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Sea Algae Supplementation of Baladi Rabbits Diet and its Implication on Certain Biochemical Parameters

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ABSTRACT

Eighteen adult male red baladi rabbits were used to evaluate the effect of feeding a diet supplemented with 1% sea algae (*Ulva lactuca* or *Gntromorpha intestinalis*) on blood plasma lipid constituents and some other selected biochemical parameters. Rabbits were weighed and divided into three groups of 6, homogeneous in age, weight and paternity. Animals in the first group were given a commercial balanced pelleted ration for breeding rabbits and served as a control (C). Animals in the second (T1) and third (T2) groups were fed the same diet as the control but supplemented with 1% sea algae (*Ulva lactuca* and *Gntromorpha intestunalis*, respectively). Blood samples were obtained from each animal every two weeks via the ear vein during the treatment period which lasted for two and half months (1st July to15th September). The results obtained revealed that cholesterol, very low density lipoprotein (VLDL), and direct to indirect bilirubin (DBl:IDBl) ratio were significantly ($P<0.01$) increased by treatments. Overall means revealed that DBl:IDBl ratio was elevated only in the T2 group. Total acid phosphatase (AcP) was significantly ($P<0.05$) increased by treatments. Meanwhile, total lipids, triglycerides (TG), high density lipoprotein (HDL), total bilirubin, (TBl) and indirect biliriubin (IDBl) were significantly ($P<0.01$) decreased during the treatment period. The overall mean for total lipids was lowered in the T2 group ($P<0.05$) compared with control group. Low density lipoprotein (LDL), direct bilirubin (DBl), prostatic acid phosphatase (PAcP), non prostatic acid phosphatase (NPAcP) and PAcP:NPAcP ratio were not significantly affected by treatments. Aspartate aminotransferase (AST) was significantly ($P<0.01$) increased by treatments, whereas alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were not significantly affected.

INTRODUCTION

Seaweeds could be a potentially valuable resource for animals feed in the future (Zalabak *et al.*, 1990). However, despite their high productivity, very little research has been carried out to evaluate their nutritive values for animals. The Japanese customarily eat seaweed, and the numbers of species eaten have been estimated at more than twenty. In particular, *Undaria pinnatifida* (wakame) is one of the most widely eaten edible brown seaweeds in Japan. Elements in wakame were found to have coagulation protective action for human blood (Hori and Nishizawa, 1982), antitumor action (Usui *et al.*, 1980) and antimutagenic activity (Okai *et al.*, 1993).

Algae are autotrophic organisms, which have potential as source of food for man and animals. It is generally accepted that seaweeds have higher contents of minerals, vitamins and non-digestible polysaccharides than terrestrial vegetables, similar or higher amounts of proteins and lower lipid contents (Norziah and Ching, 2000 and Wong and Cheung, 2000). However, studies of the digestibility of seaweeds are few and more research is needed to establish its nutritional value. Few studies have evaluated *Ulva lactuca as a feed supplement to animals, while it is a useful feed supplement* for sheep (Arieli *et al.*, 1993), it was found to be not suitable for poultry (Ventura *et al.*, 1994),

Copper is obligatory for the structure and function of the immune, nervous, skeleton systems and also for the biochemical and main physiologic functions. In addition, it is required as a cofactor of proteins and enzymes which are necessary for the constancy, healthiness and normal cellular homeostasis (Panemangalore and Bebe, 1996). Also, zinc has a role in metabolic events such as anabolism and catabolism of connective tissue, nucleic acids, protein, lipid, carbohydrate, enzymes, and is involved in the functioning of more than 300 enzymes (Prasad, 1995).

Therefore, the objective of this study was to evaluate the effect of feeding a diet supplemented with 1% seaweed of either *Ulva lactuca* (T1) or *Gntromorpha intestinalis* (T2) on blood plasma lipid constituents and some selected biochemical parameters of male red baladi rabbits.

