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THE EFFECT OF NITRATE IN DRINKING WATER ON THE LIVER FUNCTION IN LAYING HENS

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SUMMARY

Twenty weeks old Euribrid-Hisex Brown laying hens were used to st udy the effect of water pollution with nitrate on the liver function in these animals. Nitrate treatment caused an increase in total serum albumin and decreased total serum globulin and cholesterol. The activity of serum GOT and GPT enzymes were not affected by the treatment with nitrate. The results indicate that nitrate did not seem to have a negative influence on the liver function, but, it appears that nitrate might depress the immune system in these animals particularly during the early stages of exposure to the pollution. The results also indicate that laying hens are capable of adapting to high doses of nitrate in drinking water.

INTRODUCTION

The problem of environment pollution with chemical wastes is of major concern all over the world and many studies have been carried out illustrate the hazards of chemical pollutants on man, animal and plant. One of these pollutants is the nitrate ion which has been reported to be increasingly rising in level and affecting water of the rivers[1]. The sources of nitrate are sewage and industrial wastes as well as nitrogen fertilizers. In recent years, the use of nitrogen fertilizers has increased extensively as a mean of increasing the plant production. In addition, nitrate is also used as a preservative for canned meat which might be a threat to human health[2]. Nitrate becomes toxic when it is reduced to nitrite by the bacteria in the intestines of man and animal[3,4]. Nitrite is then quickly absorbed into the blood stream and oxidizes the hemoglobine to methemoglobine causing methemoglobinemia and consequently the reduction of the oxygen arriving to the tissues[4].

Local studies have shown that the rivers of Iraq are polluted with different levels of nitrate[5], and recent investigations have demonstrated that nitrate in drinking water influenced the blood picture[6], decreased the thyroid gland hormones secretion[7], and increased albumin and cholesterol contents of the egg[8]. The liver is the major site for albumin and cholesterol synthesis which might participate in the albumin and cholesterol contents of the egg. The present study was designed to investigate the effect of high levels of nitrate in drinking water on the liver function of laying hens.Total serum protein, albumin, globulin and cholesterol concentrations together with the activities of serum glutamic-oxalacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT) were measured and used as indicators for liver function.

MATERIALS AND METHODS

Twenty eight laying hens (twenty weeks old, Euribrid-Hisex Brown) were divided into four equal groups, and each bird was housed in an individual cage. Standard conditions (temperature, humidity... etc.) required for laying hens were provided for the birds which also had free access to food and water throughout the experiment.

The birds were allowed two weeks for adaptation before the commencement of the treatments and results were considered as the

pretreatment control. At the end of the adaptation period the groups were treated as follows:-

one group was given ordinary tap water and considered as the control (C) group. The second group received tap water containing 5000 ppm sodium nitrate and was designated as (T_1) group. The third group received tap water containing 7500 ppm sod. nitrate and was designated as (T_2) group. the fourth group received tap water containing 10000 ppm so. nitrate and was designated as (T_3) group.

The treatments continued for six weeks. Blood samples were collected once during the pretreatment period, and every other week during the treatment period. Blood was obtained from the wing vien and the sera were removed and analyzed for protein fractions and cholesterol concentrations as well as SGOT and SGPT activities.

Total serum protein (TSP) was determined by the Biuret Method described by *Wotton*[9], and total serum albumin (TSA) by the Bromocresol Green method described by *Tietz*[10]. Total serum globulin (TSG) was then calculated as the difference between (TSP) and (TSA). The method of Reitman and *Frankel*[11] was used to determine the (SGOT) and (SGPT) activities. Total serum cholesterol (TSC) was determined using the method described by *Franey* and *Elias*[12].

Data were analyzed by the Two-Way Analysis of Variance and Least Significant Difference tests to examine the significance of the differences in the measured parameters.

RESULTS

Throughout the experiment, birds did not show changes in behaviour or signs of illness. However, groups of the nitrate treated animals consumed more water and their droopings were soft and then watery, as the treatment progressed, compared with the pretreatment period and the control.

