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Macroscopic and microscopic study of the liver and kidneys of native kids fed with *Sargassum Angustifolium* in Bushehr province of Iran

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Abstract

To study the effect of *Sargassum Angustifolium* feeding on health and the live and kidneys, the 24 native kids with an average live weight of 15.64 ± 3/2 kg and the average age of 5-6 months in a completely randomized experimental design with three levels of algae in the edible diet (0, 10 and 20%) and 8 repetitions (kids) in each treatment were experimented in Bushehr province in 75 days. Experimental groups of 10 and 20% algae (24 kids) were fed with *Sargassum Angustifolium* in coast of Bushehr city and also control group (zero percent algae) in this period was fed with normal diet. This study aimed to examine possible changes in macroscopic and microscopic damages of the liver and kidneys, in four stages including the day zero, day 25, day 50 and day75 of the experiment. Two kids of each treatment were randomly selected and then were slaughtered. The results of the liver and kidneys showed there was found no significant symptoms showing the damage of the liver and kidneys. Microscopic examination showed there was found only the congestion and a few small symptoms of necrosis in some cases in experimental treatments in liver and kidney.

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Introduction

Identifying and introducing the new spices is necessary because of the shortage of food supplies and the increase in need of livestock products. Therefore, there have been performed a large number of researches for obtaining this value recently. Because the Bushehr province has a warm and humid climate, there are more than 2500000 of rural and nomadic livestock units and 1500000 ha of pasture that more than half of them have salty and poor vegetation (Report of Bushehr province, 1998).Hence, identifying new supplies and determining their food values have significant effects on reducing the costs and increasing the efficiency on farms. Today algae are considered as an alternative of forage for feeding animals. Using the algae as forage was conventional in some European countries such as England, Ireland, Scotland and the Maghreb and some African, American and Asian countries for horses, pigs and sheep. Algae are collected in dry and proper seasons and maintain for improper seasons (Mchugh, 2003). Sargassum Genus is excellent source of minerals, carbohydrates and some essential amino acids such as arginine, tryptophan and phenylalanine and also it full of beta-carotene and vitamins (Carrillo et. al , 2002). Today, the alga industry is highly developed so that algae as fertilizer due to their fiber plays an important role in softening the soil and also due to their micronutrients play an important role in plant nutrition. Other applications of algae are in the diet of livestock and poultry (reported study phase, 2007). It seems that there is a high imbalance of minerals and heavy metals in some species of seaweed that makes a difficulty in the possibility of their use in animal feeding. Therefore, determining the most appropriate level of algae is important to avoid poisoning. The goal of this study is to determine the most appropriate level of *Sargassum Angustifolium* in feeding of animals and its effect on the general health of native kids in Bushehr province.

Materials and methods

Materials

This study was carried out in the Animal Sciences Research station of Agriculture and Natural Resources Research center, located at 15 kilometers from the Ahrom city. *Sargassum Angustifolium* for using in animal feed had collected in the winter at the southern coast of Bushehr province and then they were dried in the sun and were crushed into small pieces by threshers (mean 2-3 cm).

Materials and methods

After that, The 24 male native kids (Adni breed) were selected with an average live weight of 15.64±3/2 kg and the average age of 5-6 months. A typical diet for a period of 15 days to adapt animals individually in the morning (8:00) and afternoon (14:00) was provided for animals in the appetite level. Fresh water was available and health care operation was performed for animals and their place. They divided into three groups of eight after numbering in the ear. Average initial weight of kids was similar in different treatments. The experiment was performed on some liver enzymes in a 75-days period in order to studying the effects of different levels of *Sargassum Angustifolium* in livestock rations. This experiment has three diets included three different layers of *Sargassum Angustifolium* (0%.10% and 20%) that was written based on NRC (table1). Each diet was assigned to a group of eight animals. Kid's weight was measured weekly before the morning meal. Kid's liver and kidneys were ready in order to studying the macroscopic and microscopic operations in four steps including: day 15, day 25, day 50, and day 75 of experiment. Samples taken from each animal separately put in plastic containers containing 10% formalin and with reports of clinical observations of animals were sent to laboratory diagnosis of the pathology department of Razi Institute. Excel software was used in order to recording and organizing the data. Statistical analysis data were analyzed using SAS (1998) and means were compared by Duncan multiple range test at the 5% level.

