J. Agric. Sci. Mansoura Univ., 25 (1): 149 - 160, 2000.

SOME PHYSIOLOGICAL AND NUTRITIONAL RESPONSES OF BROILER CHICKS TO AN ANXIOLYTIC AGENT ADMINSTRATION DURING SUMMER MONTHS.

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ABSTRACT

This experiment was conducted to study the possible beneficial effects of diazepam, a mild tranquilizer and appetite stimulator drug, on food intake and the physiological homeostasis of broiler chicks exposed to high ambient temperatures of the summer months.

A total of 200, one day old, unsexed Hubbard broiler chicks were used. The chicks were allocated into five equal groups, one of them was kept as control while the others received different gradient levels of diazepam for 6 weeks.

The results indicated that both live body weight and body weight gain at the end of the experiment (6 wk) were significantly higher for birds at 0.5 gm/L of drinking water than the control and the other treatment groups. Changes in live body weight from 0 to 6 weeks of ages were 34.3; 37.7; 43.6; 40.2 and 37.9 folds for the control; 0.25; 0.50; 0.75 and 1.0 g/L doses, respectively.

A significant increase in feed consumption was observed for birds treated by 0.50 gm/L of the drug at different ages. Similar trends were also observed for feed conversion ratio, protein consumption and efficiency of protein utilization in treated groups.

A significant dose dependent increase in T_4 and T_3 levels at 4 and 6 weeks of age was also observed. Plasma total protein; triglycerides; cholesterol; creatinine; GOT and GPT levels were significantly different among treatment groups, except for the lower dose, and the control one.

Rectal temperature was significantly higher in the control and the 0.25 gm/L of diazepam groups than the other groups. Treated birds were also more resistant to the deleterious effects of high ambient temperature, as indicated by their significantly, dose-dependent, lower H/L ratios in blood. Moreover, mortality rate was higher (10%) in the control group than in all treatment groups, where no mortalities occurred.

It could be concluded that, under hot summer conditions, water supplementation of 0.50 gm/L of diazepam is desirable to stimulate eating behaviour of broiler chicks, and to maintain their physiological equilibrium during high ambient temperatures.

Key words: heat stress, feed intake, blood picture, thyriod, growth, broilers,diazepam

INTRODUCTION

The first consequences of heat stress are a reduction in feed intake; lower growth; decreased feed efficiency and enhanced fat deposition (Geraert, *et al*., 1993, 1996). Most studies deal with heat induced hormonal changes and the endocrine control of thermogenesis. Plasma triiodothyronine (T_3) levels appear to decrease, while thyroxine (T_4) concentrations did not change (Klandorf, *et al.,* 1981; Sinurat *et al*., 1987) or even increase (Moss and Balnave, 1978).

In broilers , it has been shown that decreased rate of growth occurs when environmental temperature rises (Howlider and Rose, 1989). This negative effect was probably primarily due to reduced feed intake (Hurwuitz *et al.,* 1980). Moreover, -Zuprazil *et al.,* (1993) found that the true digestibilities of 12 amino acids were generally depressed when fed to broilers subjected to an increasing ambient temperature exposure from 21 to 32 °C. The growth reduction effect of heat stress was not related to feed intake (dale and Fuller, 1980) and, thus would have other origin. Improving feed intake by dietary fat supplementation (Dale and Fuller, 1979) or by force feeding techniques up to 16 above ad lib. Feed intake (Smith and Teeter, 1987) increased weight gain but was accompanied by, enhanced fatness. Geraert et al., (1996) reported that heat exposure appears to have an effect on growth performance independent of the decrease in feed intake. However, the reduction in growth was often greater than the reduction in feed intake.

Recent studies have been directed towards identifying specific brain sites and various neurotransmitters that were involved in food intake regulation (Denbow, 1989). Epinephrine and nor-epinephrine showed a central stimulatory effect on food intake in broilers (Denbow *et al*., 1981) but not in Leghorn (Denbow *et al*., 1983).

