2001), he found significant decreased of creatinine value in diabetic rats with the supplement of different levels of P. oleracea leaves. El-Adawi (1997), investigated that there was no significant difference in creatinine between (L) treatment at the two tested levels (5 and 10%) and control.

Tables (6) and (7) show the effect of feeding different levels of (L), (GR) or (M) on serum mineral profile and iron indices in alloxan-induced diabetic rats. In the present study, the data recorded gradual increases of serum Ca, P, Mg and Zn in diabetic rats fed the tested vegetable and their mixture in the diet. This may be due to the high content of these minerals in (L) and (GR). The data also revealed an increase of serum iron, with the increase of tested vegetables. (GR) group showed more increase in serum iron than (L) at the same level in the diet, this may be due to high content of iron in (GR).

The results of the present study recorded a gradual amelioration of ferritin, hemoglobin and hematocrit levels with the increase of (L), (GR) or (M) level in the diet of diabetic rats. The data is in accordance with El-Adawi (1997), he investigated that tomatoes and (L) highly significantly increased hemoglobin and hematocrit values.

This also agree with the results of El-Sayed (2001), he noticed that hemoglobin and hematocrit values increased significantly in diabetic rats fed diet containing 10% and 15% of p. Oleracea leaves compared to the untreated group. The reduction of hemoglobin and hematocrit values in the untreated group, may be due to the renal abnormality of diabetic rats, the kidney is unable to stimulate the production of red blood cells, causing anemia, Gyton (1981).

The effect of feeding different levels of (L), (GR) or (M), on blood glucose levels in alloxan-induced diabetic rats. The results of the present study (table 8) showed that (L), (GR) or (M), lowered blood glucose level in diabetic rats, when they were supplemented to the diet at levels 1%, 2.5% and 5%.

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Wolever *et al.,* (1988) found that the addition of up to one cup of (L), broccoli or brussels sprouts to a starchy meal has no significant effect on the blood glucose response in patients with non-insulin dependent diabetes mellitus (NIDDM). On the other hand Abo-Shadie (2002), concluded that onion, garlic and radish decreased blood glucose among diabetic rats. He suggested that the hypoglycemic effect of these vegetables could be attributed to its content of sulphur components. The reduction of blood glucose level, caused by the diet-containing radish was found also by Hala, (1990). Jenkins *et al*., (1978) reported that the addition of certain forms of dietary fiber to the diabetic diet significantly decreased post prandial hyperglycemia and would be expected improve the control of blood glucose concentration.

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تأثير تغذية الخس والجرجير على بعض المؤشرات الحيوية فى:
٢- الفئران المصابة بالسكر

احمد محمد اهاب حجازي* - شريف صبري رجب**

*** قسم الصناعات الغذائية - كلية الزراعة - جامعة القاهرة.**

**** قسم التغذية وعلوم الاطعمة - كلية الاقتصاد المنزلي - جامعة المنوفية.**

تم تغذية الفئران المصابة بمرض السكر ببعض الخضروات رخيصة الثمن مثل الخس والجرجير وبيان تأثيرها على بعض المؤشرات الحيوية. بعد تغذية الفئران بهذه الخضروات لمدة ٢٨ يوما اظهرت النتائج ما يلي:

١- أظهرت النتائج زيادة تدريجية فى الوزن النسبي لكل من الكبد والكلي والطحال.

٢-أظهرت النتائج انخفاضا تدريجيا فى نسبة الكوليسيترول ، الجلسيريدات الثلاثية، الفسفوليبدات، الاحماض الدهنية الحرة بينما زاد الكوليستيرول المرتفع الكثافة فى مصل الدم والكبد.

٣-وتبين من النتائج انخفاضا فى مستوي انزيمات الكبد ALT, AST, ALP فى مصل الدم.

٤-كما حدث انخفاض تدريجي فى مستوي كل من اليوريا والكرياتنين فى مصل الدم.

٥-أظهرت النتائج زيادة بسيطة فى نسبة كل من: الكالسيوم ، المغنسيوم ، الفسفور، والزنك فى مصل الدم.

٦-أظهرت النتائج زيادة تدريجية فى نسب الحديد ، الفيريتين ، الهيموجلوبين ، الهيماتوكريت.

٧-كما حدث انخفاض تدريجي فى مستوي سكر الدم للفئران المصابة بالسكر.

Table (1): Effect of feeding different levels of lettuce , garden rocket or a mixture of equal parts of lettuce and garden rocket on relative weight of the organs in alloxan-induced diabetic rats.