Total Serum Protein (TSP):

Results in table (1) show that means of total serum protein in all groups during the pretreatment period were close to each other. The addition of nitrate to drinking water caused a significant decrease in (TSP) of all nitrate treated groups as compared with the control. Table-1 also illustrates that the

(TSP) concentrations continued to be lower in the nitrate treated groups up to the 4th week of the treatment period as compared with the pretreatment period. Most of these differences were statistically significant. It was also noticed that by the 6th week of the treatment, the (TSP) concentrations in all treated groups returned to and exceeded the pretreatment values. Total serum protein concentration in the control group, on the other hand, showed limited and nonsignificant changes throughout the experiment.

Total Serum Albumin (TSA):

During the pretreatment period, the concentrations of total serum albumin were quite similar in all groups (Table 2). On the other hand, there was a general tendency for the (TSA) concentrations to increase in the nitrate treated groups. Some of the increments were statistically significant as compared with the control group. It was also observed that changes in (TSA) concentrations were not proportional to the supplemented levels of nitrate. Table-2 demonstrates that there were also tendencies for progressive inceases, with time, in the (TSA) concentrations of the nitrate treated groups. The inceases reached statistical significance in (T_1)and (T_3) groups at the 6th week of the treatment period as compared with pretreatment values. Negligible variations occured in the (TSA) values of the control group during the experiment.

Total Serum Globulin (TSG):

Results showed that animals of the four groups had close (TSG) concentration values during the pretreatment period (Table 3). The addition of nitrate to drinking water resulted in a statistically significant decrease in the (TSG) values of all treated groups as compared with the control. Total serum globulin values persisted to be lower in the treated groups than those of the pretreatment period with the exception of the (TSG) value of (T_3) group which returned to pretreatment level at the 6th week of the experiment. The (TSG) concentration values of the control group showed slight nonsignificant increase during the experiment.

Table 1: Total serum protein concentration (g%) in laying hens treated with different nitrate levels in drinking water.

PERIOD		GROUPS			
		C	T_1	T_2	T_3
Pretreatment		$_{A}5.04\pm0.23_{a}$	$_{A}4.84\pm0.16_{a}$	$_{A}5.02\pm0.08_{a}$	$_{A}5.16\pm0.21_{a}$
Treat-ment (weeks)	2	$_{A}4.90\pm0.23_{a}$	$_{B}4.32\pm0.09_{b}$	$_{B}4.24\pm0.16_{b}$	$_{B}4.55\pm0.36_{b}$
	4	$_{A}5.04\pm0.27_{a}$	$_{B}4.66\pm0.20_{a}$	$_{BC}4.58\pm0.23_{c}$	$_{ABC}4.86\pm0.36_{ab}$
	6	$_{A}5.20\pm0.18_{a}$	$_{A}5.08\pm0.33_{c}$	$_{A}5.20\pm0.14_{a}$	$_{B}5.78\pm0.50_{c}$

- The values represent means of 7 animals/group ± SEM.
- Different capital letters indicate significant differences ($P<0.05$) between groups.
-Different small letters indicate significant differences ($P<0.05$) within groups.

Table 2: The effect of different nitrate levels in drinking water on total serum albumin concentration (g%) in laying hens

PERIOD		GROUPS			
		C	T_1	T_2	T_3
Pretreatment		$_{A}3.08\pm0.16_{a}$	$_{A}3.04\pm0.12_{a}$	$_{A}3.02\pm0.09_{a}$	$_{A}3.06\pm0.23_{a}$
Treat-ment (weeks)	2	$_{A}2.98\pm0.19_{a}$	$_{A}2.92\pm0.20_{a}$	$_{A}3.02\pm0.10_{a}$	$_{A}3.20\pm0.30_{ab}$
	4	$_{A}2.94\pm0.31_{a}$	$_{AB}3.24\pm0.35_{ab}$	$_{AB}3.48\pm0.12_{a}$	$_{B}3.54\pm0.17_{ac}$
	6	$_{A}3.08\pm0.42_{a}$	$_{B}3.78\pm0.39_{b}$	$_{AB}3.46\pm0.30_{a}$	$_{AB}3.68\pm0.38_{bc}$

- The values represent means of 7 animals/group ± SEM.
- Different capital letters indicate significant differences ($P<0.05$) between groups.
-Different small letters indicate significant differences ($P<0.05$) within groups.