On the contrary, serotonin has an inhibitory effect (Denbow et al., 1983). Furthermore, there is evidence that gamma-Aminobutyric acid (GABA), a neurotransmitter, can modulate food intake by opposing the inhibitory effect of serotonin (Morley *et al*., 1985), and may also play a role with angiotinsin II in modulation of drinking behaviour (Swanson and Mogneson, 1981).

Pharmacological methods have been used to control appetite and body weight in broiler breeder birds (Oyawoye and Krueger, 1990). Since, recent advances in understanding the physiological control of feed intake have led to the identification of the potential veterinary products for regulating feed intake and body weight in chickens (El-Halawani *et al*., 1982). Tranquilizers have been suggested for use with poultry because of their stress ameliorating properties with possible beneficial effect on growth and feed efficiency (Huston, 1959; Premachandra and Turner, 1960; Burger and Lorenz, 1960, Kicka, 1973; Kicka and Kamar, 1977; Abdel-Hakim et al., 1986; EL-Habbak and Radwan, 1990). Since, diazepam, a mild tranquilizer, was used to stimulate eating behaviour and body weight of humans (Hoebel *et al*., 1975); pigs (Dantzer, 1976), rats (Stapleton *et al*., 1979) , Japanese quail (Shoukry, 1993), laying hens (Sushil , *et al*. , 1998) , domestic chicks (Marin,*et al*., 1998) and in mice (Rahminiwati and Nishimura, 1999).This drug is classified by Silverstone and Kyriakides . (1982) as anxiolytic agent which potentiates the inhibitory effect of GABA on the serotonergic neurons its receptors are present in both mammals and birds its binding capacity to peripheral tissues are very low compared to brain tissues, and finally it has a prolonged half life (Feldman and Quenzer, 1984). These observations suggest that, diazepam may be functioning as an appetite-stimulating drug for broiler chicks exposed to high ambient temperature stress during the summer months. Since, the percent study was undertaken to provide further information about the physiological and nutritional responses of broilers to diazepam administrations.

MATERIALS AND METHODS

Birds and management:

Total number of 200 Hubbard, one day old, unsexed broilers chicks from a commercial supplier were brooded in floor pens during the period from July, 20 to September, 3/1998. During the first week of age, the brooding temperature was maintained at 35 $\pm$1 ^oC with a constant photoperiod of 24h. feed and water provided, ad libitum, throughout the whole experimental period. Compositions of both the starter and finisher diets are shown in Table1.

The finisher diet was user after 3 weeks of age. In the second week, the chicks were asigned randomly to five treatment groups, 40 chicks each. One group was control that was freely allowed to drink tap water with no additives, and the other four groups were given graded doses (via drinking water) of diazepam (Ratiopharm GmbH Arzneimittle, 7902 Blaubeuren. Germany). The doses were 0.25; 0.50; 0.75 and 1.0 gm/L of drinking water for the four groups, respectively.

Table (1): Composition of the starter and finisher diets.

Ingredients (%)	Starter diet	Finisher diet
Yellow corn	65	74
Soybean meal (44%)	24	15
Herring fish meal	4	3
Corn gluten	4	4.5
Bone meal	1.60	2
Limestone	0.65	0.70
Premix (1)	0.25	0.25
NaCl	0.20	0.25
Lysine	0.14	0.20
DL- Methionine	0.16	0.10
Total	100	100
Calculated analysis		
CP	22.18	19.20
ME(Kcal /Kg)	3018	3110
C/P ratio	137	163
Ca%	1.12	1.10
P %	0.70	0.62
Methionine %	0.62	0.56
Lysine	1.20	1.06
Methionine + Cyst.	0.96	0.73

(1) Broiler permix , each 1 kg contained, Vit. A 12000 IU; Vit D_3 2500 IU; Vit E. 10 mg, Vit . K_3 mg, Vit B_1–1mg, Vit .B_2 . 4 mg; Pantothenic acid 10 mg Folic acid 1 mg, D-biotin 0.5 mg Niacine 40 mg, Vit. B_6–3 mg, Vit. B_{12}–20 meg; Mn-62 mg, Fe–44 mg; Zn–56 mg, Cu-5 mg and Se–100 mg.