MATERIALS AND METHODS

This experiment was carried out at the Institute of Graduate Studies and Research, Alexandria University. Eighteen adult male red baladi rabbits were weighed and divided into three groups of 6. Animals in all groups were homogeneous in age, weight and paternity. During the experimental period which lasted for two and half months (from 1st July to 15th September) the rabbits were individually housed in Universal galvanized wire batteries with feed and fresh tap water offered *ad libitum*. The first group were given a commercial balanced pelleted ration for breeding rabbits containing 18% crude protein, 14% crude fiber, 2% fat and 2600 kcal DE/ kg and served as control (C). The second (T1) and third (T2) groups were fed the same diet as control but were supplemented with 1% sea algae of two types *Ulva Lactuca* and *Gntromorpha intestinalis*, respectively. Sea algae were obtained from the Mediterranean sea in Alexandria Governorate (El-Max and Abou-Talat areas). The algae were analyzed at the Department of Soil and Water Sciences, Faculty of Agriculture, and at the Central Laboratory, Faculty of Science, Alexandria University.

Blood samples were obtained from the ear vein of each animal every two weeks in heparinized tubes and were placed immediately on ice. Plasma was obtained by blood centrifugation at 3,000 rpm for 20 min and stored at -20 °C until used for analyses. Plasma total lipids (TL) and cholesterol were determined using commercial kits obtained from Bio ADWIC, Egypt. Plasma triglyceride (TG) was determined by triglyceride-GPO kits obtained from Pasteur Lab, Egypt according to McGowan *et al.* (1983). Plasma low-density lipoprotein (LDL) and serum high-density lipoprotein (HDL) were assayed using Biosystems reagents Kits, Spain, according to Assman *et al.* (1984) and Biosystems reagents Kits, Spain, according to Burstein *et al.* (1980), respectively. Plasma very low density lipoprotein (VLDL) was calculated from triacylglycerols according to Friedwald *et al.* (1972) who reported that VLDL is present in a concentration equal to one fifth of triacylglycerols concenteration in blood plasma of less than 400 mg/dl. Total bilirubin (TBI), acid phosphatase (AcP) and prostatic acid phosphatase (PAcP) were determined using commercial kits obtained from Bio ADWIC, direct bilirubin (DBI), indirect bilirubin (IDBI), DBI:IDBI ratio, non prostatic acid phosphatase (NPAcP) and PAcP:NPAcP ratio were calculated according to Tietz (1989). Aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) were determined using commercial kits obtained from Bio ADWIC, Egypt. Lactate dehydrogenase (LDH) was determined according to Stroev and Makarova (1989).

Statistical analysis was performed using the general linear model (GLM) produced by Statistical Analysis Systems Institute (SAS, 1999). Significant differences among means were evaluated using Duncan's Multiple Range Test of SAS (1999).

RESULTS AND DISCUSSION

Table (1) shows the chemical analyses of the two algae (*Ulva lactuca* and *Gntromorpha intestinalis*). It can be seen that *Gntromorpha intestinalis* has high levels of zinc, selenium, iodine, copper, iron and manganese, whereas *Ulva lactuca* is rich in lead and cadmium.

Figure (1) and Table (2) indicate that the supplementation of sea algae has a significant ($P<0.01$) effect on total lipids, cholesterol, triglycerides (TG), high density lipoprotein (HDL) and very low density lipoprotein (VLDL). The overall mean values for total lipids significantly ($P<0.05$) decreased in animals fed on *Gntromorpha intestinalis* (T2) than that of the group fed on *Ulva lactuca* (T1) or control group. Meanwhile, the overall mean values for TG and HDL significantly ($P<0.05$) decreased in the T1 and T2 group and this reduction was more obvious in T2. On the other hand, cholesterol and VLDL significantly ($P<0.05$) increased in both treated groups (Table 2), particularly in the T2 group.

