Physiological Body Reactions and Semen Characters of Rabbit Bucks as Affected by Breed and Vitamin C Supplementation under Egyptian Summer Conditions

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Abstract: This experiment was carried out at the rabbitry of the Experimental Farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Twenty four adult rabbit males were used in this study (12 New Zealand White (NZW) and 12 Californian (Cal) rabbit bucks) to study the effect of Vit C ingestion (20 mg/kg body weight/ day for 45 days) on alleviating the effects of high housing temperature and improving semen quality parameters and some physiological traits of rabbit bucks during summer season. Generally, summer heat adversely affected the physiological performance of the two rabbit breed bucks. However, most of the studied traits were differed (P≤0.05) due to the breed effect. Cal breed had lower (P≤0.05) rectal temperature (RT), and higher (P≤0.05) WBCs values than NZW. Thus, Cal breed had lower (P≤0.05) reaction time and semen pH values than in NZW breed. Moreover, Cal breed had higher (P≤0.05) ejaculate volume, percentage of live sperm and sperm output than NZW breed. Irrespective of breed of rabbits, ingestion of Vit C improved (P≤0.05) most studied traits under summer conditions. Addition of Vit C decreased (P≤0.05) RT, respiration rate (RR) and pulse rate (PR) and increased (P≤0.05) values of Hb value, Ht % and WBCs counts compared to the control group during summer period. Furthermore, addition of Vit C decreased (P≤0.05) reaction time and semen pH and increased (P≤0.05) ejaculate volume (with and without gel) and % of forward motility of spermatozoa compared to the control group. Increased (P≤0.05) percentage of live sperm, sperm concentration and sperm output and decreased (P≤0.05) percentage of sperm acrosome alteration and sperm abnormalities was observed in Vit. C treated group compared to the control group. In conclusion this study sugessted that the Cal breed was more heat tolerant and adapted to heat stress than NZW breed under Egyptian summer condition. Also, the addition of Vit C to drinking water alleviated the stressful effects of high temperature on semen characteristics and physiological traits in rabbit bucks during summer season.

Keywords: Rabbit bucks, heat stress, physiology, semen quality, Vit C.

INTRODUCTION

All rabbit breeds at different ages can be stressed by extreme weather and no matter if it is extreme heat or extreme cold. When the weather turns extreme or is prolonged in what might be called heat waves, rabbits can suffer. The most obvious limitation to rabbit production in hot climate area is the susceptibility of this species to heat stress, which evokes a series of drastic changes in their biological functions that lead to impairment of production and reproduction (Marai *et al.,* 1991; Fernandez *et al.,* 1994). The thermoneutral zone of growing rabbits at 6-12 weeks of age is 15-18ºC (Rafai *et al.,* 1972). Rabbits can tolerate up to about 27^{o}C degrees if it isn't a 'humid' heat. Above 27^{o}C degrees in any humidity range, rabbits will suffer dramatically in those thick coats of fur. Rabbits cannot sweat, so they cannot lose excess body heat like other animals do.

High temperature in summer (for example 30^{o}C.) decreases libido and biological semen characteristics in rabbits. In such stress condition, decreases in sperm motility and percentage of live cells are reported by Marai *et al.* (2002). In addition, increasing temperature humidity index (THI) up to 38.9 resulted in significant reduction in rabbit semen quality parameters such as number of spermatozoa, motility and acrosome integrity (Marai *et al.,* 2002).

To overcome the adverse effect of heat stress, many researches had been supplementing the diet of heat stressed rabbits with Vitamin C and showed improved growth traits and some reproductive performance parameters (Abdel-Hamid and El-Adawy, 1999; Yousef *et al.,* 2003; Shebl *et al.,* 2008).

This work was planned to study the differences between two rabbit breeds on semen characteristics and physiological response of male California and New Zealand rabbit bucks during summer season. Additionally the effect of rabbit's ingestion of Vit C (20 mg/kg BW/day) on alleviating the effects of high housing temperature and humidity during summer season on the two rabbit breed bucks was also studied.

