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EFFECT OF PROLONGED ADMINISTRATION OF ESTROGEN ON SOME BLOOD COAGULATION FACTORS IN RATS

(With 7 Tables)

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تأثير اعطاء هورمون الاستروجين لمدد طويلة على بعض عوامل تجلط الدم في الفئران

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استهدفت هذه الدراسة الراهنة استبيان تأثير اعطاء هرمون الاستروجين لمدة طويلة على معايير تجلط الدم وتشمل زمن الثرمبوبلاستين الجزئي المنشط، تركيز الفيبرينوجين ، البروثرمبين الثاني، عامل تجلط الدم الثامن ، عامل تجلط الدم التاسع ، عامل تجلط الدم العاشر. وقد أوضحت الدراسةالنتائج التالية نقص في زمن الثرومبوبلاستين الجزئي المنشط بعد معالجة الفئران بالاستراديول ـ جاوات عند الأيام (٢١، ٢٨، ٣٥ في الاناث السليمة المعالجة. عند الأيام ١٤، (٢١، ٣٥ في الاناث المعالجة المستأصلة المبيض. وبعد ٣٥ يوم في الذكور. وكذلك عند الإيام (٢١، ٢٨، ٣٥ في الذكور الخصية السليمة. كذلك لوحظ ارتفاع في تركيز الفيبرينوجين بعد معالجة الفئران بالاستراديول ـ جاوات الايام : ٧،(٢١، ٢٨،٣٥ في الاناث السليمة المعالجة. الأيام ١٤، (٢١، ٢٨، ٣٥ في الاناث المستأصلة المبيض. الايام ٢٨، ٣٥ في الذكور السليمة المعالجة. والأيام ٧، ١٤، (٢١، ٢٨، ٣٥ في الذكور الخصية السليمة. كما أوضحت الدراسة ارتفاع نشاط عامل تجلط الدم الثاني بعد معالجة الفئران بالاستراديول. جاوات في الأيام (٢١، ٢٨، ٣٥ في الاناث السليمة المعالجة. الأيام ١٤، (٢١، ٢٨، ٣٥ في الاناث المستأصلة المبيض. الأيام ١٤، (٢١، ٢٨، ٣٥ في الذكور السليمة المعالجة. الأيام ٧، ١٤، (٢١، ٢٨، ٣٥ في الذكور الخصية السليمة. كما لوحظ ارتفاع نشاط عامل تجلط الدم الثامن بعد معالجة الفئران بالاستراديول ـ جاوات في الايام ١٤، (٢١، ٢٨، ٣٥ للاناث السليمة، الايام (٢١، ٢٨، ٣٥ للاناث المستأصلة المبيض. والايام ١٤، (٢١، ٢٨، ٣٥ للذكور السليمة والخصية. وأظهرت الدراسة ازدياد نشاط عامل تجلط الدم التاسع بعد معالجة الفئران بالاستراديول جاوات الأيام ١٤، (٢١، ٢٨، ٣٥ لاناث الفئران السليمة والمستأصلة المبيض. والايام ٧، ١٤، (٢١، ٢٨، ٣٥ لذكور الفئران السليمة والخصية. وأيضا ازداد نشاط عامل تجلط الدم العاشر بعد معالجة الفئران بالاستراديول ـ جاوات في الأيام ١٤، (٢١، ٢٨، ٣٥ للاناث السليمة المعالجة. الأيام ٧، ١٤، (٢١، ٢٨ ، ٣٥، للاناث المستأصلة المبيض والايام ١٤ ، (٢١، ٢٨، ٣٥ لذكور الفئران السليمة والخصية.

SUMMARY

The present study was planned to elucidate the influence of long term administration of estradiol benzoate on the blood coagulation parameters including activated partial thromboplastin time, plasma fibrinogen, prothrombin, antihaemophilic factor, christmas factor and stuart factor.

The results revealed the following finding and conclusions:

1- Activated partial thromboplastin time was significantly decreased post-estradiol benzoate administration in intact treated female rats after 21, 28 and 35 days; in ovariectomized treated rats after 14, 21, 28 and 35 days; in intact treated males after 35 days and in castrated treated rats after 21, 28 and 35 days.