Plasma cholesterol concentration is known to be influenced by the quantity and quality of fat in the diet. In human, total and low density lipoprotein (LDL) and cholesterol concentrations fall when saturated triglyceride in the diet is replaced by polyunsaturated vegetable oil (Spady and Woollett, 1990). Triglyceride is transported in blood via macromolecular particles called lipoproteins. Chylomicrons and very low density lipoprotein (VLDL) are the predominant carriers of TG and are often referred to as TG-rich lipoproteins (Kleppe *et al.*, 1988). It is well established that liver regulates plasma levels of cholesterol and TG by secretion and transport of these lipids in the VLDL and by removal of lipoproteins by receptor-mediated endocytosis, and changes in nutritional

Table (1): Chemical composition of sea algae extracts used as feed supplement for red Baladi male rabbits during the experimental period.

Items	*Ulva lactuca* (T1) ppm	*Gntromorpha intestinalis* (T2) ppm
* EC dS/m	89.0	88.0
Major Cations:		
Sodium	197.8	89.7
Potassium	97.8	148.6
Calcium	70.1	80.2
Magnesium	207.0	634.0
Major Anions:		
Carbonate	0.0	0.0
Bicarbonate	0.0	0.0
Phosphorus	312.3	90.3
Iodine	185.0	320.0
Minor Cations:		
Lead	0.070	0.000
Cadmium	0.035	0.019
Iron	2.137	4.095
Copper	0.118	0.572
Manganese	0.083	0.976
Selenium	1.150	2.500
Zinc	0.783	2.139

* EC = Electric conductivity, dS/m = deci Siemens per meter at 25°C

and hormonal status alter the rate of assembly and secretion of VLDL particles (Thomas *et al.*, 1992). The present results showed that addition of 1% sea algae to rabbit diet significantly decreased plasma TG. This is in agreement with previous findings of Murata *et al.* (1999) who found that addition of 2% wakame to rat diet significantly decreased serum triacylglycerol concentration, where the absorption of lipids from the small intestine and/or metabolism of lipids and fatty acids in the liver affect serum and liver triacylglycerol concentrations. The authors raised the possibility that dietary wakame modifies the rate of synthesis and degradation of fatty acids and lipids in the liver. The significant decreases in total lipids, TG and HDL in the present study particularly in animals fed on 1% *Gntromorpha intestinalis* (T2) indicate that selenium and other antioxidants content of the algae may play a major role in preventing lipid peroxidation.

