

**SEMEN CHARACTERISTICS; FERTILITY AND  
BLOOD PARAMETERS OF NZW RABBIT  
BUCKS AS AFFECTED BY DIETARY  
PROTEIN LEVEL UNDER MILD  
AND HOT CLIMATES**

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**ABSTRACT :** To investigate the influence of dietary protein level and environmental conditions on some semen characteristics; buck fertility and blood parameters, three groups of New Zealand White bucks (8 bucks/group) were subjected to three diets (14; 16 and 18% crude protein) during two seasons (winter and summer) of the year. The results showed that sperm concentration per ml or per ejaculate were insignificantly improved in males submitted to diets of high protein (16 or 18%), however, ejaculate volume; progressive motility and dead or abnormal spermatozoa did not show any definite trend. All semen parameters were improved significantly ( $P < 0.05$ ; 0.01 or 0.001) during winter as compared with those obtained during summer. Does served with bucks fed high protein level showed insignificantly improve in conception rate and litter size at birth (total or live). Reproductive performance of mated does was improved during winter vs. that during summer; however, the improvement was significant ( $P < 0.05$ ) only for conception rate. The effects of dietary protein levels on blood parameters were insignificant. Blood contents of total protein was increased ( $P < 0.05$ ), while total lipids and activities of alkaline phosphatase; GOT and GPT enzymes were decreased ( $P < 0.01$  or 0.001) during winter than those during summer. The interaction effects did not show any significant differences in the studied parameters except those on ejaculate volume and dead sperm percentage, which were significant (0.01 or 0.001, respectively).

Increasing dietary protein level from 14 to 16 or 18% for rabbit bucks showed some beneficial effects on semen quality; blood parameters and the reproductive performance of mated does, while they were extremely affected during summer. The interaction effects (dietary protein level with season) on the studied traits were found to be in low magnitude.

*Key words :* Semen quality – blood parameters – heat stress – dietary protein.

## INTRODCUTION

The most important economic factor in live-stock production is the efficiency of the reproductive performance (James *et al.*, 1992), especially under the intensive rabbit production and artificial insemination technique, which has been used in commercial rabbit breeding in several countries (Luzi *et al.*, 1996). Consequently, it is very important to control all factors affecting reproductive performance to optimize the sexual activity of the buck. Semen quality is influenced by several factors such as environment, especially temperature being perhaps the most important component (Marai and Habeeb, 1994 and Xylouri *et al.*, 1998); buck management and mating regime (Lopez *et al.*, 1996 and Askar, 1999) and nutrition (Homid and Tharwat, 1995; Papadomichelakis *et al.*, 2000). Few studies have analyzed the physiology of rabbit males and the effect of climatic components and nutrition on the semen quality. Using suitable male diet during the different conditions of the year to

optimize libido and semen quality is in question.

The purpose of the present work was to determine the influence of dietary protein level during mild or hot conditions (winter or summer) on semen characteristics; fertility and blood chemical components of New Zealand White bucks.

## MATERIALS AND METHODS

The experiment was performed at the rabbit farm of Department of Poultry Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. Twenty-four New Zealand White mature rabbit males (7 months of age and  $3.55 \pm 0.17$  kg live body weight) were randomly allotted in to three experimental groups of 8 bucks per group. Each group was fed *ad-libitum* one of the experimental pelleted rations, which were iso-energetic diets ( $2684 \pm 16.2$  kcal/ kg D.E.) and contained different levels of crude protein (14; 16 and 18%) as shown in Table 1.

**Table 1. Ingredients and chemical composition of the experimental diets.**

Items	Rations		
	Low protein	Milieu protein	High protein
<b>Ingredients(%)</b>			
Clover hay	31.0	32.0	33.0
Wheat bran	31.0	28.0	26.0
Barley grains	27.7	25.7	20.7
Soybean meal	3.7	9.7	15.7
Yellow corn	2.0	-	-
Molasses	3.0	3.0	3.0
Limestone	1.0	1.0	1.0
Sodium Chloride	0.3	0.3	0.3
Vitamin And mineral premix	0.3	0.3	0.3
	100	100	100
<b>Chemical analysis(%)</b>			
Crude protein**	13.97	16.14	18.20
Digestible energy* (Kcal/kg diet)	2620.0	2647.0	2676.0
Crude fiber**	13.10	13.22	13.29
Ether extract**	2.6	2.5	2.4

\*Calculated according to NRC (1977).

