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Effect of the Anabolic Agent Zeranol on Growth Performance, Testis Development and Adrenal Weight of Weaning Rabbits

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THE anabolic agent zeranol was used as subcutaneous implants for weanling male rabbits in doses of a single 1-mg- implant, two 1-mg- implants (56- day interval) or a single 2-mg- implant. Effects of the drug on growth rate, efficiency of feed utilization, adrenal weight and morphological and histological testicular development over a 16-week-period were investigated.

No significant difference in magnitude of body weight gain, average daily gain or efficiency of feed utilization could be detected between implanted and non-implanted rabbits. Neither repeating implantation on day 56 or doubling the dose could increase weight gain or improve efficiency of feed utilization.

Zeranol implants caused significant increase in adrenal weigth. Testis of Zeranol-implanted rabbits were markedly atrophied, showing decreased weight, narrower seminiferous tubules with less active germinal epithilium and reduced number of sperm reserves when compared with gonads of intact non-implanted rabbits.

Keywords : Rabbit, Zeranol, Growth performance, Testis, and adrenal

Zeranol is a synthetic derivative of the mycotoxin zearalenone which is produced by a number of *Fuzarium* species and is known to be a phytoestrogen (Mirocha *et al.*, 1977). Although it has been widely marked during the last decade as a growth promotant for cattle sheep, recent research has revealed inconsistent results about its role as a growth stimulant. Many workers reported that implanting young bulls with zeranol did not significantly affect body weight gain (Corah *et al.*, 1979;Ford and Gregory, 1983; Juniewicz *et al.*, 1985; Gray *et al.*, 1986; Silcox *et al.*, 1986; Godfrey *et al.*, 1989). In contrast, some have found zeranol to increase weight gain in bulls (Fabry *et al.*, 1983; Greathous *et al.*, 1983) while others have reported decreased growth performance due to zeranol implantation (Gray *et al.*, 1984).

Characterization of the effects of zeranol on male cattle and sheep was undertaken and involved the determination of testicular development and/or pituitary hormone secretion patterns. Generally, zeranol implants were found to inhibit testicular growth and suppress spermatogenesis and testosterone production if implanted during the preweaning growth period, while no significant effects were observed if animals were treated latter (Ralston, 1978; Elsasser *et al.*, 1983; Juniewicz *et al.*, 1985; Staigmiller *et al.*, 1985; Gray *et al.*, 1986; Silcox *et al.*, 1986; Godfrey *et al.*, 1989).

Almost no information is available about the response of male rabbit to this anabolic agent implants. Therefore, the current work was designed to evaluate the possibility of using zeranol as a growth promotant for increasing rabbit meat production and to investigate its effects on testis function and adrenal development of weaning rabbits.

Material and Methods

Animals

Forty-eight 5 to 6-week-old weanling New Zealand White male rabbits with an average body weight of 858 + 29.7 g ($\bar{x} \pm$ S.E.) were taken at random from a flock at the Poultry Research Farm of Kafr El-Sheikh Faculty of Agriculture, Tanta University, Egypt. Animals were kept in metallic batteries (3 animals/cage) and fed ad libitum a pelleted diet containing 17.38% crude protein, 10.94% crude fiber, 2.58% ether extract and 4.19 Kcal/g gross energy on dry matter basis.

Growth implant

The anabolic agent zeranol (Ralgro, International Minerals and Chemicals Corporation, Terre Haute, In, USA) was used as subcutaneous implants in the dorsal part of the neck just behind the head.

Experimental design

Animals were randomly divided into four groups (A, B, C and D) of 12 rabbits each (4 cages i.e. 4 replicates). The different groups were assigned to serve as controls or to receive implants of zeranol as follows;

Group A: served as control nontreated.

Group B: received a single l-mg-implant of zeranol on day 0.

Group C: receied two l-mg-implants of zeranol, one on day 0 and the other eight weeks latter (on day 56).

Group D: received a single 2-mg-implant of zeranol on day 0.

Characteristics studied

Growth rate and efficiency of feed utilization

In dividual weights and amount of diet fed to each cage (3 animals) were obtained on day 28, 56, 84 and 112 postimplatation for growth rate and feed/gain ratio calculations.