Parameter (g/100)	Control group	Lettuce feed level			Garden rocket feed level			Mixture feed level		
		1%	2.5%	5%	1%	2.5%	5%	1%	2.5%	5%
LW/BW	2.36 ±0.01[a]	2.43± 0.03[a]	2.48± 0.03[a]	2.53± 0.02[b]	2.46± 0.01[a]	2.52± 0.01[b]	2.57± 0.01[b]	2.52 ±0.03[b]	2.64± 0.02[c]	2.73± 0.02[d]
KW/BW	1.28 ±0.04[a]	1.33± 0.02[a]	1.40± 0.02[b]	1.48 ±0.01[b]	1.37± 0.03[a]	1.44± 0.06[b]	1.54± 0.03[c]	1.43± 0.01[b]	1.47± 0.02[b]	1.59± 0.03[c]
SW/BW	0.41± 0.02[a]	0.43± 0.01[a]	0.46 ±0.01[a]	0.48±0.02[b]	0.45± 0.03[a]	0.48 ±0.01[b]	0.53± 0.02[b]	0.45± 0.02[a]	0.52± 0.03[b]	0.62± 0.01[c]

Mean ±SE.

Different letters on the numbers mean significant difference at P <0.05

LW= Liver weight , KW= Kidney weight , SW= spleen weight , BW= body weight

Table (2) : Effect of feeding different levels of lettuce , garden rocket or a mixture of equal parts of lettuce and garden rocket on blood lipid profile in alloxan-induced diabetic rats.

Serum Lipids (mmol/l)	Control group	Lettuce feed level			Garden rocket feed level			Mixture feed level		
		1%	2.5%	5%	1%	2.5%	5%	1%	2.5%	5%
Total cholesterol	3.1±0.02 [a]	2.86 0.01	2.65 0.03	2.41 0.02	2.66 0.01	2.43 0.01	2.31 0.02	2.80 0.01	2.55 0.01	2.33 0.01
Triglyceride	1.62± 0.03[a]	1.42 0.01	1.36 0.01	1.22 0.02	1.38 0.01	1.21 0.02	1.15 0.01	1.38 0.01	1.32 0.01	1.15 0.02
Phospholipids	1.55± 0.06[a]	1.43 0.03	1.39 0.02	1.25 0.01	1.35 0.01	1.27 0.02	1.15 0.02	1.40 0.02	1.31 0.02	1.14 0.02
Free fatty acids	1.77± 0.01[a]	1.66 0.01	1.60 0.03	1.51 0.02	1.64 0.02	1.57 0.02	1.47 0.01	1.61 0.01	1.52 0.02	1.42 0.01
HDL cholesterol	0.88±0.01[a]	0.91 0.03	0.93 0.02	0.95 0.01	0.89 0.02	0.93 0.02	0.96 0.01	0.92 0.01	0.94 0.02	0.96 0.02

Table (3): Effect of feeding different levels of lettuce , garden rocket or a mixture of equal parts of lettuce and garden rocket on liver lipid profile in alloxan-induced diabetic rats.

Serum Lipids (mmol/l)	Control group	Lettuce feed level			Garden rocket feed level			Mixture feed level		
		1%	2.5%	5%	1%	2.5%	5%	1%	2.5%	5%
Total cholesterol	8.6±0.01 ^{a}	8.3± 0.02^{a}	8.1±0.02^{b}	7.6± 0.03^{b}	8.0± 0.02^{b}	7.7 ±0.01^{b}	7.1± 0.01^{c}	8.3± 0.02^{a}	7.2± 0.01^{b}	6.8± 0.01^{b}
Triglyceride	10.9± 0.03^{a}	10.3± 0.01^{a}	9.6± 0.03^{b}	9.2± 0.02^{b}	10.0± 0.01^{b}	9.4 ±0.03^{c}	9.2± 0.02^{c}	9.3± 0.01^{b}	9.2± 0.03^{b}	9.0± 0.02^{b}
Phospholipids	1.48± 0.01^{a}	14.5± 0.02^{a}	14.1 ±0.01^{a}	13.7± 0.01^{b}	14.2± 0.03^{a}	13.8± 0.01^{b}	13.5± 0.03^{b}	13.8± 0.03^{b}	13.0 ± 0.01^{b}	13.4 ±0.03^{b}
Free fatty acids	5.8± 0.02^{a}	5.6± 0.02^{a}	5.2 ±0.01^{b}	4.8 ±0.01^{b}	5.6± 0.02^{a}	5.3± 0.02^{b}	5.0 ±0.01^{b}	5.4± 0.02^{a}	5.0± 0.02^{b}	4.8 ±0.01^{c}
HDL cholesterol	0.66±0.01^{a}	0.96± 0.02^{a}	0.70± 0.03^{a}	0.72± 0.01^{b}	0.68± 0.01^{a}	0.70± 0.01^{a}	0.72± 0.02^{b}	0.96 ±0.01^{b}	0.71 ±0.01^{b}	0.73± 0.02^{b}