\*\*Determined values according to A.O.A.C. (1980).

The animals were fed the experimental diets for 45 days as a preliminary period. Fresh water was available *ad-libitum* during the whole trial period. The bucks were kept indoors, in individual wire cages under natural environmental conditions. The averages of ambient temperature degrees; relative humidity percentages and temperature humidity index (THI)

values during winter and summer (experimental seasons) are shown in Table 2. THI values were calculated according to live stock and Poultry Heat Stress Indices, Agriculture Engineering Technology Guide, Clemson University, Clemson, Sc 29634, USA. The following equation was used in THI calculation.

THI = dry bulb temperature values less than 82; moderate heat stress for 82 to < 84; severe heat stress for 84 to < 86 and very severe heat stress for the higher values.

According to this guide, there is no heat stress for THI

**Table 2. Average of ambient temperature degrees (C°); relative humidity percentages (%) and THI values during the experimental seasons.**

Trail period	Temperature (C°)	Relative humidity (%)	THI	Level of heat stress
<b>Winter</b>				
January	16.9±0.2	73.3±1.3	61.31	Null
February	17.0±0.4	64.9±2.2	61.71	Null
March	19.2±0.3	64.4±1.9	64.88	Null
<b>Summer</b>				
July	30.5±0.3	82.8±3.5	84.15	Sever
August	30.6±0.2	86.3±2.0	85.57	Sever
September	30.1±0.5	88.7±1.5	84.43	Sever

Semen was collected from the bucks by means of an artificial vagina, since they trained for artificial collection of semen using a proper artificial vagina as reported by Heidbrinik *et al.* (1980). A live doe rabbit was used as a teaser. Ejaculates were collected artificially from each buck twice a week during the two seasons of the year (winter and summer). Immediately after

collection, semen was transferred to a heated block at 35 C° and all manipulation were conducted using warmed glassware. Ejaculate volume was estimated by calibrated pipette. Progressive linear motility was determined using light microscope according to Wellett and Salisbury (1942). Concentration of spermatozoa was determined using Haemocytometer. A smear of each

ejaculate was stained with eosin-nigrosin (Weitze and Muller, 1991) to evaluate dead and abnormalities percentages of sperm cells.

Fertility of bucks was examined using a total number of 72 multiparous does in each season (24 does per group i.e. three does per buck). Estimate of fertility was based on conception rate and litter size at birth produced by the mated does.

Blood samples were collected from the ear vein of three bucks from each group during each season. Blood received from each buck was collected in non-heparinized tubes and centrifuged at 3000 rpm for 15 minutes. Serum was separated, then stored at  $-20^{\circ}\text{C}$  for chemical analysis. Serum was analyzed for total protein; albumin and total lipids content; glutamic oxaloacetic transaminase (GOT); glutamic pyruvic transaminase (GPT) and alkaline phosphatase activities using commercial kits from Diamond Diagnostics Company, Egypt.

Data were computed using GLM in SPSS program (1993) according to the following mixed model:

$$Y_{ijk} = \mu + P_i + S_j + PS_{ij} + e_{ijk}$$

Where,  $Y_{ijk}$  = observation on the  $ijk^{\text{th}}$  ejaculate,  $\mu$  = overall mean, common elements to all observation;  $P_i$  = fixed effect of  $i^{\text{th}}$  protein level ( $i = 14; 16$  or  $18\%$ );  $S_j$  = fixed effect of  $j^{\text{th}}$  season of the year ( $j =$  winter or summer);  $PS_{ij}$  = interaction effect of  $i^{\text{th}}$  protein level with  $j^{\text{th}}$  season and  $e_{ijk}$  = a random deviation of the  $k^{\text{th}}$  ejaculate, which included all the other effects not specified in the mixed model.