MATERIALS AND METHODS

Animals and husbandry

This experiment was carried out during summer season at Rabbitry of the Experimental Farm belonging to Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. A total of Twenty four mature male rabbits of the two breeds were used (12 NZW and 12 Cal). Each breed was divided into two equal groups (6 bucks each, treatment and control), the treated groups were supplemented with Vitamin C as sodium salt of L-ascorbic acid (AA) (Pharco Pharmaceutical Company, Egypt). The ascorbic acid was dissolved in distilled water to yield a final concentration of 2.0 %. The used dose of AA was 20 mg/kg body weight/ day and was given orally to rabbits once daily at 08:00 for 15 days before data collection and extended during the experimental period. This experiment was carried out throughout one month representing middle of summer season (15 July to 15 August). The animals were apparently healthy and free of any external parasites or

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skin diseases. Age of male rabbit averaged 10 months and their weight averaged 3.5±0.2 kg. Animals were individually housed in galvanized wired cages, where feed and water were provided *ad libitum*. Animals were fed on basal pellet ration contained yellow corn, soybean meal, corn gluten, minerals and vitamins premix, bone and molasses. The calculated chemical components of the diet were 17% crude protein, 2.8% fat, 10% crude fiber and 2600 kcal digestible energy/kg diet. Animals were subjected to 16 hrs light/day.

Data collected

Physiological body reaction

Rectal temperatures (RT), respiration rate (RR) and pulse rate (PR) were recorded in the morning between 08:00-10:00 am for each buck. Rectal temperature was measured by using a clinical thermometer. Respiration rate was measured by counting the movements of the chest fleece. Pulse rate was measured by counting pulses in the femoral artery with a finger for a period of one minute.

Blood sampling and analysis

Blood samples were obtained from each buck from the ear vein in heparinized tubes. Blood collection samples were carried in the morning between 8:00-10:00 am to avoid diurnal variation in the blood picture. Red blood cells (RBCs) and white blood cells (WBCs) were counted in fresh blood samples using hemocytometer. Hemoglobin concentration was determined using hemometer as the method described by Tietz (1982). Blood was withdrawn in hematocrit capillary tube and centrifuged at 6000 rpm for 15 min. Hematocrit value (%) was measured directly according to Winderobe (1965). The blood samples were centrifuged at 3000 rpm for 20 min and the plasma was stored at -20^{o}C till analysis.

Semen collection and evaluation

Semen was collected from each buck three times weekly by rabbit artificial vagina. Reaction time was done using a stopwatch to determine the time the buck spent to give an ejaculate from time of introducing the female rabbit to his cage. Semen evaluation was carried out immediately after collection. Semen evaluation included ejaculate volume, pH value, sperm forward motility, sperm concentration and sperm live and dead percentages. Ejaculate volume was measured to the nearest 0.1 ml using a graduated collection tube. Sperm forward motility was estimated microscopically at 400X. Semen pH was estimated by using pH indicator papers ranging from 6.0 to 8.0 with 0.2 grades. Sperm concentration per ejaculate was determined using the Hemocytometer technique. The percentage of dead spermatozoa was estimated using eosin-negrosin stain technique. Percentage of sperm abnormalities were determined in the same smears prepared for live/dead sperm according to the method of Blom (1983). Acrosome integrity was evaluated according to the method of Bryan and Akruk (1977).

Meteorological parameters

Ambient temperature (ºC) and relative humidity (%) inside rabbitry were recorded daily during the experimental work (summer) by using air dry bulb thermometer and hygrometer. The temperature–humidity index (THI) was estimated according to the formula of Marai *et al*. (2001) as follows:

THI = dbºC – [(0.31 – 0.31 RH) (dbºC – 14.4)],

where db ^{o}C=dry bulb temperature in Celsius and Rh=RH%/100.

The estimated values of THI were classified as follows: less than 22.2= absence of heat stress, 22.2-23.2= moderate heat stress,

23.3-25.5= severe heat stress and more than 25.5= very severe heat stress.

Statistical analysis

Data were analyzed using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc., 1998). Least Square Means (LSM) were calculated and Least Square Differences (LSD) between means was tested. Two-way analysis of variance (ANOVA-test) was carried out using the following model:

$$Y_{ijk} = \mu + C_i + B_j + CB_{ij} + e_{ijk}$$

Where:

Y_{ijk} = the observation on the k^{th} individual from the i^{th} Vit C in j^{th} breed.

μ = the overall mean.