Meteorological data:

Indoor ambient air temperature (Max.; Min) and relative humidity (RH) were recorded daily. Average of Max. ,Min. ambient air temperatures and RH during the experimental period were 36.8 ^oC; 27.4 ^oC and 59%, respectively.

Measurements:

Live body weight, weight gain;mortality rate , rectal temperature, feed consumption; feed conversion; protein consumption; and efficiency of protein utilization were determined and calculated in a biweekly basis (2,4 and 6 weeks of age). At 4 and 6 weeks of age, blood samples were withdrawn, via the wing vein, from ten birds of each treatment group. Blood serum was obtained, while EDTA was used as an anticoagulant to obtain plasma samples. All samples were centrifuged at 6000 r.p.m. for 10 min., plasma or serum was then decanted and stored frozen at -20 ºC until analyzed.

Plasma thyroxine (T_4) and triiodothyronine (T_3) were determined by the enzymatic colorimetric method, using available commercial kits (bio Merieux, 69280 Mary-I`Etoile, France). Total plasma protein, triglycerides; cholesterol, creatinine, serum glutamic oxaloacetic transaminase (GOT); and serum glutamic pyruvic transaminase (GPT) were also determined using available commercial kits. A drop of blood/bird was smeared, stained (Wright`s Stain) and then examined for the differential counts of blood leukocytes to calculate the heterophils (H) and lymphocyte (L) numbers and the H/L ratio. At the end of the experiment , six birds from each treatment were randomly taken, sacrificed and examinded macroscopically for evidence of lesions in their livers and internal organs .

Statistical analysis:

Data were subjected to the ANOVA using the SAS General Linear Models procedure (SAS institute, 1994) and to the multiple range test (Duncan, 1995) to test the differences among means.

RESULTS AND DISCUSSION

Live body weight (LBW) and weight gain (WG):

Table (2) shows the effect of diazepam on both LBW and WG of broiler chicks at 2, 4 and 6 weeks of age. It is clear from the results that the higher doses of diazepam (0.75 and 1 gm/L) gave the best LBW and WG of chicks at 2 weeks of age. However, the treated groups have significantly ($P<0.05$) higher body weights at the 4 th and the 6 th weeks of age.

At 4 weeks of age, a significant increase in LBW of birds treated with 0.25 and 0.50 gm/L of the drug was observed. However, at 6 weeks of age, birds that given the 0.50 and 0.75 gm/L doses surpassed the other treatments and recorded significantly ($P<0.05$) the highest LBW. moreover, analysis of variance data (Table 2) revealed highly significant effects of treatment age and their interaction on both LBW and WG.at 4 and 6 weeks of age

The results show also that the body weight gain increased significantly as the dose of diazepam increased. The best values obtained, however, were for birds that treated with 0.50 and 0.75 mg/L doses, respectively. It is of interest to notice that the LBW of all treatment groups increased dramatically from hatch to the marketable age (6 weeks). The percentages of change were about 34.3; 37.7; 43.6; 40.2 and 37.9 folds for the control, 0.25; 0.50; 0.75 and 1 gm/L of diazepam treated groups, respectively.

It appears from these results that diazepam administration (0.50 or 0.75 gm/L) via drinking water improved LBW and WG of broiler chicks exposed to high ambient temperatures. The averages of the maximum and minimum ambient temperature that recorded throughout the experimental period were 36.8 and 27.4 ^{o}C, respectively. Although this temperature was reported to reduce the growth of broilers (Smith and Teeter, 1987; Howlider and Rose, 1989, and Geraert *et al*., 1996), the present results clearly, indicate a pronounced increase in broilers body weight which reflects the importance of diazepam administration during hot summer months.