## RESULTS AND DISCUSSION

### 1. SEMEN TRAITS:

#### *PROTEIN EFFECT.*

The effects of dietary protein level on semen traits were insignificant (Table 3), however there was a slight improvement in sperm output (sperm concentration / ml or / ejaculate) for bucks fed high protein levels (16 or 18%) as compared with that of 14% protein. The other traits (ejaculate volume; progressive motility and dead or abnormal spermatozoa) did not show any definite trend. The present findings were closely agree with those obtained by Luzi *et al.*, (1996) and Papadomichelakis *et al.*, (2000). Since, they didn't found any significant effect for

**Table 3: Effect of dietary protein level; season and their interactions on semen characteristics (X±SE) of New Zealand White rabbit bucks.**

Items	Traits					
	Ejaculate volume (ml)	Progressive motility (%)	Sperm cell concentration (x10 <sup>6</sup> /ml)	Sperm concentration (x10 <sup>6</sup> /ejaculate)	Dead sperm (%)	Abnormal spermatozoa (%)
<b>Protein levels(%)</b>	ns	ns	ns	ns	ns	ns
14	0.78±0.04	61.01±1.53	259.28±19.52	191.24±15.79	16.31±1.27	23.80±1.26
16	0.74±0.03	62.29±1.41	277.86±15.50	200.77±12.57	15.84±0.91	22.74±1.23
18	0.74±0.02	61.64±1.61	300.57±21.45	220.23±17.99	16.16±1.03	26.59±1.57
<b>Seasons</b>	**	***	*	***	***	***
Winter	0.80±0.03	69.38±0.95	309.98±16.65	235.60±14.45	12.19±0.57	17.86±0.72
Summer	0.71±0.02	53.85±1.27	248.61±13.96	172.56±10.31	20.06±1.04	30.96±1.25
<b>Interactions</b>	**	ns	ns	ns	***	ns
14% x winter	0.90±0.07 <sup>a</sup>	68.00±1.52	281.64±28.31	229.63±24.57	8.87±0.53 <sup>d</sup>	17.13±1.12
14% x summer	0.66±0.03 <sup>c</sup>	53.82±2.41	236.91±26.76	152.86±18.71	23.97±2.15 <sup>a</sup>	30.68±1.96
16% x winter	0.73±0.04 <sup>b</sup>	68.43±1.62	298.71±16.30	223.15±17.10	13.90±1.05 <sup>c</sup>	17.77±1.16
16% x summer	0.75±0.03 <sup>b</sup>	56.14±2.08	253.86±26.38	176.34±18.24	17.77±1.45 <sup>bc</sup>	27.71±2.01
18% x winter	0.76±0.04 <sup>b</sup>	71.71±1.77	349.57±37.62	254.02±31.51	13.79±1.14 <sup>c</sup>	18.69±1.45
18% x summer	0.72±0.03 <sup>b</sup>	51.57±2.08	251.57±19.19	186.45±16.69	18.54±1.69 <sup>b</sup>	34.49±2.45

\* = Significant (P<0.05); \*\* or \*\*\* = highly significant (P<0.01 or 0.001) and ns = not significant.

protein level on these traits of semen. Improvement was observed by Nizza *et al.*, (2000) for sperm concentration/ml and ejaculate volume ( $P < 0.05$ ) and Hemid and Tharwat (1995) for sperm concentration/ml and dead or abnormal spermatozoa ( $P < 0.05$ ). Similarly, Abd-Elhady (2001), mentioned that ejaculate volume; sperm concentration and abnormal spermatozoa did not differ significantly by different protein levels, while sperm motility and dead spermatozoa percentages were significantly improved. Some investigators were also observed that increasing amino acids intake caused an increase in sperm output in pigs (Kim and Moon, 1990a,b). The increasing in sperm output may be attributed to the increasing of hypothalamic content of gonadotrophic releasing hormone (GnRH) as a result to increasing protein level. Since, it in turn increase serum content of LH (luteinizing hormone); FSH (follicle stimulating hormone) and testosterone (Louis *et al.*, 1994).