2- Plasma fibrinogen concentration was significantly increased post-estradiol benzoate administration in intact treated females at all sampling days; in ovariectomized rats after 14, 21, 28 and 35 days; in intact treated males after 28 and 35 days and in castrated treated males at all sampling days.

3- Plasma prothrombin (factor II) activity was significantly increased post-estrodiol benzoate administration in intact treated females after 21, 28 and 35 days; in ovariectomized treated rats after 14, 21, 28 and 35 days; in intact treated males after 14, 21, 28 and 35 days and in castrated treated rats at all sampling days.

4- Plasma antihaemophilic factor (factor VIII) activity was significantly increased post-estradiol benzoate administration in intact treated females after 14, 21, 28 and 35 days; in ovariectomized treated females after 21, 28 and 35 days; in intact treated males after 14, 21, 28 and 35 days and in castrated treated rats after 14, 21, 28 and 35 days.

5- Plasma Christmas factor (factor IX) activity was significantly increased post-estradiol benzoate administration in intact treated females after 14, 21, 28 and 35 days; in ovariectomized treated females after 14, 21, 28 and 35 days; in intact and castrated treated males at all sampling days.

6- Plasma Stuart factor (factor X) activity was significantly increased post-estradiol benzoate administration in intact treated females after 14, 21, 28 and 35 days; in ovariectomized treated rats at all sampling days; in intact treated males after 14, 21, 28 and 35 days and in castrated treated rats after 14, 21, 28 and 35 days.

INTRODUCTION

Estrogen is an established risk factor in the development of thromboembolic disease, and in the occurance of coronary artery thrombosis as well as other thromboembolic complications.

OWENS and CIMINO (1985) studied the combined effects of orchiectomy and estrogen administration on synthesis of selected hepatic secretory proteins factor II

(prothrombin), factor VII, antithrombin III and plasminogen. They found that pretreatment of castrated male rats with diethylstilbesterol resulted in a significant increase in cumulative synthesis of factor II (65%) and VII (76%) and reduction in antithrombin III (20%) and plasminogen (27%) compared to controls. Estrogen effect on protein synthesis by male rat liver indicates that this organ is estrogen responsive.

PLAMA, et al. (1983) have found that the administration of estrogens increased plasma fibrinogen levels in female rats submitted to tissue injury. Moreover, MARY and AVANELLE (1973), reported that administration of estrogens elevated plasma fibrinogen and prothrombin in intact and ovariectomized rats.

JOLLY, et al. (1977 a) studied the influence of sex on the plasma levels of prothrombin (II), proconvertin (VII), Christmas factor (IX) and Stuart Prower factor (X) in rats offered vit. K deficient diet. He found that male rats respond much more abruptly than female rats in synthesis and release of coagulation factors. He also added that ethynylestradiol, reduces the progressive decline of plasma prothrombin-proconvertin in non supplemented intact male rats, and affects both casterate males and females similarly, limiting the prothrombin-proconvertin decrease to about 13% below control values.

Moreover, UCHIDA, et al. (1985) reported that estrogen protects the rat against manifistation of hypothrombinemia, while androgen enhances vit. K deficiency. Estradiol administration to male rats retarded manifistation of vit. K deficiency syndromes such as increase of prothrombin time and activated part thromboplastic time, decrease of plasma factor VII and prothrombin levels. UCHIDA, et al. (1985), also added that either castration or administration of female rats with testosterone enhanced the manifistation of hypoprothrombinemia and estradiol treatment to castrated females retarded it.

JOLLY, et al. (1977 b) found that estradiol inhances the biosyn thesis of prothrombin, which was investigated by measuring incorporation of amino acids into electrophoretically separable prothrombin. The observed effect is due to prothrombinogenic mechanism.

In rats treated with ethynyl estradiol, activation of clotting factor x was accelerated (EMMS and LEWIS, 1985) and increased factor VII and factor XII and shorting of prothrombin time were reported (GORDON, et al. 1987).

MISHRA (1969) observed a striking increase in the levels of blood clotting factors II (prothrombin), V (proaccelerin), VII (proconvertin) and X (Stuart prower factor) in male and female rabbits receiving oral contraceptives containing estradiol.