It seems that diazepam enhances food intake and feed utilization of birds by a direct effect on birds thermobalance during hot weather. It may also exerts an effect on the food regulating centers in the brain either by decreasing serotonin secretion or by a main effect on the brain satiety centers. These results were supported by the findings of many authors in different avian and mammalian species (Kicka, 1973; Hoebel *et al*., 1975; Dantzer, 1977; kicka and Kamar, 1976; Stapleton *et al*., 1979, EL-Halawani *et al.,* 1982 and Marin *et al*., 1998).

Some nutritive responses to diazepam administration:

The dose response of feed consumption and efficiency to diazepam supplement were found to be significantly different during the experimental period. Table (3) shows that feed consumption was significantly ($P<0.05$) higher for the 0.25 and 1.0 gm/L groups than the other treatments and control groups at 2 weeks of age.

These differences were more obvious at 4 and 6 weeks of age, where feed consumption increased significantly ($P<0.05$) for the 0.50; 0.75 and 1.0 gm/L of drinking water groups.

At doses of 0.50 and 0.75 gm/L of drinking water, diazepam significantly improved feed efficiency of birds at 4 and 6 weeks of age. There were significant differences among the treated and the control groups, since the ANOVA data revealed a highly significant ($P<0.01$) effect of treatment, age and their interaction on all nutritional traits studied.

Similar trends were also observed for protein consumption and utilization, which may suggest that diazepam enhances the digestibility and /or absorption of dietary proteins.

Although the efficiency of protein utilization was reported to be decreased (Zuprazil *et al*., 1993) in broilers reared at high ambient temperatures, the results obtained herein indicated that diazepam can alleviate these deleterious effects. It seems that diazepam administration at 0.50 or 0.75 gm/L dose would protect birds against the anorectic effects due to heat exposure, by stimulating the eating behaviour of chickens.

That diazepam improved feed consumption and subsequently the birds performance may be due to its inhibitory effect on the serotonergic neurons in the higher brain centers, and/or its direct or indirect effect on the thyriod releasing hormone (TRH) secreting nucleus in the hypothalamus. It has been reported that diazepam potentiates GABA which in turn antagonize

the inhibitory effect of serotonin on food intake (Stapleton *et al.,* 1979 and Morley *et al*., 1985). Since the results indicate that diazepam effects were neural rather than metabolic.

Moreover, there is evidence that diazepam decreased plasma glucose concentration in birds (Dantzer, 1976; Swanson and Morgenson, 1981; and Shoukry, 1993). Since the role of plasma glucose level on food intake was reported to be mediated through its action on hepatic receptors rather than central via brain receptors.

Such a reduction in plasma glucose may cause the bird to respond by increasing food intake to obtain or to compensate for its energy requirements In agreement with the present results were the findings of Denbow, *et al.,* (1983); Denbow (1984 and 1989); Gonzalez *et al*. (1984); Ida, *et al*., (1985); Klandorf and Harvey (1985); Oyawoye and Krueger (1990) and others.

Effects of diazepam on some blood constituents:

Plasma levels of T_4 , T_3 and the T_3/T_4 ratio in both the control and diazepam supplemented groups at 4 and 6 weeks of age are presented in Table (4). It is clear that T_4 level increased significantly ($P<0.05$)in birds at the 0.50 and 0.75 mg/L groups, respectivelyThis increase reflects a stimulating effect of the drug on thyroid activity. A similar trend was also observed for T_3 levels at 4 and 6 weeks of age. The results reveal that the drug administration would be beneficial in maintaining an euthyriod status for birds exposed to high ambient temperature, which was around 36.8 ^oC during this experiment. Since, the depressing effect of an ambient temperature above 29^{o}C on the thyroid activity could be alleviated by diazepam administration.