As Table 2 has indicated (Comparing the chemical composition of *Sargassum Angustifolium* with alfalfa hay and wheat straw), *Sargassum Angustifolium* regarding to dry matter (DM) is approximately equal to alfalfa hay and wheat straw. Amounts of gross

energy (GE), crude fiber (CF), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) are less than alfalfa hay and wheat straw but they have more ash than alfalfa hay and wheat straw. *Sargassum Angustifolium* regarding to crude protein (CP) is higher than wheat straw and less than alfalfa hay. The measured amounts of minerals, *Sargassum Angustifolium* considering to mercury (Mg), sodium (Na), calcium (Ca) and manganese (Mn) are more than alfalfa hay and wheat straw, but phosphorus (P), copper (Cu), iron (Fe) are less than alfalfa hay and wheat straw. The amount of zinc (Zn) in *Sargassum Angustifolium* is less than alfalfa hay and more than wheat straw.

Results

Microscopic analyses in indigenous kids

Bleeding in the liver parenchyma (white arrow) and zonal necrosis (black arrow) are in microscopic examination of the liver in 10 % of algae treatment in four killing stages (image4).The irregular necrosis of proximal tube, the generation of the basal membrane (asterisk) and the edema among kidney's tubes (black arrow) are found by microscopic examination of the kidney in the treatment of 20 % algae in four killing stages, (image 5). Microscopic examination of the liver in 20 % algae treatment in four killing stages (image 6) shows the hemorrhage in the liver parenchyma (white arrow) and edematous areas (black arrow) parenchyma.

Table 1. Experimental diets fed to kids (shown by percent).

Treatments (%)			
3diet	2diet	1diet	Component
20	10	0	*Sargassum Angustifolium*
20	30	36/5	Wheat straw
14	14	20	Alfalfa hay
14	14	14/5	Wheat bran
30	30	27	Barelyt
1	1	1	Salt
1	1	1	Vitamin and mineral supplements
100	100	100	Total
			The chemical composition
1530	1530	1530	Metabolizable energy(k cal / kg)
75/3	75/3	75/3	Protein (g / kg)
0/67	0/43	0/38	Ca (%)
0/25	0/25	0/32	P (%)

Table 2. Comparing the chemical composition of Sargassum Angustifolium with alfalfa hay and wheat straw (٪).

DM %	GE cal/g	CP %	Mg %	CF %	Na %	Ash %	EE %	NDF %	ADF %	Ca %	P %	Mn Ppm	Cu Ppm	Fe Ppm	Zn Ppm	Analysis Feed
92/62	2316	4/87	1/32	7/1	3/06	41/29	0/3	16/96	14/48	3/57	0/04	64/92	5/69	205	14/75	Alga
93/56	4206	14/57	0/32	31/29	0/1	9/97	1/1	40/81	33/4	1/55	0/22	35/94	14/17	306/7	22/69	Alfalfa hay
94/57	3976	3/22	0/14	40/16	0/05	9/59	0/79	71/7	46/31	0/39	0/08	46/63	6/93	325/93	10/8	Wheat straw

Treatment analysis

According to the microscopic lesions observed in the kidney and liver tissues in three experimental treatments (control , 10 and 20 % algae treatments) , lesions of all three treatments are relatively similar with no significant difference. Therefore, it can be concluded that *Sargassum Angustifolium* up to 20 percent of the diet can't make certain pathological lesions in the kid's liver and kidney tissues.

The results of the microscopic and macroscopic analyses in indigenous kids fed with *Sargassum Angustifolium* in coast of Bushehr province have been shown separately in Tables 3 to 6 and images 1 to 6. There was not found any significant sign indicating

the damage of liver and kidney tissues in four steps of killing. Microscopic examination of kidneys in control treatments (zero percent algae) in four killing stages according to image1 are irregular necrosis of proximal tube. The generation of basal membrane (asterisk), edema between kidney's tubes (black arrow) and bleeding between tubular renal cortexes (white arrow) are visible.

Table 3. The macroscopic analyses of kidney and liver in control, 10 and 20 % algae treatments in the first slaughter (15 days after feeding).