### **SEASON EFFECT.**

The effects of hot climate (during summer) in comparison with mild condition (during winter) on seminal characteristics are presented in Table 3. All

parameters of semen were improved significantly ( $P < 0.05$ ; 0.01 or 0.001) during winter vs. those during summer season. The present results were similar to those achieved by Amin *et al.*, (1987); Tharwat *et al.*, (1994); Finzi *et al.*, (2000) and El-Maghawry and Soliman, (2002). On the other hand, some investigators failed to find any significant effect for season on ejaculate volume; progressive motility, abnormal sperm percentage (Virag *et al.*, 1992 and EL-Masry *et al.*, 1994) and sperm concentration (Marai *et al.*, 1996). The deterioration of semen quality during summer may be due to the low level of testosterone in blood during the hot conditions (Katongole *et al.*, 1974; EL-Masry *et al.*, 1994). Since the function of the accessory glands are controlled by testosterone (Hammond *et al.*, 1983). Testosterone can also maintains the balance of hormones and prolongs the life of sperm and promotes its motility (Hsu *et al.*, 1987). It was reported that sperm concentration was paralleled with the level of testosterone in blood (El-Masry *et al.*, 1994). Also, gonadotropin releasing hormone therapy improved the reproductive performance of hot stressed bucks (Hsu *et al.*, 1987 and El-Gaafary, 1994).

The interaction effects between protein level and season did not show any significant effect on semen parameters (Table 3) except those on ejaculate volume and dead sperm percentage, which were significant ( $P < 0.01$  or  $0.001$ , respectively). Bucks fed the low level of protein during summer had the lowest value of ejaculate volume and the highest percentage of dead spermatozoa in comparison with the other groups of males. This may be attributed to the bad effect of decreasing the dietary protein level and the stress of the hot conditions during summer.

## **2. REPRODUCTIVE PERFORMANCE**

### ***PROTEIN EFFECTS.***

The results of fertility trial are presented in Table 4. Does served with bucks fed high protein level showed insignificant improvement in conception rate and litter size at birth (total or live). The present results are in agreement with those of Hemid and Tharwat (1995) and Nizza *et al.*, (2000). The improvement in fertility parameters may be due to the increasing of sperm output by feeding the high level of dietary protein (Table 3).

### ***SEASON EFFECTS.***

Data presented in Table 4 show that the reproductive performance of mated does was improved during winter as compared with that of summer. However, the improvement was significant ( $P < 0.05$ ) only for conception rate. This improvement could be attributed to the comfortable condition (temperature degree and relative humidity) during winter. Similar results were mentioned by El-Masry *et al.* (1994).

The interaction effect (protein level with season) did not show any significant difference in the reproductive performance of the mated does (Table 4). Although, a slight increase was observed in conception rate and total litter size at birth for does mated with bucks fed 18% protein during winter season.

## **3. BLOOD COMPONENTS:**

### ***PROTEIN EFFECTS.***

Values of total protein were insignificantly increased, while activity of alkaline phosphatase enzyme was insignificantly decreased as a result of increasing dietary protein level in the diets. Protein levels (Table 5) did not



consistently influence the other parameters of blood. Same results were observed also by Abd El-Hady (2001) in mature rabbits and Ayyat (1991) and Ismail (1999) in growing rabbits. On the other hand, Omar *et al.* (1997) and Abou-Zeid *et al.*, (1999) reported a significant effect of dietary protein level on total protein and globulin values. The slight improvement in total protein may be attributed to the increase of thyroxin production that stimulate protein synthesis (Habeeb *et al.*, 1989). Results presented in Table 5 indicate that dietary protein level did not affect liver function (albumin and globulin content and enzymes activity), since biochemical parameters of blood used as indicators of physiological and nutritional status of rabbits (Abou-Zeid *et al.*, 1999). Furthermore, whole albumin and most of globulin are formed intrahepatically (Sorensen, 1982).

### SEASON EFFECTS.

Data presented in Table 5 show that blood contents of total protein and its fractions (albumin and globulin) were increased during winter than during summer, however the differences between the two seasons in these parameters were significant only

for total protein ( $P<0.05$ ). These results are in agreement with those of Ayyat and Marai(1996) and Ismail (1999), who reported that the concentrations of total protein and albumin in rabbits were lower in summer than in winter. Furthermore, Marai *et al.*: (1998) mentioned that high temperature in summer inversely affected blood total protein; albumin and globulin in broiler male rabbits. The significant decrease in blood protein content due to heat stress during summer seems to be as a result to a depression of anabolic hormonal secretion such as growth hormone (Yousef and Johnon, 1966). Opposite results were reported by Abd El Hakeam *et al.*, (1991) who found that total protein; albumin and globulin were significantly ( $P<0.05$ ) higher in bucks exposed to high air temperature ( $33.8\text{ C}^\circ$ ) vs. those of the control group ( $16.4\text{ C}^\circ$ ). Total lipids content increased significantly ( $P<0.001$ ) during the hot season (summer) than that of winter (Table 5). These results were in the same line of the findings obtained by Abd El Hakeam *et al.* (1991) who observed an increase in plasma cholesterol level of male rabbits at high air temperature ( $P<0.05$ ) than