In lambs, estrogen-progesterone implants affect the blood coagulation system (AL-BAKER and KEETON, 1982). They reported that fibrinogen concentration and factors II, VII, IX and X activities were increased and that such changes were due

to the estrogenic, rather than progestogenic content of the preparation. The prothrombin time and activated partial thromboplastin time were decreased. They also found that the use of estrogen preparations as growth promoters induced a hypercoagulable state in these ewe lambs.

The fact that oral contraceptives are associated with high levels of certain blood clotting factors was investigated by POLLER, et al. (1968). They found that anticonceptive therapy with ethynyl estradiol in healthy women was followed by shortensed plasma prothrombin time and activated partial thromboplastin time, marked increase in the activity of the antihaemophilic A factor (factor VIII) and a slight increase in proconvertin (factor VII) activity. No significant changes were observed in blood samples collected at intervals during one menstrval cycle in non-treated normal women.

KIM, et al. (1981) reported that using estrogen-containing oral contraceptive pills to women, significantly increase the fibrinogen level, with a significant decrease in the (PT) prothrombin time and APTT time. Antithrombin III levels decreased gradually. Thus, increased estrogen levels appear to induce the so called hypercoagulable state through an increase in the blood coagulation factors and a decrease in antithrombin III, a potent natural inhibitor of activated blood coagulation factors.

The aim of this work is to study the effect of prolonged administration of estrogen on APTT, Fibrinogen; prothrombin; antihaemophilic, Christmas F., and Stuart factor in albino rats.

MATERIAL and METHODS

Seventy-four albino rats at the age of 68 to 80 days, weighing between 180 and 220 g. were used in the present research. The animals were housed separately in cages, each cage contains one group of rats (Table 1) and individual food and water supplies ad libitum.

Some animals were gonadectonized (Table 1) one weeks prior to experimentation to minimize the influence of endogenous estrogen on the blood coagulation. Ovariectomy was performed under light ether anaesthesia through the bilateral flank incision (GORDON, et al. 1985) vaginal smears were taken from all ovariectomized rats prior to assignment to groups. Any animal that did not show diestrus was discarded. The male rats were castrated under light ether anaethesia through bilateral incision (OWENS and CIMINO, 1985).

All treated rats were injected subcutaneously daily with 2 ug estradiol in 0.1 ml olive oil daily at 10 AM for 35 days. Control rats were injected subcutaneous with 0.1 ml oil. Effectiveness of estradiol benzoate treatment was checked by vaginal smears to detect the cornified epithelial cells.

Blood samples were collected 30 minutes before injection by orbital sinus puncture using citrated capillary tubes, under light ether anaesthesia from all experimental groups on days 7, 14, 21, 28 and 35. Blood was collected into 3.8% sodium citrate solution. Immediately, the citrated blood samples were centrifuged at 3000 r.p.m. for 15 minutes and plasma were separated. The collected plasma used for determination of activated partial thromboplastin time, fibrinogen, factor VIII and factor IX activity, factor II and factor X, plasma samples were stored at -20°C untill used.

Coagulation factors were determined using kits. Activated partial thromboplastin time (APTT) was determined according to ZELDIS, et al. (1972), Fibrinogen "Factor I" according to Von-Clauss (1957), Prothrombin "Factor II" according to OWREN (1949), Antihaemophilic F. "Factor VIII" according to BARROW and GRAHAM (1974), Christman F. "Factor IX" according to MANNHALTER, et al. (1984) and Stuart-Prower F. "Factor X" according to DANSON (1961). The bioMerux fibrometer was used in performing the blood coagulation factor.

RESULTS

Concerning the effect of estradiol benzoate administration on the activated partial thromboplastin time in rats; the obtained results (Table 2) showed a significant decrease in intact treated females after 21, 28 and 35 days; in ovariectomized treated rats after 14, 21, 28 and 35 days; in intact treated males after 35 days and in castrated treated males after 21, 28 and 35 days in comparison with that of their respective controls.

Concerning the effect of estradiol benzoate administration on plasma fibrinogen concentration in rats; the results displayed in (Table 3) revealed a significant increase in intact treated females at all sampling days; in ovariectomized rats after 14, 21, 28 and 35 days; in intact males after 28 and 35 days and in castrated males at all sampling days in comparison with that of their respective controls.

The data presented in (Table 4) revealed that, plasma prothrombin activity was significantly increased post-estradiol administration in intact treated females after 21, 28 and 35 days; in ovariectomized treated rats after 14, 21, 28 and 35 days; in intact treated males after 14, 21, 28 and 35 days and in castrated treated rats at all sampling days in comparison with that of their respective controls.