C_i = the fixed effect of the i^{th} Vit C.

B_j = the fixed effect of the j^{th} breed.

CB_{ij} = the interaction between i^{th} Vit C and j^{th} breed.

e_{ijk} = the random error associated with the ijk^{th} individual.ijk^{th} individual.

RESULTS AND DISCUSSION

1. Meteorological parameters

Data in Table (1) showed the averages of ambient temperature (^{o}C), relative humidity (%) and temperature-humidity index (THI) in the rabbitry during summer season conditions. Average of temperature-humidity index (THI) value during experiment was 28.65 which represent a very severe heat stress during summer season as reported by Marai *et al.* (2008).

Table (1): Averages of ambient temperature (^{o}C), relative humidity (%) and temperature-humidity index (THI) in the rabbitry during the summer season.

Season	Ambient temperature (^{o}C)			Relative humidity (%)	Temperature-humidity index (THI)
	Max	Min	Mean		
Summer	36.15±1.38	25.28±0.89	30.71±0.89	58.12±7.79	28.65

2. Physiological body reaction responses

Table (2) showed environmental effects of summer season on male rabbit's physiological body reactions and the effect of Vit C ingestion on alleviating heat stress during summer season. Bucks of Cal breed were significantly lower in RT value than that in NZW bucks. Treatment with Vit C decreased (P≤0.05) RT, RR and PR values in treated groups compared to the control group (without Vit C). These results are in agreement with Shebl *et al.* (2008) who found that administration of Vit C to pregnant rabbits reduced the increase in all physiological body reactions and alleviated the effect of heat stress. Abdel-Kariem *et al.* (2002) reported that rectal temperature of male rabbits was significantly (P<0.05) lower in the treated groups with vitamins (Vit A & E) than the control group under Egyptian conditions. Furthermore, the beneficial effect of Vit C in alleviating heat stress was reported by Alam (2000) and Marai *et al.* (2002). Alam (2000) stated that Vit C is involved in the synthesis of some stress hormones such as epinephrine. This hormone controls respiration rate, blood flow and pressure and maintains body temperature almost constant during heat stress.

Table (2): Physiological body reactions of New Zealand White (NZW) and California (Cal) rabbits as affected by breed and Vit C supplementation under Egyptian summer conditions (Means ± S.E.)

Items		Hemoglobin (g/100ml)	Hematocrit (%)	RBCs ($x10^6$/ml)	WBCs ($x10^3$/ml)
Breed (B)					
	NZW	9.33±0.13	25.77±0.51	3.51.±0.08	5.05±0.29 [b]
	Cal	9.80±0.18	26.00±0.59	3.72±0.07	6.33±0.23[a]
Vit C (C)					
	+ Vit C	10.30±0.18[a]	27.92±0.43 [a]	3.66±0.07	7.00±0.24 [a]
	- Vit C	8.84±0.12 [b]	23.85±0.55 [b]	3.57±0.08	4.38±0.21 [b]
(BxC)					
	NZW + Vit C	10.11±0.19	27.82±0.83	3.45±0.12	6.33±0.31
	NZW – Vit C	8.55±0.23	23.73±0.81	3.48±0.11	3.77±0.34
	Cal + Vit C	10.48±0.25	28.02±0.82	3.77±0.12	7.66±0.32
	Cal – Vit C	9.13±0.27	23.97±0.79	3.66±0.12	5.00±0.29

[ab]Means bearing different superscripts in the same column, differ (P≤0.05)

3. Hematological parameters of rabbit bucks

The results of hematological parameters of rabbit bucks as affected by both breed and addition of Vit C are presented in Table (3). Cal breed had higher (P<0.05) WBCs values than those in NZW breed under summer season condition.

The ingestion of Vit C increased (P<0.05) values of Hb, Ht % and WBCs counts in treated animals compared to control group (without Vit C). The increase in some hematological parameters in treated bucks due to ingestion of Vit C indicted more resistance of animals to adverse effects of summer stressful condition. In addition, the high WBC count trend in Cal breed than in NZW was more pronounced in treated bucks with Vit C than in controls during summer period. The present results indicated that addition of Vit C alleviated the stressful effect of heat stress during summer months.