Morphological and histological studies

Three animals with the average body weight in each group were chosen on days 56 and 112 for studying the influence of the growth implant on testicular and adrenal development. Animals were sacrificed then testes and adrenals immediately removed and weighed. The testis of the right side was fixed in 10% buffered formalin, dehydrated in a series of graded alcohol, embedded in paraffin, sectioned serially at 7 microns, mounted and stained with hematoxylin and eosin for histological evaluation at light microscope level. Seminerferous tubule diameters were determined on 100 tubules/rabbit of different size and shape. The widest and narrowest diameter of each tubule was measured and the average was calculated. Differentiation of germ cells in the various stages of the seminiferous epithelial cycle was also noted. Tunica albuginea of the left testis was carefully removed and weighed, while the parenchyma was processed according to the method described by Orgebin-Crist (1968) for the determination of testicular sperm reserve.

Statistical analysis

All data obtained were statistically analyzed for differences due to treatment by least squares analysis of variance (Snedecor and Cochran, 1976).

Results and Discussion

Effect of zeranol on growth and efficiency of feed utilization is shown in Table 1. No significant difference in magnitude of body weight gain or average daily gain over the 16-weeks experimental period between implanted and nonimplanted weanling rabbits could be detected ($P< 0.05$).

Similarly, zeranol did not alter feed consumption or efficiency of feed utilization. Neither repeating implantation on day 56 (group C) nor doubling the dose implanted (group D) could increase weight gain or improved efficiency of feed utilization. These results are in consistent with previous reports on bulls (Baker and Arthaud, 1972; Corah *et al.*, 1979; Ford and Gregory, 1983; Staigmiller *et al.*, 1985; Juniewicz *et al.*, 1985; Gray *et al.*, 1986; Silcox *et al.*, 1986) and rams (Wiggins, 1976). However our results are in contrast with many others who claimed that zeranol increased the growth rate of both growing and fattening animals (Perry et al, 1970; Thomas and Armitage, 1970; Thomas *et al.*, 1970; Staigmiller *et al.*, 1983).

TABLE 1. Effect of zeranol implants on growth, feed consumption and efficiency of feed utilization.

Experimental groups	Treatments	Experimental days Animals age (wks) No. of animals/grp.	0 5 12	28 9 12	56 13 12	84 17 9	112 21 9	Av.Values over 112-d period.
A	control non-implanted	Live body wt. Feed consumption, g/animal/day. Weight gain, g/animal/day. Efficiency of feed utilization	860±27.1 ---------- ----------	1656±63.9 105.0 28.4 3.69	2620±89.3 106.2 34.4 3.08	3374±146.9 104.3 26.9 3 86	3625±160.3 99.1 9.0 11.01	---------- 103.7 24.7 4.20
B	Given one 1-mg implant on day 0	Live body wt. Feed consumption, g/animal/day. Weight gain, g/animal/day. Efficiency of feed utilization	853±25.3 ---------- ----------	1656±62.5 103.7 29.0 3.58	2584±101.3 104.8 32.8 3.19	3345±159.7 101.9 27.2 3.75	3605± 176.4 100.6 9.3 10.82	---------- 102.8 24.6 4.19
C	Given two 1-mg implants on day 0 and 56.	Live body wt. (g) Feed consumption, g/animal/day. Weight gain, g/animal/day. Efficiency of feed utilization	881±30.5 ---------- ----------	1681±59.7 106.4 28.6 3.72	2623±110.1 107.2 33.6 3.19	3316±137.2 104.3 24.8 4.21	3695±149.9 100.7 13.5 7.46	---------- 104.7 25.1 4.17
D	Given one 2-mg implant on day 0.	Live body wt. (g) Feed consumption, g/animal/day. Weight gain, g/animal/day. Efficiency of feed utilization	843±29.4 ---------- ----------	1676±63.4 104.9 29.6 3.54	2661±116.2 105.6 35.2 3.00	3358±121.1 105.3 24.9 4.23	3704±135.2 101.2 12.4 8.16	---------- 104.5 25.5 4.10

