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REPRODUCTIVE PERFORMANCE OF FILANDER RABBITS TREATED WITH RECOMBINANT BOVINE SOMATOTROPIN

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ABSTRACT

The objective of this study was to determine the impact of recombinant bovine somatotropin (rbST) on reproductive performance of rabbits. The experimental flock consists of 120 females and 45 males of Filander rabbits. They were injected by rbST at a dose of 0, 0.75 and 1.5 mg/kg biweekly basis throughout the experiment. Experimental rabbits were fed ad libitum pelleted reproductive commercial ration to serve their nutritional requirements for mature period of rabbits. In accordance to semen quality, it could be speculated that the semen volume of injected rabbits was significantly higher than that of control-group. Also, both advance motility and semen concentration were significantly improved. Relative weight of both testes and pituitary gland was significantly increased by rbST injection at both dosages.

With respect to testosterone hormone concentration and mating activity, the present results showed that the rabbits treated with rbST were significantly higher both testosterone concentration and mating activity compared with control-group. The litter size at birth was significantly increased in rabbits treated with rbST when compared with control-group. The

injected rabbits with rbST produced significantly higher milk yield compared to control-group. Concerning pre-weaning mortality rate, it could be noticed that the pre-weaning mortality rate was significantly decreased in injected rabbits. In conclusion, we conducted that the reproductive performance and sexual behavior of rabbits were significantly improved by injection of rbST at both dosages.

Key words; Rabbits, reproductive parameters, rbST

INTRODUCTION

Rabbits provide an excellent source of protein for human consumption and may play a significant role in solving a part of meat shortage in Egypt. Adding several products from different sources to rabbits feed now is widely used for improving performance (Boulous et al., 1992). Growth promoters (chemical products, antibiotics, enzymes, hormones, etc) play an essential role in the rabbit industry. Although, good results were obtained with these substances, their use might have unfavorable effect and it may result some unfavorable residual in the tissues resulted many problems for consumers. Recently, using antibiotics as a growth promoters and many artificial feed additives in animal production become international refused due to their adverse and side effects on human health (Marzo, 2001).

Bovine somatotropin (rbST), a natural growth hormone secreted by the anterior pituitary in all animals, has a major effect on the regulation of growth. It is now possible to transfer the DNA sequences responsible for somatotropin synthesis into bacteria and to produce large quantities of somatotropin commercially at economic levels. Interest in increasing growth performance by administering somatotropin (ST) has been sparked by the *in vitro* synthesis of mammalian ST using recombinant DNA techniques (Bauman et al., 1989) and by the observations that injections of ST enhance growth in pigs (Etherton and Bauman., 1998) and increase milk production in cows (Peel et al., 1985). The treatment of dairy cows with recombinant bovine somatotropin (rbST) has been shown to have marked effects on milk yield (Burton et al., 1994; Chilliard et al., 1998; Etherton and Bauman, 1998).

It is also well known that the effects of rbST are associated with, and mediated by, many physiological and metabolic changes, such as

enhanced cardiac output, mammary blood flow, alveolar cell secretion activity, endocrine changes, lipolysis, glucose sparing, and gluconeogenesis (Bauman et al., 1989; Burton et al., 1994; Chilliard et al., 1998; Etherton and Bauman, 1998). However, authors have generally concluded that only few effects on blood metabolites and hormone levels, due to the rbST treatment, can be seen (Burton et al., 1994; Chilliard et al., 1998).

Nevertheless, looking at the experimental papers, there is evidence for marked variations in blood concentration of some metabolites and hormones, namely during the first days of rbST treatment. In addition, in dairy cows treated daily with rbST Piccioli-Cappelli et al., 1989 observed a significant increase in prefeeding plasma concentrations of non-esterified fatty acids (NEFA), cholesterol and bilirubin, whereas the urea level and the activity of the lactate dehydrogenase (LDH) were significantly reduced. Similar results have also been observed by Oldenbrock et al. (1989), Rosi et al. (1990), and consistent, but smaller variations have been shown by Sechen et al. (1989). Moreover, with respect to hormones, the concentrations of insulinlike growth factor (IGF-I) have been observed to increase after rbST administration (Ronge and Blum, 1989), but the rbST-IGF axis seems strictly dependent on the cow's nutritional status (Burton et al., 1994; Ronge et al., 1988).