The results displayed in table 5 revealed that plasma antihaemophilic factor (Factor VIII) activity was significantly increased post-estradiol benzoate administration in intact treated females after 21, 28 and 35 days; in ovariectomized treated rats after 14, 21, 28 and 35 days; in intact treated males after 14, 21, 28 and 35 days and in castrated treated rats at all sampling days in comparison with that of their respective controls.

Concerning the effect of administration of estradiol benzoate on plasma Christman factor (factor IX) in rats, the results presented in table 6 showed a significant increase in factor IX activity in intact treated females after 14, 21, 28 and 35 days; in ovaricctomized treated rats after 14, 21, 28 and 35 days; in intact and castrated treated males at all sampling periods in comparison with that of their respective controls.

In the present study, the results displayed in table 7 revealed that treatment of rats with estradiol benzoate resulted in a significant increase in Stuart factor (factor X) in intact treated females after 14, 21, 28 and 35 days; in ovariectomized treated rats at all sampling days; in intact treated males after 14, 21, 28 and 35 days and in castrated treated rats after 14, 21, 28 and 35 days in comparison with that of their respective controls.

DISCUSSION

The decrease in the activated partial thromboplastin time of rats following estradiol benzoate administration might be due to increase of clotting factors involved the intrinsic and extrinsic common pathways of blood coagulation.

AL-BAKER and KEETON (1982) studied the effects of estrogen-progestrone implants on the coagulation system of lambs. Such preparation caused an increased fibrinogen concentration and factores II, VII and IX activities, whereas the prothrombin time and activated partial thromboplastin time were decreased. These changes were due to the estrogenic, rather than theprogestogenic content of the preparation. The effect of estrogen implantation on blood coagulation in ewe lambs indicated that the uses of hormonal growth promotors induced a hypercoagulable state.

The data of plasma firinogen agree with those obtained by MARY and AVANELLE (1973) who found that plasma fibrinogen increased by oral contraceptives containing estrogen substances and by dietary lipids. Our results also agree with KIM, et al. (1981) who concluded that elevated endogenous estradiol resulted in an increased fibrinogen level with a significant positive correlation between estrogen, and fibrinogen level; decreased partial thromboplastin time.

These data coincided with the data of JOLLY, et al. (1977 a) who studied the effect of estrogen on absorption of vitamin K and prothrombin synthesis and activity. They also concluded that estrogen can assist in maintaing plasma prothrombin-proconvertin levels during vitamin K deficiency. Moreover, prothrombin derived from vitamin K depleted animals (dicumarol treatment or dietary) does not have the ability to bind calcium "inactive" in blood coagulation (STENFLO and GANROT, 1973).

The results concerning factor VIII might be due to direct effect of estrogen on protein synthesis by liver with emphasis principal coagulation proteins and on their release "factors I, II, V, VII, VIII, IX, X, XI, XII and XIII" (VILADIU, et al. 1975; MANNI, et al. 1978 and OWENS and CIMNO, 1985). The obtained results of factor IX could be explained in that estrogen may have an indirect mechanism where in estrogen may modify the metabolism of vit. K in rats of both sexes, in turn, would then regulate the blood Christmas factor "factor IX" (JOLLY, et al. 1977 a).

Concerning factor X these findings coincided with the results of (MISHRA, 1969) who found that administration of oral contraceptive, Anovular (norethindrone and ethinylestradiol) in rabbits, resulted in increased activity of factor II, V, VIII and X (Stuart Prower factor). Our results also agree with the data of EMMS and LEWIS (1985) who concluded that blood clotting factor X activity in rats was accelerated following estradiol administration.

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Table (1): Animal groups

Group No.	No. of rats	Groups of rats
1	11	Intact female rats (treated)
2	10	Intact famale rats (control)
3	8	Ovariectomized female rats (treated)
4	8	Ovariectomized female rats (control)
5	12	Intact male rats (treated)
6	8	Intact male rats (control)
7	9	Castrated male rats (treated)
8	8	Castrated male rats (control)

Table (2): Effect of estradiol benzoate administration on activated partial thromboplastin time (seconds) in rats.