4. Semen quality characters of rabbit bucks

4.1. Reaction time, pH, ejaculate volume and initial motility percent

The effects of breed and addition of Vit C on reaction time, pH, ejaculate volume and forward motility % of spermatozoa are presented in Table (4). Cal breed had lower (P<0.05) reaction time and semen pH and higher (P<0.05) ejaculate volume than those found in NZW breed. However, Abd Elhakeam *et al.* (1992) reported significant high values of ejaculate volume, higher pH and low sperm motility in NZW than in that in Cal breed during November and December. The difference between the two results are mainly due to environmental conditions.

Several investigators reported the phenomenon of semen quality differences between breeds, where the average of ejaculate volume, pH values and sperm motility % of semen varied according to breed (Abd Elhakeam *et al.,* 1992). In addition, Ibrahim (1994) reported that Cal breed had insignificantly lower values in pH than those in NZW rabbit bucks. The difference in pH values between two breeds could be due to the differences in chemical constituents of seminal plasma and semen motility, in which Cal had higher semen motility % than NZW breed. The high rate of sperm motility decreases the pH of semen more rapidly than that of lower motility samples (El-Sheikh and Mahmoud, 1967). Moreover, Ibrahim (1994) recorded that Cal breed had insignificantly higher sperm motility % than NZW rabbit bucks. The present results disagreed with the results of Ibrahim (1994) who found

insignificant differences between Cal and NZW in reaction time and pH values during summer and winter seasons.

Moreover, addition of Vit C decreased (P<0.001) reaction time and semen pH and increased (P<0.001) ejaculate volume and forward motility % of spermatozoa in comparison with the control group. These results are in agreement with those reported previously (Salem *et al.,* 2001; Yousef *et al.,* 2003). Shebl *et al.* (2008) reported that addition of Vit C to rabbits exposed to heat stress maintained the level of Triiodothyronine (T_3) to be at normal levels and alleviated significantly the effect of thermal heat stress. In adult animals, the thyroid gland is considered to play an important role in the adaptive phenomena associated with changes in environmental temperatures (Bobek *et al.,* 1996). On the other hand, many authors reported the beneficial effects of Vit C in detoxification of different toxic material and enhancing the immune system (Head, 1998) and in prevention of cellular free radical damage (Dumitrescu *et al.,* 1993). Thus, the oral supplementation of Vit C, which is a free radical scavenger, may protect the animals from the harmful effects of environment such as severe heat stress.

Table (3): Hematological parameters of New Zealand White (NZW) and California (Cal) rabbits as affected by breed and Vit C supplementation under Egyptian summer conditions (Means ± S.E.)

Items		Rectal temperature (^{o}C)	Respiration rate (R/m)	Pulse rate (P/m)
Breed (B)				
	NZW	39.21± 0.11 [a]	117.70±0.98	127.45±0.99
	Cal	38.69± 0.10 [b]	115.37±0.99	126.20±0.96
Vit C (C)				
	+ Vit C	38.62± 0.10 [b]	113.70± 0.93 [b]	121.83±0.95 [b]
	- Vit C	39.28± 0.11 [a]	119.37±0.98 [a]	131.83±0.97 [a]
(BxC)				
	NZW + Vit C	38.85±0.15	113.75±1.42	122.66±1.39
	NZW – Vit C	39.58±0.17	121.66±1.41	132.25±1.42
	Cal + Vit C	38.47±0.15	113.66±1.40	121.00±1.40
	Cal – Vit C	38.95±0.13	117.08±1.41	131.41±1.41

[a,b]Means bearing different superscripts in the same column, differ (P≤0.05)

Table (4): Reaction time, ejaculate volume, semen pH and forward motility of New Zealand White (NZW) and California (Cal) rabbits as affected by breed and Vit C supplementation under Egyptian environmental conditions (Means ± S.E.)