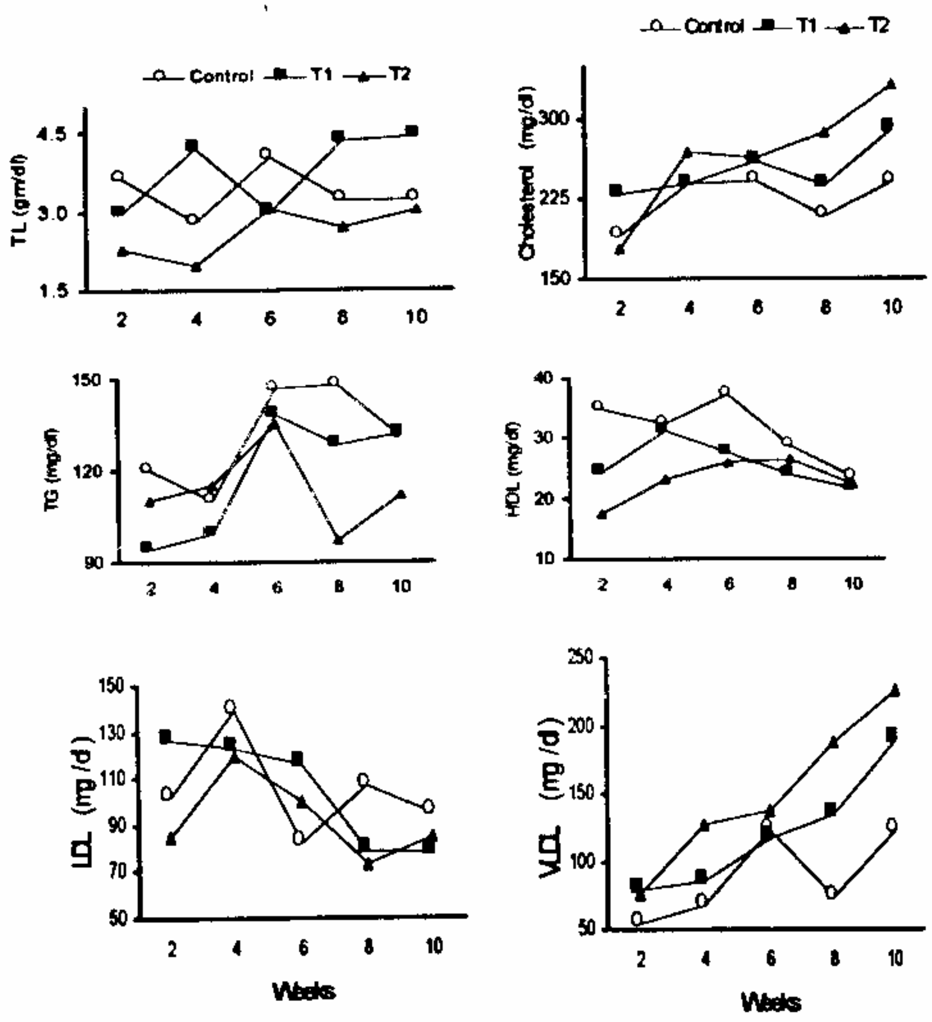

Figure 1: Effect of feeding sea algae (*Ulva sp. and Gentromorpha sp.*) on blood plasma total lipids (TL), cholesterol, triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) of red Baladi male rabbits during treatment period.

Table 2 : Overall means ± SEM of blood plasma biochemical constituents of male rabbits during treatment with sea algae *Ulva lactuca* and *Gntromorpha intestinalis*.

Parameters	Control	Treatment		SEM
		T_1	T_2	
TL (gm/dl)	3.38^a	3.78^a	2.60^b	0.20
Cholesterol (mg/dl)	224.2^b	251.2^a	266.0^a	8.14
TG (mg/dl	131.0^a	118.3^b	113.7^b	4.56
HDL (mg/dl)	31.2^a	25.7^b	23.0^c	1.08
LDL (mg/dl)	105.1	104.4	92.4	6.81
VLDL (mg/dl)	87.9^c	121.1^b	150.5^a	8.98
TBl (mg/dl)	1.63^a	1.45^b	1.35^b	0.05
DBl (mg/dl)	0.63	0.55	0.60	0.03
IDBl (mg/dl)	1.00^a	0.89^b	0.76^c	0.04
DBl: IDBl ratio	0.66^b	0.67^b	0.83^a	0.05
AcP (IU/L)	43.9^b	46.7^{ab}	50.1^a	2.19
PAcP (IU/L)	12.2	11.9	13.9	1.01
NPAcP (IU/L)	31.7	34.8	36.2	1.83
PAcP:NPAcP ratio	0.40	0.37	0.42	0.04
AST (IU/L)	162.4^b	164.6^{ab}	170.9^a	3.36
ALT (IU/L)	48.7	52.3	48.8	1.92
AST/ALT ratio	3.3^b	3.2^b	3.6^a	0.08
ALP (IU/L)	66.9	68.6	77.3	4.03
LDH (IU/L)	2.4	2.5	2.3	0.07

a,b,c Within rows, means with different superscript letters differ significantly ($P<0.05$)

Total bilirubin (TBl), indirect bilirubin (IDBl) and direct to indirect bilirubin ratio (DBl/IDBl) were significantly ($P<0.01$) affected by treatment (Fig. 2). The overall mean values revealed that TBl and IDBl were significantly ($P<0.05$) decreased in both treated groups compared with the control one, while DBl/IDBl ratio was significantly ($P<0.05$) elevated in the

T2 group only (Table 2). In obstructive Jaundice there is an increase in total bilirubin, but this increase is primarily in the bilirubin glucuronide. In hemolytic Jaundice the unconjugated (indirect) bilirubin fraction is elevated.