Most studies deal with heat induced hypothyriodism in broilers which improved the physiological adaptation of birds to heat stress by the modulation of metabolic rates (Klandorf *et al*., 1981; Sinurat *et al*., 1987 and Geraert, *et al.*, 1996). Although the results of Shoukry (1993) indicate that plasma T_4 decreased by a single dose of diazepam injection (in crop) in Japanese quail, no such decrease was observed in the present study. This may be due to the different magnitude and duration of the drug administration and also to the different avian species used.

Concerning plasma total protein (TP) level, the data indicate a significant increase in TP of birds treated with 0.25; 0.50 and 0.75 mg/L of the drug than those treated with 1.01 gm/L and the control group. In addition, a significant dose dependent increase in plasma triglycerides; cholesterol and creatinine levels were also observed. these results indicate considerable changes in the nutrients metabolism as a consequence of the drug supplementation. This may also indicate that diazepam can affect the lipolytic action of the adrenergic neurons. Diazepam administration has been reported to affect the α – adrenergic receptors and lipid and protein mobilization and degradation in birds which confirms the results the present study (Denbow, *etal*., 1981; Colasanti, 1982 ,Feldman and Quenzer, 1984).,and Sushil , *et al.*, 1998).

There were also significant differences in the serum GOT and GPT among the control and the treated groups. A dose-dependent increases in

both enzymes were observed at 4 and 6 weeks of age. The increased GOT and GPT levels may be attributed to the effect of diazepam on liver function.

This was evident as some birds of the higher doses groups of the drug (above 0.5mg/L) show signs (at autopsy) of liver subcutaneous blood hematomases, and hypertrophy of liver spleen and the right kidney. These signs, however, did not cause any mortality losses in all the treated groups, although the mortality rate of the control group was higher (10%) as a result of heat stress emposed during the experimental period.

Table (4) shows also that the drug administration improved the resistance of birds to heat stress as indicated by the lower H/L ratio obtained for groups treated with doses of 0.50; 0.75 and 1 gm/L of diazepam at 4 and 6 weeks of age. This effect was also accompanied by a lower rectal temperature of birds in the higher doses groups. The desirable effects of the drug in decreasing both the H/L ratio and RT may suggest the drug administration during the summer months where the air temperature rises. It is also suggested that H/L ratio may be used as a criterion to measure the resistance of birds to stress conditions. This was supported by the findings of Zulkifli and Siegel (1995) who reported that an H/L ratio lower than 0.5 is a good indicator that the bird is more heat tolerant.

It is concluded, from the present results, that diazepam administration at 0.50 gm/L of drinking water during the hot summer months would be a suitable appetite stimulator and a growth enhancing agent for broilers. It would be suggested to use diazepam in the drinking water of broilers to maintain their physiological equilibrium and productive performance under stressful conditions. The role, that the drug plays at the brain receptors and opioid peptides need, however, further investigations.

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الإستجابة الفسيولوجية والغذائية عند معاملة بدارى اللحم بأحد المواد المهدئة خلال شهور الصيف

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أجريت هذه التجربة لدراسة التأثيرات الايجابية المحتملة عند إضافة أحد المواد المهدئة الخفيفة والتى لها تأثير منشط لإستهلاك الغذاء فى مياه الشرب عند تربية بدارى اللحم خلال شهور الصيف الحارة واستخدم فى التجربة عدد ٢٠٠ من كتاكيت الهبرد حيث قسمت إلى خمسة مجاميع إحداها للمقارنة والباقى حسب الجرعة المعطاة وهى ٠,٢٥، ٠,٥٠، ٠,٧٥ و١جرام لكل لتر من ماء الشرب.