Results concerning the effect of zeranol on adrenal and testis development are presented in Table 2. Adrenal weight was markedly affected by zeranol implants. Intact (non-implanted) rabbits had significantly lighter adrenal glands than zeranol-implanted ones on day 56 and 112 after implantation ($P < 0.05$). No significant differences among the three treated group (B, C and D) could be detcted ($P > 0.05$). These results confirm those reported by Borger *et al.* (1973) on steers and Wiggins *et al.* (1976) on lambs. The larger adrenal glands of implanted rabbits may reflect androgen production by the steroidogenic cells of the adrenal cortex. It is possible that zeranol through its estrong-like effect may increase adrenal cortical steroids production, either due to direct action of estrogen on the adrenal cortex or through increased ACTH release.

Testicular development was drastically influenced by zeranol implants. After 56 days of implantation paired testis weight for control animals was about 187.2%, 182.0% and 185.5% of that for treated groups B, C and D respectively. On day 112 the corresponding values were 181.6%, 189.8% and 162.9% consequently. Besides, weight of tunica albuginea as percentage of testis weight in implanted animals was significandly greater that that in controls ($P < 0.05$). Statistical analysis could not reveal any significant differences among the three treatments which may indicate that the single 1-mg-implant was sufficient enough to cause the adverse effects observed.

Under histological examination gonads of zeranol implanted rabbits appeared to have much less active germinal epithelium than intact animals. Sections in samples taken after 56 days of implantaion (animals age about 13 wks.) are shown in Fig.1. Testis of intact males showed apparently larger seminiferous tubules compared with those of treated animals. All tubules had spermatogonia and primary spermatocytes. Some primary spermatocytes were undergoing meiotic division to form the secondary spermatocytes which were present in some tubules (Fig. 1, A). Sections of zeranol-implanted males showed significantly narrower tubules ($P < 0.05$) with less number of spermatogonia and few number of primary spermatocytes. Nuclei of spermatogonia and Lyding cells appeared pyknotic (Fig. 1; B, C and D).

By the day 112 after implantation (animals age about 21 wks.) testis sections of intact males showed the typical apperance of full active gonads. All stages of spermatogenesis were present. Remarkable lumen in the middle of each tubule was noticed with bundles of spermatozoa around (Fig.2, A). The average diameter of seminiferous tubules in this group was nearly 166% of that in treated groups. Testis sections of zeranol-implanted rabbits were characterized by less diameter of seminiferous tubules and the absence of spermatozoa bundles found in case of intact animals. Spermatogenesis developed up to the formation of spermatids. Metamorphism of spermatids to form spermatozoa have not begun yet (Fig. 2; B,C and D). This indicates that gonads of these animals were still unable to produce mature spermatozoa.

TABLE 2. Effect of zeranol implants on adrenal weight, testis weight, diameter of seminiferous tubules and testicular sperm reserve.