Mean ± SE

Different letters on the numbers mean significant differences at P < 0.05

Table (4) : Effect of feeding different levels of lettuce , garden rocket or a mixture of equal parts of lettuce and garden rocket on liver functions in alloxan- induced diabetic rats.

Parameter	Control group	Lettuce feed level			Garden rocket feed level			Mixture feed level		
		1%	2.5%	5%	1%	2.5%	5%	1%	2.5%	5%
AST (U/L)	32.1±0.10^{a}	30.1± 0.10^{a}	27.1±0.11^{b}	24.1± 0.35^{b}	29.3± 0.35^{b}	26.1 ±0.20^{b}	23.1± 0.20^{c}	27.1± 0.22^{b}	23.4± 0.10^{b}	21.3± 0.30^{c}
ALT (U/L)	29.4±2.50^{a}	26.4± 2.01^{b}	24.8± 2.20^{b}	22.1± 2.10^{c}	25.3± 4.16^{ab}	22.8 ±1.12^{c}	19.4± 2.11^{c}	24.0± 2.52^{a}	21.8± 3.25^{b}	17.3± 1.10^{c}
ALP (U/L)	96.4± 2.51^{a}	88.1±2.05^{b}	83.1 ±2.10^{b}	79.1± 2.30^{c}	85.1± 1.10^{b}	82.1± 1.40^{b}	75.1± 2.03^{c}	83.1± 1.40^{a}	79.1 ± 1.25^{b}	73.1 ±1.10^{b}

Mean ± SE

Different letters on the numbers mean significant differences at P < 0.05

Table (5): Effect of feeding different levels of lettuce , garden rocket or a mixture of equal parts of lettuce and garden rocket on kidney functions in alloxan- induced diabetic rats.

Parameter (mg/100 mL)	Control group	Lettuce feed level			Garden rocket feed level			Mixture feed level		
		1%	2.5%	5%	1%	2.5%	5%	1%	2.5%	5%
Creatinine	0.74±0.10^{a}	0.70± 0.20^{b}	0.66±0.20^{b}	0.63± 0.10^{c}	0.68± 0.2^{b}	0.63 ±0.2^{c}	0.59± 0.1^{c}	0.65± 0.1^{b}	0.60± 0.10^{b}	0.56± 0.2^{c}
Urea	47.35 ±0.1^{a}	44.15 ±0.2^{a}	42.10 ±0.3^{b}	38.25 ±0.2^{b}	43.25 ±0.1^{b}	40.25±0.2^{b}	37.28 ±0.2^{c}	40.1±0.1^{b}	37.1± 0.3^{b}	34.2± 1.10^{c}

Mean ± SE

Different letters on the numbers mean significant differences at P < 0.05

Table (6) : Effect of feeding different levels of lettuce , garden rocket or a mixture of equal parts of lettuce and garden

rocket on liver functions in alloxan- induced diabetic rats.

Serum mineral	Control group	Lettuce feed level			Garden rocket feed level			Mixture feed level		
		1%	2.5%	5%	1%	2.5%	5%	1%	2.5%	5%
Ca (mmol/L)	1.7±0.02^{a}	1.9± 0.02^{b}	2.0±0.02^{b}	2.1± 0.01^{b}	1.8± 0.03^{b}	1.9 ±0.02^{b}	2.1± 0.02^{b}	1.9± 0.02^{a}	2.1± 0.03^{b}	2.23± 0.01^{b}
P(mmol/L)	0.66±0.01^{a}	0.69± 0.01^{a}	0.71± 0.2^{b}	0.76± 0.1^{c}	0.71± 0.2^{b}	0.74 ±0.1^{b}	0.76±0.1^{c}	0.73± 0.3^{b}	0.75± 0.2^{b}	0.78± 0.2^{c}
Mg(mmol/L)	0.68± 0.3^{a}	0.71±0.02^{a}	0.73 0.01^{b}	0.77± 0.02^{c}	0.71± 0.03^{a}	0.73± 0.02^{b}	0.76± 0.03^{b}	0.74± 0.02^{b}	0.75 ± 0.03^{b}	0.78 ±0.02^{c}
Zn(umol/L)	12.6± 0.3^{a}	12.9 ±0.2^{a}	13.2 ± 0.1^{b}	13.7 ±0.2^{b}	12.8± 0.1^{a}	13.2 ± 1.3^{b}	13.6 ±1.2^{b}	13.4 ±0.2^{b}	13.8± 2.5^{b}	13.9 ±2.5^{b}