Insulin increments due to rbST treatment were also observed (Ronge and Blum, 1989; West et al., 1991), while other authors showed variable insulin changes after rbST treatment (Davis et al., 1988). Many other authors have reported small changes of insulin and thyroid hormone concentrations (Chilliard et al., 1998), and Johnson et al. (1991) observed a significant reduction of 3,5,3'-triiodothyronine (T3) and cortisol concentrations.

Rabbits are characterized by rapid growth, more efficient feed conversion, low cost per breeding. In addition, the rabbit meat is nearly white, fine grained, mild flavored, high in good quality protein content, low in fat and caloric content, nearly of the same nutritive value as beef meat and is acceptable to the general consumer in the most countries of the world (Galal and Khalil, 1994).

Therefore, the objective of the present study was to evaluate the effect of rbST injection on reproductive performance of rabbits under prevailing environmental Egyptian conditions.

MATERIALS AND METHODS

This experiment lasted 4 months and was carried out on 120 multiparous does and 45 sexual mature bucks of Filander rabbits aged eight months. All rabbits were individually housed in wired battery cages supplied with feeders and stainless steel nipples for feeding and drinking. The pelleted diets covered the nutritional requirements of the mature phases of rabbits according to NRC (1977) recommendations. Ingredients and chemical composition of the pelleted ration are summarized in Table (1). All batteries were located in a windowed Rabbitry with natural ventilation. Fresh tap water was automatically available all the time in each cage.

All the experimental animals were healthy and clinically free from internal and external parasites and were kept under the same managerial and hygienic conditions. The rabbits were divided into three comparable groups (15 bucks and 40 does in each group). The first group was left untreated as a control and fed commercial diets according to NRC (1977) recommendations, while the second and third groups injected with 0.75 and 1.5 mg/kg rbST, respectively (every three days).

Blood samples of rabbit bucks were taken in less than two minutes from the marginal ear vein of six rabbit bucks within each experimental group monthly. Blood serum testosterone hormone concentration of the rabbit bucks was determined using RIA Kits (Immunotech, A Coulter Co., France) according to the manufacturer information.

Libido (sexual desire) was measured in terms of reaction time in seconds that was estimated just from the time of introducing doe to the buck until the buck start to mount (Daader *et al.*, 1999 and Seleem, 2003). Semen was collected artificially twice a week for up to five weeks by means of an artificial vagina as described by Seleem (1996 and 2003). Semen samples ejaculated from each rabbit buck were evaluated individually microscopically and then semen ejaculate volume (ml); advanced sperm motility (%); dead spermatozoa (%); sperm

abnormalities (%); acrosomal damages (%) sperm-cell concentration ($N \times 10^6/ ml$) and total -sperm output ($N \times 10^6/ ejaculate$) were estimated according to Salisbury *et al.* (1978) and Seleem (1996 and 2003).

Table (1): The ingredients and chemical composition of the pelleted diets fed to rabbits, during the experimental work.

Ingredients	%
Artificially dried Alfalfa (IBEX Alfalfa)	26.95
Wheat bran	27.50
Yellow corn	20.70
Soybean meal (44%)	18.00
Molasses	3.40
Calcium carbonate (lime stone)	0.75
Sodium chloride	0.30
Vitamins & Mineral Premix *	0.30
DL-Methionine	0.10
Di-Calcium phosphate	2.00
Total	100.00
ated chemical composition **	
Crude protein (CP)	18.0
Ether extract (EE)	2.87
Crude fiber (CF)	10.52
Digestible energy (Kcal/Kg)	2600