Group	Sampling periods (days) 7	14	21	28	35
Intact females (treated)	40.12 ±0.72	39.63 ±0.55	38.01* ±0.53	36.18** ±0.40	35.36** ±0.31
Intact females (control)	40.60 ±0.75	39.90 ±0.89	39.90 ±0.80	39.70 ±0.78	40.40 ±0.75
Ovariectomized females(treated)	40.50 ±1.03	39.38* ±0.86	38.63** ±0.92	37.75** ±0.72	37.50** ±0.78
Ovariectomized females(control)	42.50 ±0.67	42.33 ±0.71	42.67 ±0.61	42.17 ±0.94	42.00 ±0.73
Intact males (treated)	41.10 ±0.95	40.55 ±0.69	40.03 ±0.73	39.13 ±0.46	38.03* ±0.48
Intact males (control)	39.91 ±1.26	39.99 ±1.27	40.98 ±0.88	39.76 ±0.66	40.40 ±1.17
Castrated males (treated)	40.80 ±0.98	40.78 ±0.81	40.20* ±0.57	38.44** ±0.47	38.34** ±0.33
Castrated males (control)	41.33 ±1.08	41.67 ±0.99	41.83 ±0.70	41.50 ±0.72	42.00 ±0.85

± Standard error

* Significantly different from control values at ($P < 0.05$)

** Significantly different from control values at ($P < 0.01$).

Table (3): Effect of estradiol benzoate on plasma fibrinogen concentration (mg/dl) in rats.

Group	Sampling periods (days) 7	14	21	28	35
Intact females (treated)	320.64** ±9.07	336.18** ±10.80	341.00** ±0.32	368.00** ±8.07	378.73** ±6.39
Intact females (control)	291.60 ±5.38	286.80 ±6.31	303.10 ±8.38	290.20 ±8.66	295.30 ±8.64
Ovariectomized females(treated)	307.88 ±9.94	324.00** ±9.10	339.25** ±6.22	357.00** ±10.18	369.75** ±10.32
Ovariectomized females(control)	306.17 ±12.08	283.50 ±8.41	292.00 ±9.32	296.00 ±7.92	306.17 ±12.07
Intact males (treated)	298.25 ±8.62	305.92 ±7.55	315.25 ±7.29	329.17** ±9.63	334.83** ±9.79
Intact males (control)	293.00 ±10.48	298.50 ±8.04	300.25 ±11.88	295.13 ±7.56	301.38 ±9.83
Castrated males (treated)	304.11** ±8.26	319.33* ±13.36	345.33** ±8.19	338.56** ±10.09	353.78** ±8.98
Castrated males (control)	275.00 ±8.06	283.50 ±8.41	287.50 ±7.76	283.50 ±8.41	279.50 ±8.64

± Standard error

* Significantly different from control values at ($P < 0.05$)

** Significantly different from control values at ($P < 0.01$)

Table (4): Effect of estradiol benzoate on Prothrombin % activity in rats:

Group	Sampling period (days) 7	14	21	28	35
Intact females (treated)	86.36 ±1.63	89.01 ±1.94	107.18** ±3.39	112.00** ±3.27	116.90** ±3.86
Intact females (control)	85.20 ±2.60	86.20 ±2.43	86.20 ±2.43	84.10 ±2.20	84.20 ±2.72
Ovariectomized females(treated)	78.50 ±2.82	84.75** ±2.54	90.00** ±3.48	94.00** ±2.99	95.40** ±2.84
Ovariectomized females(control)	72.50 ±2.01	71.00 ±1.90	72.50 ±2.01	72.60 ±3.22	72.50 ±2.01
Intact males (treated)	95.50 ±2.78	98.25* ±2.45	101.16** ±2.53	103.30** ±3.13	110.60** ±3.20
Intact males (control)	91.25 ±2.80	88.87 ±3.63	91.25 ±2.80	88.63 ±3.11	91.25 ±2.80
Castrated males (treated)	87.22* ±2.48	93.44** ±3.78	102.44** ±4.03	105.00** ±3.60	114.89** ±3.15
Castrated males (control)	78.83 ±2.95	75.67 ±2.89	80.50 ±3.20	80.67 ±3.47	75.67 ±2.89

± Standard error

* Significantly different from control values at ($P < 0.05$)

Table (5): Effect of estradiol benzoate on factor VIII % activity in rats.