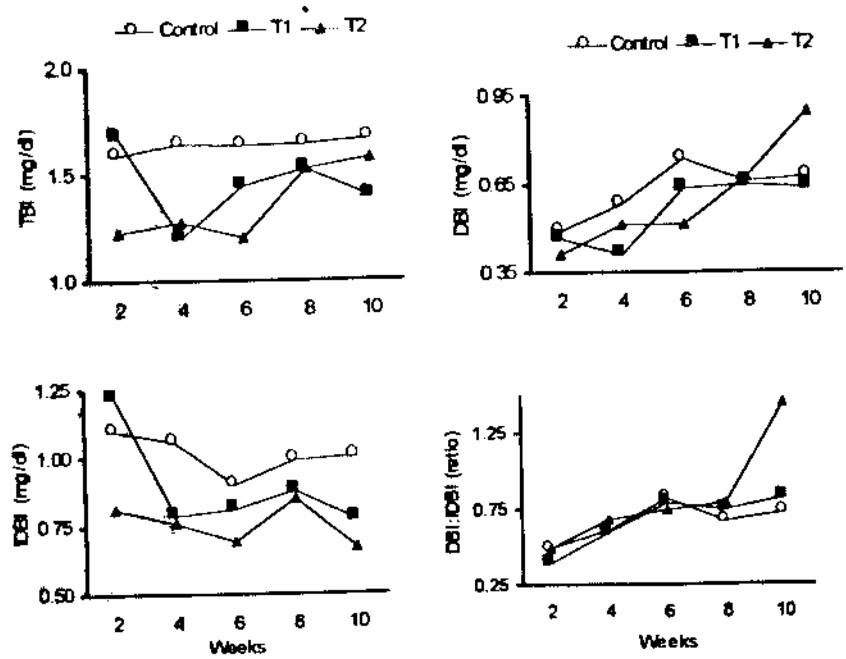

Figure 2: Effect of feeding sea algae (*Ulva sp. and Gentromorpha sp.*) on blood serum total bilirubin (TBI), direct bilirubin (DBI), indirect bilirubin (IDBI) and direct bilirubin to indirect bilirubin (DBI:IDBI) ratio of red Baladi male rabbits during treatment period.

Both conjugated (direct) and unconjugated (indirect) serum bilirubin are increased in hepatitis (Routh, 1976). A major function of the liver is the formation of bile and hepatobiliary excretion of endogenous substances and xenobiotics, such as chemicals, drugs and their metabolites (Georing, 2003). Biliary lipid secretion involves the exocytosis of cholesterol and phospholipids-containing vesicles (Crawford *et al.*, 1995). Although the exact vesicular mechanism has not been revealed, the following sequence of events for biliary lipid secretion has been proposed. Cytosolic delivery of lipid vesicles to the internal hemileaflet of the canalicular membrane, transfer from the internal to the external hemileaflet of the canalicular membrane, and formation and detachment of unilamelar vesicles from the canalicular membrane (Kunze and Rustow, 1993). Movement of cholesterol into bile is a well-described process (Crawford *et al.*, 1995), but mechanisms by which cholesterol is distributed in the cell are still poorly understood. Nevertheless, it is thought that cholesterol can be transported from its site of synthesis to plasma membrane by several distinct mechanisms. Some evidence indicates that intracellular cholesterol trafficking could occur by vesicular means (Reinhart *et al.*, 1987), while other studies render it conceivable that a lipoprotein-like particle between source and target membrane in the cell may be involved (Kaplan and Simoni, 1985 and Duguay *et al.*, 2000). The present results showed a clear reduction of total and indirect bilirubin, while the direct bilirubin was not affected by the treatment. Therefore, it may be suggested that sea algae may be useful as a food for adjusting liver functions, as it decreased TBI and IDBI in both treated groups compared with the control one.