Vitamins and minerals premix per kilogram contains*

Items	Concentration	Items	Concentration
Vit.A	10,000 IU	Biotin	0.2mg
Vit.D3	900 IU	Choline	1200.0mg
Vit.E	50.00mg	Niacine	50.0mg
Vit.K	2.00 mg	Zn.	70.0mg
Vit.B1	2.00mg	Cu.	0.1mg
Vit.B2	6.00mg	Mn.	8.5mg
Vit.B6	2.00mg	Fe.	75.0mg
Vit.B12	0.01mg	Folic acid	5.0mg
		Pantothenic acid	20.0mg

Calculated according to NRC (1977) for rabbits

Acrosome status was determined using a Giemsa stain procedure as described by Watson (1975). At the end of experiment, four rabbit bucks from each experimental group were randomly chosen and slaughtered. Scrotal circumference was measured as the method described by Mickelsen *et al.* (1982). Testicular index (length x width x depth) was calculated in cubic centimeters as recorded by Daader *et al.* (2003). Mating activity (frequency of mating within 15 minutes) of each buck was determined using sexual receptive doe.

Data were subjected to analysis of variance according to Snedecor and Cochran (1982) using the General Linear Model Program of SAS (2001). Percentage values were transformed to Arc. Sin values before being statistically analyzed. Duncan's new multiple range tests was used to test the significance of the differences between means (Duncan, 1955). Conception and kindling rates were analyzed using the Contingency Tables according to Everitt (1977).

RESULTS AND DISCUSSION

Data presented in Table (2) indicated that, injection of rabbits at 0.75 and 1.5 mg improved significantly ($P \leq 0.05$) the reproductive capability of Filander rabbit bucks represented by libido (sexual desire) and physical semen characteristics (semen-ejaculate volume; advanced-sperm motility; live and normal spermatozoa; acrosome status; sperm-cell concentration and total-sperm output). Dhami and Kodagali (1987) suggested a positive correlation between AST activity in seminal plasma and sperm concentration, live sperm percent, motility, semen volume and fertility rate of semen. Also, they reported that AST enzyme plays an important role in sperm metabolism through its involvement in the vital cellular process. So, we suggest that increased in AST enzyme in blood (Abd El-Motaal *et al.*, 2008) may be responsible to the increase of AST in seminal plasma which would explain the improvement of semen quality recorded in the present study of rabbits treated with rbST. As a result of the improvement in some mineral absorption, the activity of some essential enzyme was increased, which could result in development and maturation of sperm in the testes.

Table (2): Libido and physical semen characteristics of Filander rabbit bucks treated with rbST

Trait	rbST (mg/kg)		
	0	0.75	1.5
Sexual desire –libido- (sec.)	24.9a±2.10	19.2b±2.00	19.0b±1.80
Semen-ejaculate volume (ml)	0.41b± 0.01	0.87a±0.02	0.91a±0.02
Advanced-sperm motility (%)	71.9b±2.70	79.8a±2.60	82.1a±3.50
Alive spermatozoa (%)	79.1b±2.10	85.6a±2.50	88.1a±2.10
Normal morphological sperm (%)	82.2b±2.30	86.1a±1.90	87.3a±2.00
Acrosomal damages (%)	14.1a±1.10	11.9b±1.60	11.2b±0.80
Sperm-cell concentration (Nx10 ⁶ /ml)	599.7b±33.5	751.8a±29.8	770.7a±31.50
Total-sperm output (Nx10 ⁶ /ejaculate)	245.9c±21.6	654.1b±24.5	701.3a±28.8

^{a,b} Means within the same row are bearing different superscripts, differ significantly (P<0.05).

rbST: recombinant bovine somatotropin

Tables (3) showed that the effect of rbST injection on body, gland and pituitary weights of Filander rabbits. The presented results indicated that the gonads represented by (testes; epididymis and sexual accessory glands) and pituitary gland weight; scrotal circumference and testicular index of rabbits treated with rbST were significantly higher, while live body weight was insignificantly higher than those of untreated group. The improvement in reproductive performance of bucks in treated group may be due to the powerful role of rbST the process of sperm formation, sperm maturation, and the maintenance of sperm quality.

Data presented in Table (4) showed that the rabbits treated with rbST at 0.75 and 1.5 mg/kg were significantly higher testosterone concentration in the blood compared to control-group. Similar trend was noticed for mating activity, whereas the mating activity of treated rabbits was significantly higher than that of control-group.