Mean ± SE

Different letters on the numbers mean significant differences at P < 0.05

Table (7): Effect of feeding different levels of lettuce , garden rocket or a mixture of equal parts of lettuce and garden rocket on iron indices in alloxan-induced diabetic rats.

Iron Indices	Control group	Lettuce feed level			Garden rocket feed level			Mixture feed level		
		1%	2.5%	5%	1%	2.5%	5%	1%	2.5%	5%
Serum Fe (Ug/dl)	58.4±3.2^{a}	60.2± 2.0^{a}	62.3±2.1^{b}	65.7±2.4^{b}	62.1± 2.3^{b}	64.2 ±2.5^{b}	67.20± 1.3^{c}	63.30± 2.5^{b}	65.35± 1.6^{b}	68.35± 1.2^{c}
Serum ferritin(Ug/l)	48.2±3.1^{a}	49.8± 1.1^{a}	51.3±2.2^{b}	51.9± 2.0^{b}	51.4± 1.3^{b}	52.6 ±2.3^{b}	52.9± 3.5^{b}	52.3± 2.5^{b}	53.4± 2.3^{b}	53.5± 2.1^{b}
Hemoglobin (g/L)	10.3± 0.1^{a}	10.7± 0.2^{a}	11.3 ±0.3^{b}	11.5± 0.2^{b}	10.7± 0.2^{a}	11.1± 0.3^{b}	11.7± 0.3^{b}	10.9± 0.3^{a}	11.3 ± 0.2^{b}	12.4 ±0.2^{c}
Hematocrit (%)	37.2±2.5^{a}	37.6±1.1^{a}	38.8 ±2.1^{a}	38.3 ±1.0^{b}	37.6± 3.1^{a}	38.3± 2.4^{a}	38.5 ±1.5^{a}	37.9± 2.4^{a}	39.2±1.5^{b}	39.9 ±2.4^{b}

Mean ± SE

Different letters on the numbers mean significant differences at P < 0.05

Table (8): Effect of feeding different levels of lettuce , garden rocket or a mixture of equal parts of lettuce and garden rocket on blood glucose level (mg/dl) in alloxan-induced diabetic rats.

Intervals (week)	Control group	Lettuce feed level			Garden rocket feed level			Mixture feed level		
		1%	2.5%	5%	1%	2.5%	5%	1%	2.5%	5%
1st week	392.7±3.5^{a}	388.2± 1.5^{a}	368.4±3.5^{a}	305.2±5.5^{b}	372.2± 2.1^{a}	352.2 ±2.5^{a}	276.4± 5.3^{a}	360.1± 2.5^{a}	340.3± 3.1^{a}	226.2± 2.1^{b}
2nd week	402.2±1.2^{a}	361.4± 2.6^{b}	345.2±3.2^{b}	288.4± 3.3^{b}	355.3± 3.5^{a}	334.2 ±3.0^{b}	255.3±5.5^{c}	342.2± 4.0^{b}	314.4± 2.3^{b}	205.2± 2.1^{c}
3rd week	368.3± 2.7^{a}	327.2± 5.5^{a}	313.5 ±3.6^{a}	260.2± 1.1^{b}	318.2± 2.6^{a}	288.5± 1.0^{b}	238.6±3.8^{b}	286.5±3.2^{b}	270.5± 5.5^{b}	178.3 ±2.5^{c}
4th week	388.2±4.5^{a}	288.3±3.5^{b}	257.7 ±1.7^{b}	218.5 ±3.5^{b}	276.2± 2.7^{b}	237.5± 3.4^{b}	200.6 ±1.5^{c}	236.3± 1.2^{b}	227.2±1.2^{b}	150.3 ±4.5^{c}

Mean ± SE

Different letters on the numbers mean significant differences at P < 0.05