Total acid phosphatase (AcP) was significantly ($P<0.05$) affected by treatment, the overall mean values increased slightly in the treatment groups (Table 2 and Figure 3). On the other hand prostatic acid phosphatase (PAcP), nonprostatic acid phosphatase (NPAcP) and PAcP/ NPAcP ratio were not significantly affected by treatment (Fig. 3). Acid phosphatase is an important enzyme distributed in many tissues of the mammalian body and related mainly to cell metabolism. Gill *et al.* (1990) stated that the decrease in the activity of AcP could be attributed to the structural damage of the cellular machinery concerned with enzyme production, which is in contrast with our results.

Aspartate amino transeferase (AST) and AST / alanine amino transeferase (ALT) ratio were significantly ($P< 0.01$) increased by treatment, while ALT, LDH and ALP were not significantly affected by treatment (Table 2 & Fig. 4). Zinc and copper accumulate mostly in the liver (Saito, 1996). However, there are other studies which suggested that zinc and copper loading which may lead to intoxication can increase liver enzyme levels (Saito 1996 and Levengood *et al.*, 2000) which may support the finding of the present study especially in T2. The increase of AST in the present study also agrees with the finding of Bag *et al.* (1999) on rats.

In summary, dietary sea algae (*Ulva lactuca* and *Gntromorpha intestinalis*) supplementation to rabbit diet decreased plasma total lipids, triglycerides, high density lipoprotein, total bilirubin, indirect bilirubin but has no significant effects on low density lipoprotein, direct bilirubin, prostatic acid phosphatase, non prostatic acid phosphatase, prostatic to non prostatic acid phosphatase ratio, alanine aminotrasferase, lactate dehydrogenase and alkaline phosphatase. So it can be concluded that dietary sea algae particularly *Gntromorpha intestinalis* has positive effect on major lipid parameters and liver functions tested in this study. Thus, sea algae may be useful as a food to prevent hyperlipidemia.