Table (3): Body, glands and pituitary weights, scrotal circumference and testicular index of Filander rabbit bucks treated with rbST

Trait	rbST (mg/kg)		
	0	0.75	1.5
Live body weight, g	3211.5±52.9	3117.9±49.0	3222.2±47.0
Testes weight, g	5.5b±0.30	6.40a±0.20	6.80a±0.40
Testes weight, %	0.17b±0.01	0.21a±0.01	0.21a±0.01
Sexual accessory glands weight, g	3.10b±0.02	3.90a±0.01	4.10a±0.01
Sexual accessory glands weight, %	0.10b±0.01	0.13a±0.01	0.14a±0.01
Epididymis weight, g	0.85b±0.001	0.97a±0.01	0.99a±0.02
Epididymis weight, %	0.03±0.001	0.03±0.001	0.03±0.00
Pituitary weight, g	22.3b±0.70	26.5a±0.40	27.0a±1.00
Pituitary weight, %	0.69b±0.02	0.85a±0.02	0.84a±0.04
Scrotal circumference, g	7.10b±0.60	7.80a±0.70	8.00a±0.20
Scrotal circumference, %	0.22±0.02	0.25±0.01	0.25±0.02
Testicular index (cm ³)	5.9b±0.40	6.7a±0.60	7.0a±0.50
Testicular index, %	0.18b±0.01	0.21a±0.01	0.22a±0.01

^{a,b} Means within the same row are bearing different superscripts, differ significantly ($P \leq 0.05$).

rbST: recombinant bovine somatotropin

Table (4): Testosterone concentration and mating activity of Filander rabbit bucks treated with rbST

Trait	rbST (mg/kg)		
	0	0.75	1.5
Testosterone concentration, ng/ml	4.3b±0.30	5.2a±0.40	5.7a±0.30
Mating activity (frequency of mating/15 min.)	4.0b±0.10	5.7a±0.20	6.1a±0.20

^{a,b} Means within the same row are bearing different superscripts, differ significantly ($P \leq 0.05$).

rbST: recombinant bovine somatotropin

Data presented in Tables (5 and 6) showed that, rabbit does and mated naturally by using subjected to bucks the same treatment recorded significantly ($P \leq 0.05$) higher kindling rate, litter size and weight at birth; milk yield / doe. In this respect, this may lead to increased milk secretion and its yield in treated rabbits. Beside that, the increase in milk production may be due to increase in litter size at birth, where there was a positive correlation between the litter size at birth and milk yield (Lebas *et al.*, 1997 and Rommers *et al.*, 2001).

Finally, the improvement in litter traits proved that, the rbST treatment is capable to improve the milking ability of the doe which is reflected in her care and ability to suckle her young till weaning.

Interest in increasing growth performance by administering somatotropin (ST) has been sparked by the *in vitro* synthesis of mammalian ST using recombinant DNA techniques (Bauman et al., 1989) and by the observations that injections of ST enhance growth in pigs (Etherton and Bauman., 1998) and increase milk production in cows (Peel et al., 1985). The treatment of dairy cows with recombinant bovine somatotropin (rbST) has been shown to have marked effects on milk yield (Burton et al., 1994; Chilliard et al., 1998; Etherton and Bauman, 1998).

The comparison between control and treatment groups indicates a significant ($P \leq 0.05$) decrease in pre-weaning mortality rate (%) in treatment group (Table 5). In addition to, rbST may have an essential role in reduced mortality rate by its role in modify pH of rabbit digestive tract promoting useful bacteria and inhibit the harmful ones (Pinheiro *et al.*, 2004).

On the other hand, the milk available per kit may also have a pronounced effect on the mortality of young rabbits (Rommers *et al.*, 2001 and Szendro *et al.*, 1995). The lowest and pronounced decrease of mortality percentage in offspring's treated rabbit does may be discussed from the view which demonstrated by Fortun-Lamothe and Boullier (2004) who showed that, in the young rabbit a passive immunity occurs due to mother's immuno-globulins transmission by the colostrum and to a lesser extent by the milk. In addition, it has been shown that injected of rbST may be induced significant improves the immune system (Savage *et al.*, 1996). So, this is sufficient to provide protection against infections.