Necropsy observations	Appearance	Experimented organ	Age (month)	Genus	Animal number	Experimental treatments
The heart doesn't have a natural thickener. Other members have no signs or symptoms (normal).	Animal is thin. It had already been treated for diarrhea. Eating food well. Stool is normal. The initial, final and carcass weight are 11.600, 12.300 and 3.500kg, respectively.	Liver and kidney	8 months	Male	56	Control
Lung necrosis is exist and he liver and kidneys are healthy. Heart tissue is soft.	Animal is thin. Appearance is healthy. The initial, final and carcass weight are 8.600, 10.400 and 3kg, respectively.	Liver, heart, lung and kidney	7months	Male	52	
Lung is Abnormal (necrosis). There are symptoms of severe pneumonia. The liver and kidneys are healthy.	Animal is very thin. The initial, final and carcass weight are 10, 12.300 and 3.500kg, respectively.	Liver, lung and kidney	7months	Male	58	10% algae
The lung is severely necrotic. The liver and kidneys are normal.	Livestock is very skinny. It was slaughtered before dying due to infected to Pneumonia. The initial, final and carcass weight are 10.400, 10.600 and 4kg, respectively.	Liver, lung and kidney	6months	Male	60	
Some part of the lung tissue is necrosis. The liver and kidneys are normal.	The animal is skinny. It have the weight decrease rather than 15 previous days	Liver and kidney	7months	Male	61	20% algae
The lung, liver and kidneys are normal	The animal has good appearance. The initial, final and carcass weight are 10.800, 13.200 and 5kg, respectively.	Liver and kidneys	7months	Male	55	

Microscopic examination of the liver in control treatments (zero percent algae) in four killing stages, according to image 2, edematous areas in the liver parenchyma have been presented (black arrows). Microscopic examination of the kidneys of 10 % algae treatments in four killings (image 3), degeneration of basal membrane (asterisk), irregular necrosis of proximal tube , and the tubes renal edema (black arrows) are visible.

Discussion

Although algae are not the main source of energy, they have a high food value in terms of vitamin and protein (Norziah & Ching, 2000). Using the algae as forage was conventional in some European countries such as England, Ireland, Scotland and the Maghreb and some African, American and Asian countries for horses, pigs and sheep. (Mchugh, 2003). Laboratory studies about sheep and seaweed have been limited. Han (1989) pointed out that *ulva lacluca* (Linnaeus) as an animal feed is categorized with high nitrogen and low energy and this alga is a useful supplement for sheep. Al-Shorep et *al* (2001) found an exponential increase in water and urine concentrations of kids after eating 20% and 30% *Macrocystis pyrifra*. The high concentration and misbalancing the minerals was well as presenting the heavy metals in some spices of algae are important

factors for making adverse conditions on feeding. Hence, determining the most appropriate level of feeding for prohibiting poisoning is important (Marin *et al,* 2009). Liver and kidneys are important organs in studying the poisoning with heavy metals. Some heavy metals can make pathological effects on kidney and liver tissues (Telisman *et.al*). Stoycho *et al.* (2003) claimed that copper make significant histopathological effects in the liver, kidneys and lungs. Necrosis , apoptosis , fibrosis , and congestion are symptoms of kidneys resulted by the authors.The study of heavy metals on algae in five areas of the coast of Kuwait showed that these elements were highest in iron, zinc, copper, manganese, nickel, vanadium, and lead and their concentration in these areas had significant differences (Buo- Olayan *et al,* 1996). A study on deposits and six dominant species of algae was done in the coastal of Bushehr city and *Sargassum Vulgar* was introduced as an appropriate indicator of the biological monitoring of lead metal in that area (Qamarzadeh, 2008). Chandra and Manjunaresh (1997) reported that *Sargassum Angustifolium* can make analyses of Tissue Particularly in the brain and liver of mice . The results of this study were not consistent with the results of Chandra and Manjunaresh (1997) because in this study up to 20 percent of the kids were fed by Sargassum algae but there was found no significant pathological effects in macroscopic and microscopic observations on the liver and kidneys. This can be due to the type of animal and algae.

Table 4. The macroscopic analyses of kidney and liver in control, 10 and 20 % algae treatments in the first slaughter (15 days after feeding).

Necropsy observations	Appearance	Experimented organ	Age (month)	Genus	Number	Treatments
The lung, liver and kidneys are normal.	Appearance is healthy. The initial, final and carcass weight are 12, 12.400 and 5.500kg, respectively.	Liver and kidney	8	Male	49	Control
Some part of lung is hepatic. Liver and kidneys are normal.	Animal is normal and eat well. The initial, final and carcass weight are 13, 12.600 and 6.200kg, respectively.	Liver, lung and kidney	8	Male	54	
Liver is normal. Lung has been hepatic. Kidneys sound normal	Animal is normal and eat well. The initial, final and carcass weight are 12.400, 11.600 and 4.400kg, respectively.	Liver and kidney	7	Male	62	10% algae
Some part of lung is hepatic. Liver and kidneys are normal	Animal is normal. The initial, final and carcass weight are 15.800, 14.600 and 7.200kg, respectively.	Liver, lung and kidney	9	Male	48	
Specific symptoms were not observed in all tissues and all of them have normal consistency.	The appearance is good and big spots. The initial, final and carcass weight are 8.600, 10.400 and 3kg, respectively	Liver and kidney	8	Male	53	20% algae
The liver and kidneys look normal.	The appearance is good and it has been improved than past. The initial, final and carcass weight are 15, 12.400 and 5.800kg, respectively	Liver, lung and kidney	9	Male	63	