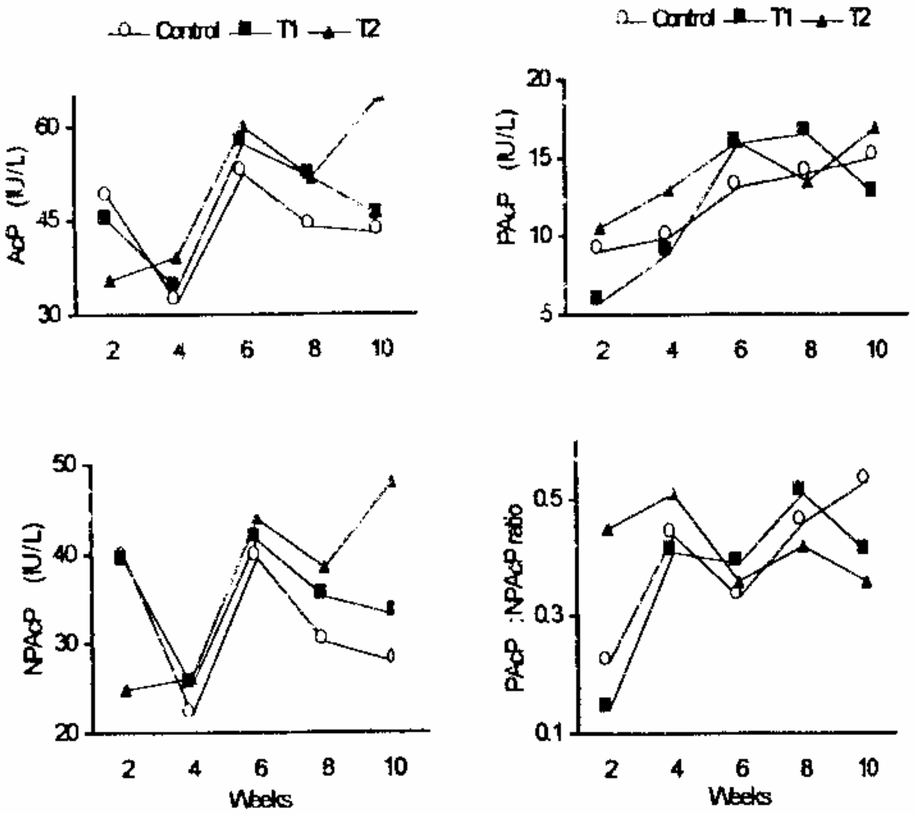

Figure 3: Effect of feeding sea algae (*Ulva sp. and Gentromorpha sp.*) on blood plasma Total acid phosphatase AcP, prostatic acid phosphatase (PAcP), nonprostatic acid phosphatase (NPAcP) and prostatic to nonprostatic acid phosphatase (PAcP:NPAcP) ratio of red Baladi male rabbits during treatment period.