Table 3: The effect of supplementing drinking water with different nitrate levels on total serum globulin concentration (g%) in laying hens.

PERIOD		GROUPS			
		C	T_1	T_2	T_3
Pretreatment		$_{A}1.96\pm0.16_{a}$	$_{A}1.80\pm0.20_{a}$	$_{A}2.00\pm0.13_{a}$	$_{A}2.10\pm0.25_{a}$
Treat-ment (weeks)	2	$_{A}1.92\pm0.40_{a}$	$_{B}1.40\pm0.29_{b}$	$_{B}1.22\pm0.21_{b}$	$_{B}1.35\pm0.39_{b}$
	4	$_{A}2.10\pm0.22_{a}$	$_{B}1.42\pm0.22_{b}$	$_{B}1.10\pm0.14_{b}$	$_{B}1.32\pm0.23_{b}$
	6	$_{A}2.12\pm0.31_{a}$	$_{B}1.30\pm0.16_{b}$	$_{C}1.74\pm0.25_{b}$	$_{A}2.10\pm0.40_{a}$

- The values represent means of 7 animals/group ± SEM.
- Different capital letters indicate significant differences ($P<0.05$) between groups.
-Different small letters indicate significant differences ($P<0.05$) within groups.

Table 4: Serum GOT and GPT activity (IU/L) in laying hens treated with different nitrate levels in drinking water.

PERIOD			GROUPS			
		Enz-yme	C	T_1	T_2	T_3
Pretreatment		GOT	112±3.0	110±5.4	116±4.0	113±7.2
		GPT	34±1.4	35±2.1	32±2.9	33±1.8
Treat-ment (weeks)	2	GOT	112±4.4	115±5.1	114±3.8	109±6.5
		GPT	36±2.2	34±2.7	35±2.7	33±2.4
	4	GOT	118±3.5	109±2.8	110±5.0	115±6.0
		GPT	33±2.0	33±1.6	36±1.5	36±2.8
	6	GOT	108±4.3	113±5.0	115±4.6	111±5.4
		GPT	35±2.7	36±3.0	34±2.3	35±2.6

- The values represent means of 7 animals/group ± SEM.

Serum GOT and Serum GPT:

Table-4 demonstrates that neither the supplemented levels of nitrate, nor the duration of treatment have had any effect on the actvity of these two enzymes in animals sera of all groups.

Total Serum Cholesterol (TSC):

Data of Table- 5 show that there were no significant differences in the (TSC) concentrations between the four groups during the pretreatment period. The table also demonstrates that the (TSC) values decreased in all treated groups as compared with values of the control group and the pretreatment period. The decreases persisted in the treated groups throughout the experiment, whilst the (TSC) values in the control group showed nonsignificant variations.

DISCUSSION

Results of the present study reveal that the addition of nitrate to drinking water increased the (TSA) concentration in the treared group.It is possible to suggest that albumin synthesis was stimulated by nitrate treatment. This stimulation might be attributed to the expected increase in growth hormone. It has been reported that the nitrate treatment had influenced the anterior pituitary gland and increased the concentration of growth hormone in heifers blood[13]. A more recent study showed that hyperalbuminemia in hens was associated with increased growth hormone secretion due to pituitary gland hypertrophy[14]. The observed reduction in (TSP) values during the first four weeks of treatment was probably due to the marked decrease in (TSG) values. The decrease of (TSG) concentration in the nitrate treated groups might indicate that nitrate has a depressing effect on the immune system of laying hens. Therefore, safety precautions are recommended to minimize the losses by diseases in laying hens in areas suspected to be polluted with nitrate. The recovery of the (TSP) concentration values by the 6th week of the treatment period could be due to the adaptation of the laying hens to the supplemented levels of nitrate. Previous studies have demonstrated that sheep[15,16], dogs[17] and Japanese quail[18] could adapt to high levels of nitrate, and that laying hens were more adaptable to nitrate than other poultry[19].