A study in order to test the effect of brown Sargassum algae on renal interstitial fibrosis of kidneys induced by unilateral ureteral obstruction in rats was performed. Results showed that Sargassum algae can improve unilateral ureteral obstruction induced (Lei Zuoxi *et al,* 2005). The results of this study were consistent with the results obtained by Lei Zuoxi *et al* (2005), because in this study, to 20 percent of Sargassum algae were fed the kids but there was found no significant pathological effects on macroscopic and microscopic. This value can be due

to the similarity of two cited studies according to using same algae.

Table 5. The macroscopic analyses of kidney and liver in control, 10 and 20 % algae treatments in the first slaughter (15 days after feeding).

Necropsy observations	Appearance	Experimented organ	Age (month)	Genus	Animal number	Treatments
There hasn't found any special symptom. The little effect of pneumonia are observed on the liver.	The appearance is healthy. The initial, final and carcass weight are 14, 17.600 and 7.500kg, respectively.	Liver and kidney	10	Male	51	Control
The liver and kidneys are healthy.	The animal is completely healthy. The initial, final and carcass weight are 9.200, 10.400 and 4.800kg, respectively.	Liver and kidney	10	Male	66	
The pneumonia effects has been shown on lung. Some part of lung was hepatic	The appearance is good. The initial, final and carcass weight are 10.800, 12 and 5.400kg, respectively.	Liver, lung and kidney	10	Male	57	10% algae
All organs were healthy and there was found no special symptom	The appearance sounds healthy. The initial, final and carcass weight are 12.600, 14.200 and 6.100kg, respectively.	Liver and kidney	10	Male	67	
The liver and kidneys are completely healthy.	The physical body is healthy. The initial, final and carcass weight are 12.800, 11.600 and 5.300kg, respectively.	Liver and kidney	10	Male	59	20% algae
The liver and kidneys are completely normal.	The appearance is healthy. The initial, final and carcass weight are 11.400, 13.600 and 5.900kg, respectively.	Liver and kidney	10	Male	50	

Table 6 . The macroscopic analyses of kidney and liver in control, 10 and 20 % algae treatments in the first slaughter (15 days after feeding).

Necropsy observations	Appearance	Experimented organ	Age (month)	Genus	Number	Treatments
The liver and kidneys are healthy	The physical body is healthy. The initial, final and carcass weight are 10.800, 12.600 and 4.800kg, respectively.	Liver and kidney	11	Male	70	Control
There hasn't found any special symptom	The animal is completely healthy. The initial, final and carcass weight are 12.600, 13.600 and 5.800kg, respectively.	Liver, lung and kidney	11	Male	71	
The liver and kidneys are healthy	The appearance is good. The initial, final and carcass weight are 15.200, 15.800 and 6.500kg, respectively.	Liver and kidney	11	Male	68	10% algae
All organs were healthy and there was observed no symptom	The appearance is good. The initial, final and carcass weight are 11.800, 15.400 and 6.100kg, respectively.	Liver and kidney	11	Male	69	
The liver and kidneys are completely normal	The appearance sounds good. The initial, final and carcass weight are 12.200, 13.400 and 6.700kg, respectively.	Liver and kidney	11	Male	65	20% algae
The liver and kidneys are completely normal and healthy	The animal is completely healthy. The initial, final and carcass weight are 12.200, 15.600 and 6.100kg, respectively.	Liver and kidney	11	Male	64	

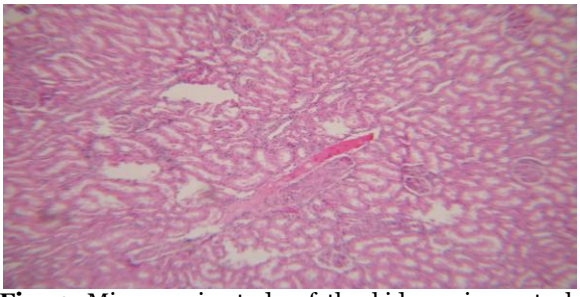

Fig. 1. Microscopic study of the kidneys in control treatments (zero percent algae) in four stages of killing (the dying of Hematoxylin & eosin × 100).