Experimental group treatment	A Control non-implanted	B Given one 1-mg implant on day 0.	C Given two 1-mg implant s on day 0 and 56.	D Given ame 2-mg implant on day 0.
		I - On day 56.		
Body weight (g)	2610.0 ± 10.29	2590.0 ± 11.47	2631.0 ± 12.63	2615.0 ± 9.96
Adrenal weight, (mg)	348.0 ±19.83b	476.0 ± 20.08a	452.0 ± 16.66 a	503.0 ± 21.11a
Paired testis weight, (mg)	1848.0± 86.43a	987.0 ±51.45b	1015.0 ± 73.82 b	996.0 ± 62.73b
Weight of tunica albuginea of the left testis, (mg)	132.0 ±11.14	146.0 ± 10.88	169.6 ± 12.33	164.0 ± 11.67
(as% of testis wt.)	13.2 ± 1.13b	29.2 ± 2.21a	32.2 ± 2.98a	30.0 ± 2.87 a
Diameter of semineferour tubules, (microns)	79.6 ± 6.35a	47.4 ± 7.11b	41.9 ± 6.85b	39.9 ± 5.82b
Testicular sperm reserve	0	0	0	0
		II - On day 112		
Body weight (g)	3632.0 ± 18.73	3608.0 ± 19.91	3652.0 ± 12.56	3690.0 ± 18.73
Adrenal weight, (mg)	357.0 ± 12.90b	439.0 ± 21.70 a	471.0 ± 20.85a	428.0 ± 19.77a
Paired testis weigh, (mg)	2938 ± 93.09a	1618.0 ± 85.15b	1548.0 ± 77.23b	1804.0† ± 71.11b
Weight of tunica albuginea of the left testis, (mg) (as%	88.0 ± 9.15 b	117.0 ± 8.81ab	134.0 ± 7.25a	115.0 ± 8.86ab
of testis wt.)	5.4 ± 0.68b	12.8 ± 1.11a	16.3 ± 1.42a	12.1 ± 1.03a
Diameter of semineferous tubules, (microns)	163.9 ± 13.43a	98.8 ± 10.44b	93.9 ± 8.79b	96.7 ± 9.99b
Testicular sperm reserve for	284.2 ± 19.99a	45.5 ± 6.36b	39.8 ± 4.67b	43.5 ± 8.44b
the left testis X 10^6,	173.5 ± 13.52a	49.5 ± A.73b	48.2 ± 4.59 b	45.8 ± 4.39b
(total) (/g tissue)				

a, b, c, Means in the same row that do not have a common superscript letter are significantly different at P≤ 0.05.

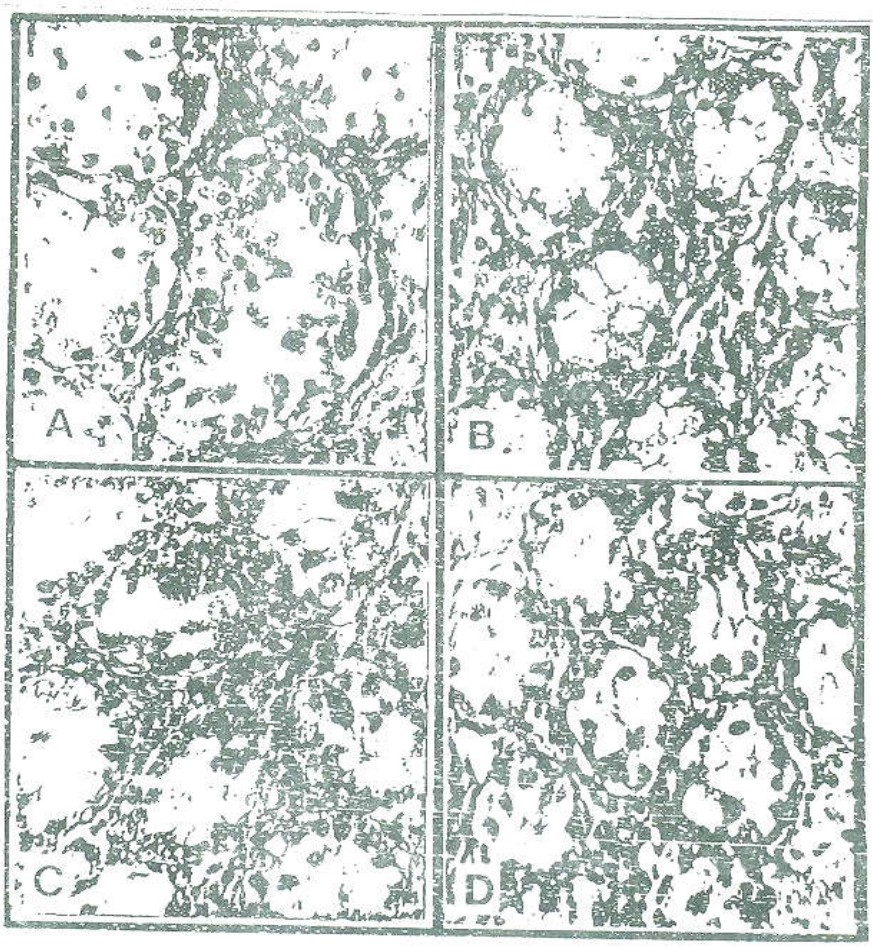

Fig. 1 . Cross sections in the testis of control and zeranol-implanted groups 56 days after implantation. Hematoxylin and eosin stain, X 400;
(A) Control (non-implanted)group,
(B) Received a single-1-mg implant on day 0.
(C) Received two 1-mg-implants on days 0 and 56,
(D) Received a single 2-mg- implant on day 0
Note that sections of implanted groups are characterized by decreased seminiferous tubular diameter and almost inactive monolayered seminiferous epithelium as compared with sections of control group.