The results showed that the addition of nitrate to drinking water of laying hens did not affect the liver enzymes activity. Furthermore, serum albumin which is synthesized mainly by the liver tissue, was significantly increased in the nitrate treated groups. This might indicate that nitrate treatments had no apparent negative effect on the liver function as far as SGOT and SGPT enzymes are concerned.

The decrease in total serum cholesterol concentration of the nitrate treated groups might be due to a decrease in carbohydrate and fat metabolism. A recent investigation has shown that nitrate treatment depressed the thyroid function in laying hens[7]. Thyroid hormones play a significant role in the metabolism of carbohydrate and fat in various organs of the body. A decrease in carbohydrate and fat metabolism would result in a reduced supply of acetyl coenzyme - A, the building unit of cholesterol, and hence, depressed cholesterol synthesis. this, however, is unlikely to be the main reason for the marked decrease in (TSC), since the metabolism of carbohydrate and fat is also regulated by other hormones. Another possibility, is that the supplemented levels of nitrate might disturb cholesterol intestinal absorption. The observed watery feces of the nitrate treated groups could refer to increased passage rate of the digesta. Consequently, cholesterol absorption was possibly affected, which was then reflected on the serum cholesterol level.

Table 5: The concentration of total serum cholesterol (mg%) in treated and untreared hens with different levels of nitrate supplements in drinking water.

PERIOD		GROUPS			
		C	T_1	T_2	T_3
Pretreatment		$_{A}137\pm3.4_{a}$	$_{A}145\pm5.6_{a}$	$_{A}149\pm5.8_{a}$	$_{A}150\pm7.7_{a}$
Treat-ment (weeks)	2	$_{A}151\pm5.8_{a}$	$_{A}145\pm8.9_{a}$	$_{B}106\pm6.1_{b}$	$_{B}98\pm11.8_{b}$
	4	$_{A}147\pm9.1_{a}$	$_{A}136\pm10.1_{a}$	$_{AB}134\pm8.5_{a}$	$_{B}111\pm9.4_{b}$
	6	$_{A}147\pm6.5_{a}$	$_{B}109\pm6.0_{b}$	$_{B}110\pm3.9_{b}$	$_{B}107\pm8.7_{b}$

- The values represent means of 7 animals/group ± SEM.
- Different capital letters indicate significant differences ($P<0.05$) between groups.
-Different small letters indicate significant differences ($P<0.05$) within groups.

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تأثير النترات في مياه الشرب على وظيفة الكبد في دجاج البيض

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الخلاصة

استخدمت ثمان وعشرون دجاجة من الضروب المستخدمة لانتاج البيض في العراق وبعمر عشرين اسبوعا لدراسة تأثير تلوث مياه الشرب بنسب مختلفه من النترات على فعالية الكبد. ادت اضافة النترات لمياه الشرب الى زيادة في مستوى الالبومين الكلي وانخفاض في مستويات كل من الكلوبيولين والكوليستيرول في مصل الدم. لم تتأثر فعالية انزيمي الكلوتاميك اوكسال اسيتيك ترانساميناز والكلوتاميك بايروفيك ترانساميناز في مصل دم الطيور المعامله بالنترات مقارنة مع مستويات هذين الانزيمين في مجموعة السيطرة . اظهرت نتائج هذه الدراسه عدم وجود تأثير سلبي على فعالية الكبد الوظيفيه ولكن يبدو ان للنترات تأثيرا سلبيا على الجهاز المناعي لهذه الطيور خصوصا في المراحل المبكره من التعرض للتلوث , مما يستوجب اتخاذ الاجراءات الوقائيه اللازمه خلال هذه الفتره لتلافي الخسائر التي قد تنتج عن الاصابه بامراض معدية. اشارت النتائج الى امكانية دجاج البيض على التعود على تناول النترات بالتركيز المضاف الى مياه الشرب