that in the control group. Other results indicated that season effect was insignificant on blood content of total lipids in mature rabbits (Ibrahim, 1994), while El-Maghawry *et al.*, (2000) reported that blood total lipids content was increased significantly ( $P < 0.01$ ) during winter than that during summer. The activities of enzymes (alkaline phosphatase and serum transaminases (GOT and GPT)) were increased significantly ( $P < 0.01$  or  $0.001$ ) during summer than during winter (Table 5). Dissident results were reviewed in the literature in this respect, since Ismail (1999) and El-Maghawry *et al.*, (2000) failed to find any significant effect for season on GOT or GPT activities in growing rabbits. While, the activity of GOT enzyme was increased significantly ( $P < 0.05$ ) during winter than that during summer in mature doe rabbits (Ibrahim, 1994). A slightly increase in the activity of GPT enzyme (Ibrahim,

1994) and GOT (El-Maghawry *et al.*, 2000) during summer season compared with that of winter in mature rabbits was also demonstrated.

The interaction effects (protein level with season) did not show any significant difference in blood parameters among the different groups of bucks fed high or low level of dietary protein during winter or summer season.

Conclusively, It could be concluded that increasing dietary protein level from 14 to 16 or 18% for rabbit bucks showed some beneficial effects on semen quality; Blood parameters and the reproductive performance of mated does, especially under the hot climate. These traits were extremely affected during summer. The interaction effects (dietary protein level with season) on the studied traits were found to be in low magnitud.

**Table 5: Effect of dietary protein level and season of the year and their interactions on some blood chemical composition ( $\bar{X} \pm SE$ ) of New Zealand White rabbit bucks.**

Items	Traits						
	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total lipids (mg/dl)	Alkaline phosphatase (u/l)	GOT (u/l)	GPT (u/l)
<b>Protein levels(%)</b>	ns	ns	ns	ns	ns	ns	ns
14	6.56±0.26	4.59±0.35	1.97±0.30	347.77±21.68	7.49±0.35	32.32±2.30	35.83±2.11
16	6.59±0.29	4.45±0.31	2.14±0.25	335.25±20.26	7.46±0.36	32.44±2.60	33.67±2.10
18	6.67±0.26	4.85±0.26	1.82±0.12	354.00±17.76	7.24±0.37	37.02±3.02	33.80±2.53
<b>Seasons</b>	*	ns	ns	***	**	**	**
Winter	7.01±0.25	4.82±0.31	2.18±0.25	299.24±14.17	6.69±0.20	30.31±1.24	30.85±1.11
Summer	6.21±0.13	4.42±0.16	1.78±0.09	391.47±7.62	8.11±0.26	37.54±2.05	38.01±1.24
<b>Interactions</b>	ns	ns	ns	ns	ns	ns	ns
14% x winter	7.08±0.40	4.88±0.71	2.21±0.64	279.60±13.99	6.87±0.36	31.14±2.44	33.34±1.69
14% x summer	6.12±0.25	4.35±0.31	1.77±0.17	404.50±13.22	8.01±0.50	33.50±3.42	38.32±2.67
16% x winter	7.10±0.50	4.77±0.62	2.34±0.50	278.33±21.34	6.65±0.36	29.02±2.05	30.18±1.78
16% x summer	6.08±0.11	4.13±0.85	1.95±0.12	392.17±7.41	8.26±0.43	35.86±3.71	37.15±2.04
18% x winter	6.84±0.43	4.84±0.41	2.01±0.14	336.50±28.37	6.58±0.35	30.78±2.28	29.04±2.10
18% x summer	6.47±0.29	4.87±0.37	1.60±0.15	375.00±18.00	8.04±0.52	43.26±2.26	38.56±1.90

\* = Significant ( $P < 0.05$ ); \*\* or \*\*\* = highly significant ( $P < 0.01$  or  $0.001$ ) and ns = not significant.

Table 4: Reproductive performance (X±SE) of mated dose.