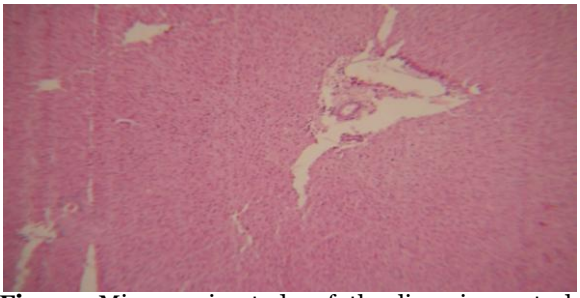

Fig. 2. Microscopic study of the liver in control treatments (zero percent algae) in four stages of killing (the coloration of Hematoxylin & eosin × 100).

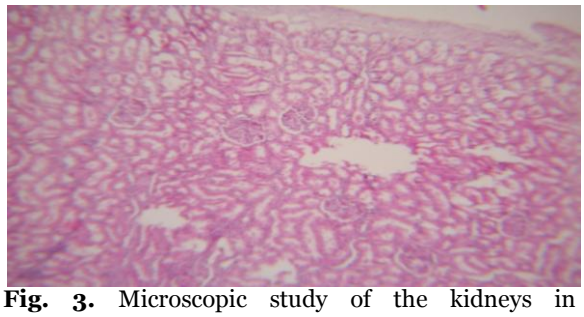

Fig. 3. Microscopic study of the kidneys in treatments of 10% algae in four stages of killing (the coloration of Hematoxylin & eosin × 100).

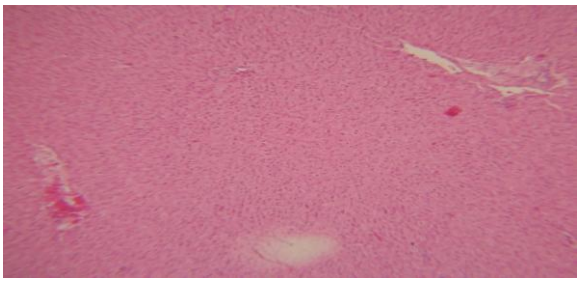

Fig. 4. Microscopic study of the liver in treatments of 10% algae in four stages of killing (the coloration of Hematoxylin & eosin × 100).

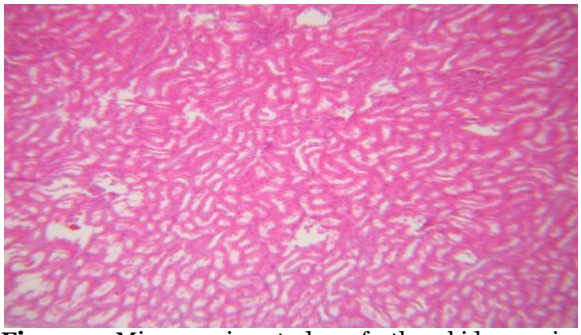

Fig. 5. Microscopic study of the kidneys in treatments of 20% algae in four stages of killing (the coloration of Hematoxylin & eosin × 100).

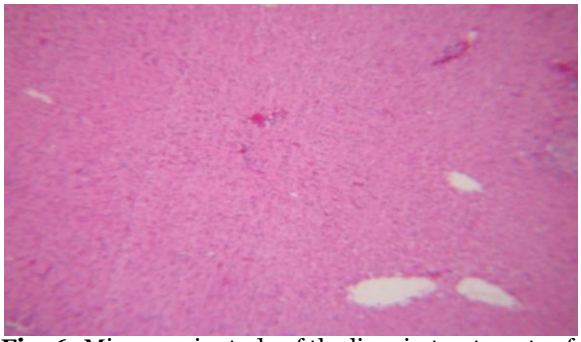

Fig. 6. Microscopic study of the liver in treatments of 20% algae in four stages of killing (The coloration of Hematoxylin & eosin × 100).

Conclusion

As there was shown in the results of this study, the use of *Sargassum Angustifolium* with used doses (10 and 20 percent), according to heavier elements in it, it didn't have any harmful effects on the kid's liver and kidneys. Consequently, this food supply can be used as an important option in drought conditions, high prices and scarcity of forage up to 20 percent for the diet of kids. In the bais of this results author suggested: I. Because our country, Iran, is facing a shortage of forage and food, algae can, II. Higher percentage of algae can be replaced in the diet in order to find its effects on different characteristics, III. In addition to use this huge supply of food for livestock; it can be used for poultry and fish widely, IV. Other spices of algae will be tested in diet of different animals like sheep and cow. V. Other spices of algae will be tested in diet of different animals like sheep and cow. VI. Another study will be performed about using the algae in diet and measuring the heavy metals in different organs such as kidney and liver.

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