Table 5: Fertility traits of Filander does naturally mated and treated with rbST

Trait	rbST (mg/kg)		
	0.00	0.75	1.50
Number of mated does	40 X 2	40 X 2	40 X 2
Number of conceived does	52	70	74
Kindling rate (%)	65	87.5	92.9
Litter size at birth	6.7b±0.70	7.8a±0.80	8.1a±0.70
Litter weight at birth (gm)	249.9b±11.9	301.7a±18.8	316.9a±19.1

^{a,b} Means within the same row are bearing different superscripts, differ significantly ($P \leq 0.05$).

rbST: recompanant bovine somatotropin

Table (6): Milk and pre-weaning mortality rate of Filander rabbit does naturally mated and treated with rbST

Trait	Period		rbST (mg/kg)		
	From	To	0.00	0.75	1.50
Milk yield (g/ doe)	Birth	7 days	570.9c±15.9	690.3b±21.1	760.6a±31.3
	Birth	14 days	1850.7c±19.7	2001.4b±30.3	2505.2a±29.9
	Birth	21 days	2790.2b±16.6	3219.3a±27.7	3260.2a±17.8
	Birth	28 days	3101.1c±24.6	4009.1b±21.5	4101.1a±26.2
Pre-weaning mortality rate (%)	Birth	7 days	4.1	3.0	2.6
	Birth	14 days	6.1	4.2	3.1
	Birth	21 days	7.0	5.0	3.8
	Birth	28 days	7.9	5.5	4.2

^{a,b and c} Means within the same row are bearing different superscripts, differ significantly ($P \leq 0.05$).

rbST: recombinant bovine somatotropin

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الأداء التناسلي للارانب الفلندر المعاملة بهرمون النمو البكتيرى (rbST) علاء الدين محمد عبد المتعال* - ايمن محمد حسن احمد - طارق جابر زكى*** - طارق عبد الغفار الأعصر***

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صممت هذه التجربة لدراسة تأثير هرمون النمو البكتيرى (rbST) على الاداء التناسلى فى الارانب. استخدم فى هذه الدراسة 120 انثى و 45 ذكر من سلالة الفلندر. تم تقسم الارانب الى ثلاث مجاميع، كانت المجموعة الاولى كمنترول بينما حقنت المجموعة الثانية والثالثة بهرمون النمو البكتيرى بتركيز 0.75 و 1.5 مللجم/كجم وزن حتى كل ثلاث ايام. تم تغذية الارانب خلال الفترة التجريبية بصورة حرة على عليقة توفى الاحتياجات الغذائية للارانب. سجلت الارانب المحقونة بهرمون النمو البكتيرى حجم سائل منوى اعلى من الكمنترول. كما ادى الحقن الى زيادة الحركة التقدمية وتركيز الحيوانات المنوية. شوهد زيادة معنوية فى الوزن النسبى للخصيتين والغده النخامية وذلك فى الارانب المعاملة بهرمون النمو البكتيرى مقارنة بالكمنترول. سجلت الارانب المعاملة بالهرمون زيادة معنوية فى تركيز هرمون التستسترون وكذلك زيادة معنوية فى النشاط الجنسى مقارنة بالكمنترول.

يصاحب الحقن بالهرمون زيادة معنوية فى عدد الخلفة عند الميلاد وذلك مقارنة بالكمنترول. انتجت الارانب المعاملة بهرمون النمو البكتيرى كمية لبن اعلى معنويا من الاخرى الغير معاملة. كما سجلت الارانب المعاملة معدلات نفوق قبل الفطام اقل بصورة معنوية من الارانب الكمنترول.

الخلاصة، اوضحت النتائج المتحصل عليها ان المعاملة بهرمون النمو البكتيرى اداء تناسلى اعلى معنويا من الارانب الغير معاملة (الكمنترول).