Items	Traits			
	Conception rate(%)	Litter size at birth	Live litter size at birth	Stillbirths
<b>Protein levels(%)</b>	ns	ns	ns	ns
14	54.17±7.27	5.88±0.37	5.58±0.33	0.31±0.12
16	54.66±7.25	6.02±0.39	5.78±0.41	0.25±0.12
18	61.87±7.17	6.42±0.38	6.06±0.41	0.37±0.11
<b>Seasons</b>	*	ns	ns	ns
Winter	66.58±5.65	6.21±0.30	6.00±0.31	0.21±0.07
Summer	47.22±5.92	6.02±0.32	5.62±0.34	0.40±0.13
<b>Interactions</b>	ns	ns	ns	ns
14% x winter	66.67±9.83	5.81±0.53	5.50±0.47	0.31±0.15
14% x summer	41.67±10.28	6.00±0.52	5.70±0.47	0.30±0.21
16% x winter	63.50±10.10	6.13±0.53	6.00±0.55	0.13±0.09
16% x summer	45.83±10.39	5.91±0.59	5.55±0.64	0.36±0.24
18% x winter	69.57±9.81	6.69±0.52	6.50±0.53	0.19±0.10
18% x summer	54.17±10.39	6.15±0.58	5.62±0.65	0.54±0.22

\* = Significant (P<0.05) and ns = not significant.

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## صفات السائل المنوي والخصوبة وصفات الدم لذكور أرانب النيوزلندي الأبيض تحت تأثير مستوى بروتين العليقة فى الطقس المعتدل والحر

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لدراسة تأثير مستوى بروتين العليقة والظروف البيئية على صفات السائل المنوي وخصوبة الذكر وقياسات الدم. تم تغذية ثلاثة مجاميع من ذكور أرانب النيوزلندي الأبيض (٨ ذكور / المجموعة) على ثلاث علائق (١٤ ، ١٦ ، ١٨% بروتين خام) خلال موسمين (الشتاء - الصيف). أظهرت النتائج أن تركيز الحيوانات المنوية فى الملى أو فى القذفة قد تحسن بدرجة غير معنوية فى الذكور المغذاة على علائق عالية فى البروتين الخام (١٦ أو ١٨%) ولو أن حجم القذفة والحركة التقدمية ونسبة الحيوانات المنوية الميتة والشاذة لم تظهر أى اتجاه محدد نتيجة لتأثير مستوى البروتين. تحسنت كل صفات السائل المنوي معنوياً (على مستوى أقل من ٠.٠٥ و ٠.٠١ أو ٠.٠٠١) أثناء فصل الشتاء مقارنة بمثلتها أثناء الصيف. تأثير مستوى البروتين فى العليقة على صفات الدم كان غير معنوي. محتوى الدم من البروتين الكلى زاد معنوياً (على مستوى أقل من ٠.٠٥) بينما قل المحتوى من الدهن الكلى وكذلك نشاط إنزيم الألكالين فوسفاتيز ، GOT ، GPT (على مستوى أقل من ٠.٠١ ، ٠.٠٠١) أثناء الشتاء مقارنة بتلك التى كانت أثناء الصيف. أظهرت الإناث الملقحة من الذكور المغذاة على علائق عالية فى مستوى البروتين تحسن فى نسبة الإخصاب وحجم البطن عند الميلاد (الكلى والحي). الأداء التناسلى للإناث الملقحة قد تحسن أثناء الشتاء مقارنة بتلك الذى حدث أثناء الصيف ولو أن التحسن كان معنوي (على مستوى أقل من ٠.٠٥) فقط فى نسبة الإخصاب. لم يظهر الأثر المتداخل بين مستوى البروتين والموسم أى اختلاف معنوي فى الصفات المدروسة فيما عدا حجم القذفة ونسبة الحيوانات المنوية الميتة والذى كان معنوياً (على مستوى أقل من ٠.٠١ أو ٠.٠٠١ على التوالى).

زيادة مستوى البروتين فى العليقة من ١٤ إلى ١٦ أو ١٨% لذكور الأرانبي أظهر تحسن طفيف فى جودة السائل المنوي، وصفات الدم والأداء التناسلى للإناث الملقحة. هذه الصفات تأثرت بدرجة كبيرة أثناء فصل الصيف ولكن الأثر المتداخل على هذه الصفات كان محدود.