- أتضح من النتائج وجود زيادة معنوية (عند مستوى ٠,٠٥) فى وزن الجسم ومعدل الزيادة فى وزن الجسم للمجموعات التى عوملت بمادة الديازيبام ، كما أتضح أن أفضل الجرعات كانت ٠,٥ يليها ٠,٧٥ جرام/لتر وبصفة عامة فإن تأثير المعاملة كان معنويا جدا (٠,٠١) بالنسبة للزيادة فى وزن الجسم من عمر يوم حتى ٦ أسابيع حيث وجد أن وزن الجسم قد تضاعف خلال تلك الفترة بمعدلات ٣٤,٣ ، ٣٧,٧، ٤٣,٦ ، ٤٠,٢ ثم ٣٧,٩ مرة فى مجموعة المقارنة ثم عند إضافة الديازيبام بجرعات ٠,٢٥ ، ٠,٥ ، ٠,٧٥ ثم ١ جرام/لتر من ماء الشرب على التوالى.
- كان للمعاملة بالديازيبام تأثير معنوى منشط لإستهلاك الغذاءرغم إرتفاع درجة حرارة الجو وذلك فى جميع الأعمار كما أن كفاءة التحويل الغذائى كانت مرتفعة وبصفة خاصة عند مستوى ٠,٥ ، ٠,٧٥ جرام/لتر وبالتالى ازدادت كفاءة الطائر فى معدل استهلاك الغذاء ومعدل الإستفادة منه حيث كان تأثير المعاملات معنوى جدا (مستوى ٠,٠١) ٠
- كان للمعاملات تأثير معنوى مرتفع على خفض درجة حرارة المستقيم وخاصة فى الجرعات العالية (بداية من ٠,٥ جرام/لتر) كما لم يحدث نفوق لأى من كتاكيت المعاملات بينما كانت نسبة النفوق فى المجموعة المقارنة (١٠%).
- بالنسبة لمستوى هرمونات الغدة الدرقية فلقد زاد مستواها معنويا فى الدم بزيادة الجرعة سواء فى عمر ٤ أو ٦ أسابيع كما ازداد مستوى البروتين فى البلازما والجلسيريدات الكلية والكولستيرول والكرياتينين والأنزيمات الناقلة لمجموعات الأمين وذلك بزيادة الجرعة.
- من الناحية التشريحية أوضحت النتائج عدم وجود أى اختلاف فى أعضاء الجسم الهامة سوى بعض الارتشاحات الدموية على الكبد وزيادة وزنه كما وجد فى نسبة قليلة تضخم فى الكلى اليمنى والطحال وكل هذه المظاهر كانت موجودة بنسبة قليلة فى بعض طيور المجموعات التى أعطيت جرعات عالية من الديازيبام (٠,٧٥ ، ١ جرام/لتر).

من النتائج السابقة يتضح أن إضافة هذه المادة إلى مياه الشرب بمعدل ٠,٥ جرام لكل لتر يعطى تأثيرا جيدا عند إرتفاع درجات الحرارة صيفا حيث ينشط عملية استهلاك الغذاء كما يحافظ على الاتزان الفسيولوجى للطائر وبالتالى لا تتأثر عمليات التمثيل الغذائى عند ارتفاع الحرارة صيفا وخاصة فى العنابر المفتوحة، فمن الناحية الفسيولوجية لم يتأثر نشاط الغدة الدرقية وكذلك درجة حرارة الجسم وباقى المقاييس الأخرى بارتفاع درجات الحرارة ٠

Table (2): Body weight and weight gain (WG)in grams of broiler chicks as affected by treatments at different ages.

Age (wk) Dose (g/L)	One day	2	WG((g)	4	WG(g)	6	WG(g)
0	43.2±0.53 ^{a}	310.6±30.2 ^{b}	267..8.3±15.1b	896.3±86.5 ^{b}	586.2±36.6b	1480.2±116.2 ^{d}	583.9±46.6^{e}
0.25	42.6±0.75 ^{a}	303.2±18.8 ^{b}	262.±18.6b	1060.2±104.2 ^{c}	756.4±58.3b	1604.8±131.8 c	546.8±40.2^{c}
0.50	42.8±0.55 ^{a}	318.5±23.4 ^{bc}	277.8±20.8a	1126.0±88.2 ^{a}	810.5±51.3a	1866.5±128.2 a	740.4±50.2a
0.75	43.0±0.64 ^{a}	330.1±29.8 ^{a}	286.3±19.7a	968.8±65.8 ^{b}	647.8±41.2c	1730.3±139.8 ^{b}	761.2±67.4a
1.0	42.8±0.66 ^{a}	321.7±16.6 ^{ac}	278.81±19.8a	987.3±80.6 ^{b}	666.5±44.6c	1620.4±108.5 ^{c}	633.2±41.6b
Significance of effects (n = 200)							
Treatment (T)	NS	*	*	**	***	***	**
Age (A)	NS	**	*	***	**	**	**
T × A	NS	NS	NS	**	**	**	**