Group	Sampling period (days) 7	14	21	28	35
Intact females (treated)	97.00 ±0.70	106.00** ±0.87	106.00** ±0.87	109.64** ±0.95	109.64** ±0.95
Intact females (control)	97.00 ±0.77	96.70 ±0.70	97.00 ±0.77	98.20 ±1.11	97.30 ±0.83
Ovariectomized females(treated)	97.38 ±1.32	99.63 ±1.32	106.88** ±1.23	110.13** ±1.03	113.00** ±0.80
Ovariectomized females(control)	98.50 ±1.50	97.50 ±1.63	98.50 ±1.28	98.50 ±1.28	97.00 ±1.10
Intact males (treated)	99.00 ±0.93	105.33** ±1.34	120.33** ±1.04	121.00** ±1.00	122.16** ±0.96
Intact males (control)	98.88 ±1.26	98.13 ±1.13	99.25 ±1.24	79.75 ±1.24	97.38 ±1.05
Castrated males (treated)	100.00 ±0.87	104.89** ±1.14	120.00** ±1.15	121.67** ±1.11	122.89** ±0.99
Castrated males (control)	98.50 ±1.69	97.50 ±0.92	98.50 ±1.28	97.00 ±1.10	99.50 ±1.20

± Standard error

* Significantly different from control values at ($P < 0.05$).

Table (6): Effect of estradiol benzoate on factor IA & activity in rats.

Group	Sampling periods (days) 7	14	21	28	35
Intact females (treated)	100.91 ±0.55	105.91** ±0.64	114.55** ±0.81	117.09** ±0.95	118.27** ±1.19
Intact females (control)	101.10 ±0.57	100.80 ±0.55	100.70 ±0.70	101.10 ±0.71	100.90 ±0.84
Ovariectomized females(treated)	97.50 ±0.73	102.50** ±0.76	110.25** ±0.88	114.00** ±0.85	116.00** ±0.53
Ovariectomized females(control)	96.33 ±0.67	97.33 ±0.95	97.67 ±0.99	97.00 ±0.89	96.67 ±0.61
Intact males (treated)	99.83* ±0.67	103.00** ±0.58	106.50** ±0.87	111.17** ±0.72	112.83** ±0.72
Intact males (control)	97.25 ±0.80	97.25 ±0.70	98.25 ±1.13	98.75 ±1.16	97.75 ±1.19
Castrated males (treated)	100.33** ±0.82	102.56** ±0.67	106.22** ±1.26	112.22** ±0.85	112.89** ±1.01
Castrated males (control)	95.67 ±0.84	96.67 ±0.95	97.00 ±1.03	97.67 ±0.99	97.67 ±0.99

± Standard error

* Significantly different from control values at ($P < 0.05$)

** Signivicantly different from control values at ($P < 0.01$)

Table (7): Effect of estradiol benzoate on factor IA & activity in rats.

Group	Sampling period (days) 7	14	21	28	35
Intact females (treated)	87.00 ±1.40	92.73** ±1.62	106.64** ±1.87	114.36** ±2.47	126.73** ±3.54
Intact females (control)	87.00 ±1.47	85.20 ±1.28	85.20 ±1.56	83.40 ±0.98	84.00 ±1.00
Ovariectomized females(treated)	87.75* ±1.77	91.75** ±2.02	103.75** ±3.09	111.38** ±3.77	120.63** ±4.68
Ovariectomized females(control)	79.67 ±1.95	77.67 ±1.67	82.33 ±2.01	75.17 ±2.00	79.67 ±2.65
Intact males (treated)	104.35 ±2.09	109.50* ±3.07	115.10** ±2.36	116.33** ±1.93	127.17** ±3.27
Intact males (control)	103.75 ±3.09	101.100 ±2.87	101.88 ±2.96	101.88 ±2.96	103.63 ±2.37
Castrated males (treated)	105.56 ±1.63	106.44** ±1.94	116.78** ±2.22	116.78** ±2.22	126.44** ±3.80
Castrated males (control)	105.00 ±3.64	102.50 ±3.57	100.17 ±3.75	100.17 ±3.75	102.50 ±3.57

± Standard error

* Significantly different from control values at ($P < 0.05$)

** Significantly different from control values at ($P < 0.01$)