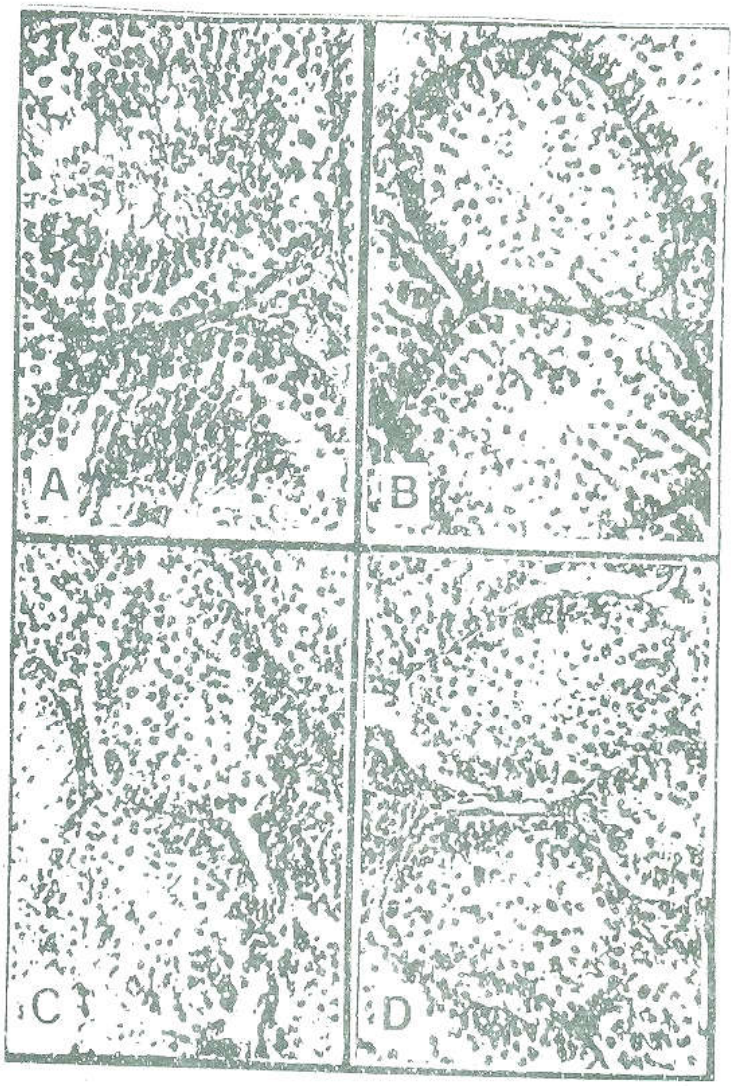

Fig. 2 . Cross sections in the testis of control and zeranol-implanted groups 112 days after implantation. Hematoxylin and eosin stain, X 400;
(A) Control (non-implanted)group,
(B) Received a single-1-mg implant on day 0.
(C) Received two 1-mg-implant on days 0 and 56,
(D) Received a single 2-mg- implant on day 0 .
Note that sections of control group showed fill active seminiferous epithelium. Spermatogenic cells are successively arranged from spermatogonia to spermatozoa. Sections of implanted groups are characterized by smaller seminiferous tubular diameter and absence of spermatozoa.

Estimates of testicular sperm reserves presented in Table 2 support the histological findings mentioned above. The average total testicular sperm reserve for animals of the zeranol-treated groups B, C and D were only about 16%, 14% and 15% resp. of that for control group A. Almost no difference could be observed due to repeated implantation on day 56 (group C) or to doubling the dose implanted (group D).