1* P≤ 0.05 ; **P≤ 0.01; *** P≤ 0.001; NS – not significant a, b,… Means with different superscripts within a column are significantly different (P≤0.05)

Table (3): Effect of treatments on feed consumption (FC gm) feed efficiency (FE *, gm feed / gm gain); Protein consumption(PC) and efficiency of protein utilization (EPU; gm protein / gm gain) of chicks at 2,4, and 6 weeks **of age .

Age (wk)	2				4				6			
Dose(9/L)	FC9	FE	PC9	EPU	FC9	FE	PC9	EPU	FC9	FE	PC9	EPU
0	350±28^{b}	1.12±0.5^{b}	77±9.2^{b}	0.29±0.03^{b}	1318±64^{c}	1.47±0.15^{b}	290±21^{b}	0.49±0.13^{b}	3285±82^{d}	2.23±0.09^{c}	690±43^{d}	1.31±0.20^{b}
0.25	371±22^{a}	1.23±0.09^{a}	81.8±8.6^{a}	0.32±0.06^{a}	1420±53^{b}	1.30±0.12^{a}	312±18^{a}	0.41±0.09^{a}	3461±89^{c}	2.14±0.12^{b}	727±38^{c}	1.35±0.18^{c}
0.50	366±18^{ab}	1.15±0.10 ^{a}	80.6±10^{ac}	0.29±0.09^{b}	1481±48^{a}	1.28±0.09^{a}	326±24^{a}	0.40±0.14^{a}	3660±101^{a}	1.96±0.08^{a}	769±56^{a}	1.03±0.12^{a}
0.75	355±26^{b}	1.07±0.06^{b}	78.5±11.2^{bc}	0.27±0.10^{b}	1340±66^{c}	1.38±0.14^{a}	295±20^{b}	0.46±0.12^{b}	3559±74^{b}	2.05±0.14^{b}	747±62^{b}	0.98±0.20^{a}
1.0	380±26^{a}	1.18+0.12^{a}	83.6±8.5^{a}	0.29±0.08^{b}	1375±58^{c}	1.40±0.12^{b}	303±27^{b}	0.48±0.12^{b}	3491±93b	2.15±0.12^{b}	733±42^{c}	12.6±0.21^{b}
ANOVA Significance of effects												
Treatment (T)	*	*	*	*	**	*	*	*	**	**	*	**
Age (A)	NS	**	*	*	**	**	**	*	**	*	**	*
T×A	NS	*	*	*	**	*	*	NS	**	*	*	*

a-c : Means within a column having different superscripts are significantly different (P≤ 0.05)

Table (4): Effect of diazepam administration on some blood parameters at 4 and 6 weeks of age.