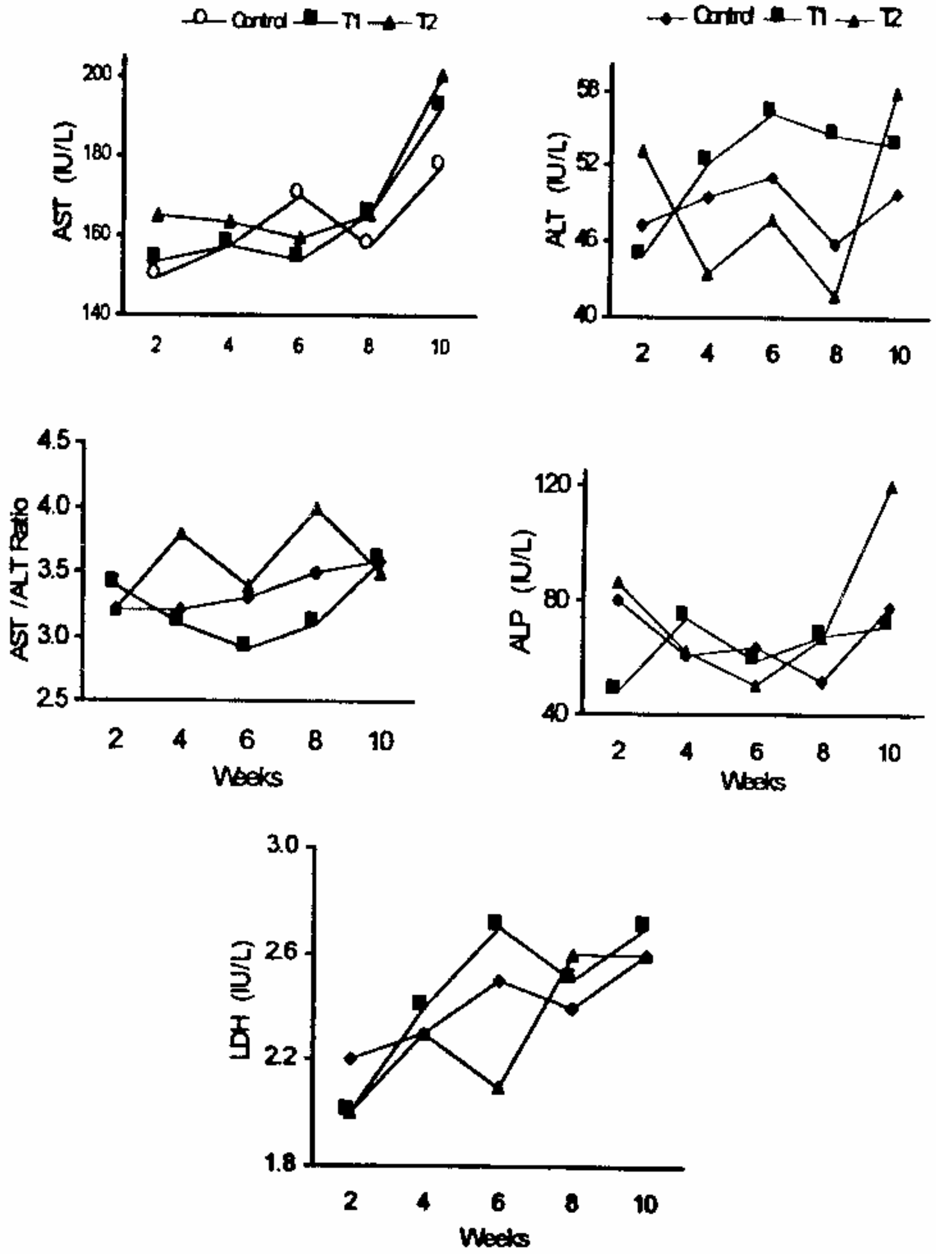

Figure 4: Effect of feeding sea algae (*Ulva sp. and Gentromorpha sp.*) on blood plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) AST:ALT ratio, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) of red Baladi male rabbits during treatment period.

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تأثير إضافة الطحالب البحرية للغذاء علي بعض المعايير البيوكيميائية في الأرانب البلدي الحمراء

صباح جابر البنا
قسم الدراسات البيئية بمعهد الدراسات العليا والبحوث جامعة الإسكندرية

استخدم في هذه الدراسة ١٨ من ذكور الأرانب البلدي الحمراء الناضجة وقسمت لثلاث مجموعات متماثلة الوزن والعمر:
المجموعة الأولى: غذيت على العليقة التجارية المتوازنة للأرانب واستخدمت كمجموعة المقارنة.
المجموعة الثانية والثالثة: غذيت على نفس العليقة السابقة مع إضافة ١٪ من الطحالب البحرية (*Ulva lactuca* or *Gntromorpha intestinalis*) على التوالي واستمرت التجربة لمدة شهرين ونصف خلال موسم الصيف من أول يوليو حتى منتصف سبتمبر أخذت خلالها عينات الدم من كل حيوان من خلال الوريد الأذني مرة كل أسبوعين.

بينت النتائج زيادة مستويات كل من تركيز الكولسترول، الليبوبروتين منخفض الكثافة (VLDL)، النسبة بين البيليروبين المباشر وغير المباشر (خاصة في المعاملة الثانية) والفوسفاتيز الحامضى خلال فترة المعاملة. على العكس من ذلك انخفضت مستويات كل من الدهون الكلية (في المعاملة الثانية) ، الجلسريدات الثلاثية (TG)، الليبوبروتينات العالية الكثافة (HDL) والبيليروبين الكلى (TBI) والبيليروبين الغير مباشر(IDBI) . بينما لم تتغير مستويات كل من الليبوبروتين منخفض الكثافة (LDL) والبيليروبين المباشر (DBI) والفوسفاتيز الحامضى البروستاتى (PAcP) و الغير بروستاتى (NPAcP) والنسبة بينهما (PAcP: NPAcP) .

وعلى الجانب الأخر زاد نشاط إنزيم اسبارتيت أمينوترانسفيريز (AST) زيادة معنوية، بينما لم يتأثر نشاط كل من إنزيمات الانين ترانسفيريز (ALT) لاكتيت ديهيدروجينيز (LDH) والفوسفاتيز القاعدي (ALP) أثناء فترة المعاملة.

من النتائج المتحصل عليها في هذه الدراسة يتبين أن استخدام هذه الطحالب بالنسبة المستخدمة كمضاف غذائي لها تأثيرات ايجابية في معظم المعايير التي تم تقديرها .