The inhibitory effect of zeranol on testicular growth and spermatogenesis observed in the present study was reported by many workers in cattle and sheep (e.g. Wiggins *et al.*, 1976; Corah *et al.*, 1979; Gregory and Ford, 1983; juniewicz *et al.*, 1985; Godfrey *et al.*, 1989). Several authors reported that zeranol implants remarkably suppressed serum testosterone concentration (Fabry *et al.*, 1983; Staigmiller *et al.*, 1985; Gray *et al.*, 1986; Silcox *et al.*, 1986; Godfrey *et al.*, 1989). The lower concentrations of testosterone in implanted males suggest that zeranol may interfere with normal luteinizing hormone (LH) secretory episodes and likely is recognized as an estrogen by the hypothalamus (Gray *et al.*, 1986) Silcox *et al.* (1986) reported that Leyding cells of zeranol-treated bulls had less smooth endoplasmic reticulum which may be the reason for the lowest testosterone concentration in the implanted males. This inhibitory effect on concentrations of androgens in the peripheral circulation in implanted males may explain the absence of response in body growth to zeranol obtained in the present study since androgens have been implicated in causing increased body growth rates in males (Gortsema *et al.*, 1974).

In conclusion, results of the present study indicate that using zeranol implants for weanling male rabbits in doses of a single 1-mg-implanted, two 1-mg-implants (56-day interval) or a single 2-mg-implant showed no beneficial effects on either body weight gain or efficiency of feed utilization over a period of 16 weeks. Moreover, testis of zeranol-implanted animals were markedly atrophied, while adrenals showed hypertrophy.

Better results may be obtained if other schedules of implantation are examined at different ages.

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تأثير العامل البنائى " زيرانول" على كفاءة النمو وتطور الخصية ووزن غدة جار الكلية فى الأرانب حديثة الفطام

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مستحضر الزيرانول أحد منشطات النمو الشائعة الأستخدام فى مجال انتاج الماشية والأغنام وتتناول هذه الدراسة بحث إمكانية استخدام هذا العقار فى مجال الأرانب وخاصة مع الذكور حديثة الفطام وما قد يصحب ذلك من آثار جانبية على تطور الغدد الجنسية وغدة جار الكلية.

ولقد أجريت التجارب على ٤٨ من ذكور النيوزيلاندى الأبيض حديثة الفطام (عمر ٥ - ٦ أسابيع) تم تقسيمها عشوائيا الى أربع مجموعات متساوية وتم معاملتها كالآتى : المجموعة الأولى أستخدمت كمجموعه مقارنة غير معاملة ، المجموعة الثانية : أعطيت جرعة مقدارها ١ مجم / أرنب من الزيرانول فى بداية التجربة ، المجموعة الثالثة : أعطيت جرعتان كل منها ١ مجم/ ارنب الأول عند بداية التجربة والثانية بعد ٥٦ يوم ، أما المجموعة الرابعة فقد أعطيت جرعة واحدة مضاعفة مقدارها ٢ مجم / أرنب فى بداية التجربة.

وقد أعطيت الجرعات بواسطة الزرع تحت الجلد فى الناحيه الظهرية خلف الرقبة. وعلى مدى ١٦ أسبوعا ثم دراسة تاثير المعاملات المذكورة على معدل النمو وكفاءة التحويل الغذائى والتطور المورفولوجى والهستولوجى للخصية ومخزونها من الحيوانات المنويه الى جانب وزن غدة جار الكلية.

ولقد أوضحت نتائج التجربة أن مادة الزيرانول بالجرعات المستخدمه لم يكن لها أى تاثيرمعنوى على كل من معدل النمو وكفاءة التحويل الغذائى.

وعلى الجانب الآخر سببت هذه المادة زيادة معنوية فى وزن غدة جار الكلية وانخفاض واضح فى تطور الخصيتين حيث قل وزنهما معنويا وكذلك انخفض بشدة مخزونها من الحيوانات المنويه . وعند الفحص الميكرسكوبى تلاحظ صغر أقطار الأنابيب المنوية وقلة نشاط النسيج الطلائى الجرثومى اذا ماقورن بنظيره فى الأرانب الغير معاملة.