Dose	0		0.25		0.50		0.75		1.0	
Item	**4wk**	**6**	**4**	**6**	**4**	**6**	**4**	**6**	**4**	**6**
T4*	1.26 ± 106^{a}	14.2 ± 2.2^{b}	12.9 ± 1.4^{b}	15.4 ± 1.8^{a}	20.9 ± 2.4^{a}	20.5 ± 3.4^{a}	17.5 ± 3.2^{a}	18.7 ± 3.5^{a}	10.9 ± 4.2^{b}	15.9 ± 2.8^{b}
T3*	$3.9\pm.91^{a}$	2.8 ± 1.2^{b}	4.2 ± 1.0^{a}	3.2 ± 1.2^{b}	$4.4\pm.9^{a}$	4.2 ± 1.3^{a}	3.7 ± 1.2^{b}	4.2 ± 1.2^{a}	3.7 ± 1.7^{b}	3.9 ± 1.4^{a}
T3/T4	$0.30\pm.06^{a}$	$.19\pm.03^{a}$	$.32\pm.08^{a}$	$.20\pm.04^{b}$	$.12\pm.06^{b}$	$.20\pm.05^{b}$	$.22\pm.03^{a}$	$.21\pm.05^{ab}$	$.35\pm.02^{b}$	$.24\pm.06^{a}$
T.Protein**	4.2 ± 1.0^{a}	3.9 ± 1.01^{ab}	$4.2\pm.93^{a}$	$4.01\pm.9^{ab}$	4.7 ± 1.0^{a}	4.9 ± 1.1^{a}	4.7 ± 1.2^{a}	4.4 ± 1.1^{a}	3.9 ± 1.1^{b}	3.7 ± 1.3^{b}
Triglyc.***	320.6 ± 52.8^{b}	380.8 ± 46.6^{b}	375.6 ± 44.6^{a}	396.0 ± 60.2^{b}	430.5 ± 56.4^{a}	465.1 ± 70.0^{a}	395.6 ± 62.2^{a}	474.2 ± 73.2^{a}	418.8 ± 74.3^{b}	484.5 ± 81.2^{a}
Cholest.***	82.5 ± 18.8^{ab}	91.6 ± 15.4^{c}	100.6 ± 16.8^{a}	94.5 ± 20.1^{c}	125.8 ± 23.2^{b}	134.8 ± 22.5^{b}	118.9 ± 19.8^{b}	142.6 ± 29.2^{a}	102.5 ± 21.0^{a}	198 ± 25.2^{a}
Creatinine***	$.64\pm.08^{b}$	$.72\pm.11^{c}$	$.71\pm.12^{b}$	$.77\pm.14^{c}$	$.86\pm.09^{a}$	$.88\pm.16^{b}$	$.85\pm.16^{b}$	$.98\pm.2^{b}$	$1.1\pm.32^{b}$	$1.26\pm.28^{a}$
GOT****	60.1 ± 9.2^{b}	61.8 ± 11.3^{c}	78.3 ± 10.5^{a}	81.8 ± 12.4^{c}	92.2 ± 18.6^{a}	98.5 ± 13.1^{b}	96.4 ± 16.2^{a}	101.4 ± 19.3^{b}	106.8 ± 21.8^{b}	126.5 ± 20.8^{a}
GPT****	7.8 ± 1.4^{a}	$10.2\pm.94^{c}$	10.8 ± 1.1^{ab}	12.0 ± 1.2^{c}	22.3 ± 2.6^{b}	29.9 ± 3.2^{b}	18.9 ± 1.2^{b}	31.4 ± 3.3^{b}	32.7 ± 3.6^{b}	43.5 ± 4.9^{a}
H/L ratio	$.46\pm.02^{a}$	$.68\pm.09^{a}$	$.53\pm.1^{a}$	$.58\pm.06^{b}$	$.50\pm.12^{a}$	$.37\pm.08^{c}$	$.35\pm.09^{b}$	$.35\pm.12^{c}$	$.31\pm.05^{b}$	$.24\pm.06^{b}$
RT(°C.)	$41.3\pm.08^{a}$	$42.8\pm.08^{a}$	$41.2\pm.07^{a}$	$42.3\pm.12^{a}$	$41.2\pm.10^{a}$	$41.5\pm.06^{b}$	$40.8\pm.13^{b}$	$41.2\pm.09^{b}$	$40.5\pm.11^{b}$	$41.03\pm.08^{b}$

a-d, Means with different superscripts within a row in the same ages are significantly different ($P \leq .05$)

*** (ng/ml)   ** (g/100ml)   *** (mg/dl)   **** (μ/l)**