Items		Reaction time (Sc)	Ejaculate volume (ml) (+) gel	Ejaculate volume (ml) (-) gel	pH	Forward motility (%)
Breed (B)						
	NZW	30.33±3.48 [a]	0.33±0.02 [b]	0.31±0.02 [b]	7.91±0.04 [a]	68.02±1.52
	Cal	19.87±3.42 [b]	0.45±0.04 [a]	0.44±0.03 [a]	7.69±0.06 [b]	71.66±1.48
Vit C (C)						
	+ Vit C	17.37±3.35 [b]	0.48±0.05 [a]	0.45±0.01 [a]	7.69±0.05 [b]	76.04±1.52 [a]
	- Vit C	32.83±3.48 [a]	0.32±0.02 [b]	0.30±0.02 [b]	7.91±0.04 [a]	63.64±1.57 [b]
(BxC)						
	NZW +Vit C	21.08±4.92	0.39±0.04	0.38±0.03	7.77±0.06	73.12±2.25
	NZW – Vit C	39.58±4.85	0.27±0.03	0.24±0.04	8.06±0.07	62.91±2.15
	Cal + Vit C	13.66±5.12	0.53±0.05	0.52±0.03	7.62±0.06	78.95±2.18
	Cal - Vit C	26.08±4.79	0.37±0.03	0.36±0.02	7.77±0.05	64.37±2.19

[a,b]Means bearing different superscripts in the same column, differ (P≤0.05)

4.2. Sperm abnormalities, acrosome integrity, sperm concentration and sperm output:

Data presented in Table (5) showed the effects of breed and addition of Vit C on % of live spermatozoa, acrosome integrity, abnormalities, sperm concentration and sperm output of rabbit bucks under summer conditions. Cal breed had higher (P<0.05) values of % of live sperm and sperm output than NZW. In contrast, Ibrahim (1994) reported that, Cal breed had significantly (P<0.05) lower sperm concentration/ml and higher sperm abnormalities than NZW rabbit bucks during summer and winter season.

In addition, significant differences (P<0.05) were found between Vit C treatments in live spermatozoa %, acrosome integrity %, abnormalities %, sperm concentration of rabbit bucks under summer conditions (Table 5). Treatment with Vit C increased (P<0.05) live spermatozoa and sperm concentration and sperm output and decreased (P<0.05) acrosome integrity % and abnormalities % when compared to the control group. These results are in agreement with those obtained by Harris *et al.* (1979), Dawson *et al.* (1986) and Salem *et al.* (2001) who found that the administration of Vit C caused significant improvements in rabbit semen characteristics and male fertility. Moreover, Salem *et al.* (2001) and Yousef *et al.* (2003) reported that addition of Vit C improved significantly sperm concentration, and decreased significantly dead and abnormal sperm than in control group in male rabbits. Moreover, Abd Elhalim (2008) and Shebl *et al.* (2008) found that supplementation of Vit C to pregnant rabbits improved productive and reproductive performance under summer conditions.

Table (5): Live spermatozoa, acrosome integrity, abnormalities, sperm concentration of New Zealand White (NZW) and California (Cal) rabbits as affected by breed and Vit C supplementation under Egyptian environmental conditions (Means ± S.E.).

Items		Live spermatozoa (%)	Acrosome integrity (%)	Abnormalities (%)	Sperm concentration ($x10^6$/ml)	Sperm concentration ($x10^6$/ejaculate)
Breed (B)						
	NZW	72.27±1.75 [b]	11.02±0.48	24.50±1.33	463.33±25.54	155.77±15.21 [b]
	Cal	78.93±1.85 [a]	10.37±0.52	22.00±1.31	495.00±25.04	230.16±15.41 [a]
Vit C (C)						
	+ Vit C	81.58±1.58 [a]	9.64±0.50 [b]	17.41±1.31 [b]	560.20±24.67 [a]	255.06±16.34 [a]
	- Vit C	69.62±1.52 [b]	11.7±0.48 [a]	29.11±1.33[a]	398.12±25.04 [b]	130.87±15.67 [b]
(BxC)						
	NZW + C	77.71±1.78	10.08±0.59	17.92±1.79	389.58±35.67	207.21±21.34
	NZW- C	69.84±1.81	11.95±0.68	30.96±1.84	583.33±35.59	104.33±22.04
	Cal + C	84.45±1.87	9.20±0.61	16.88±1.86	409.66±36.12	302.91±21.68
	Cal - C	72.42±1.86	11.54±0.63	27.13±1.81	409.66±36.12	157.41±21.54

[a,b]Means bearing different superscripts in the same column, differ (P<0.05).

CONCLUSION

It could be concluded that, Cal breed was significantly better in most studied traits than that of NZW breed under summer season, and it indicated that the Cal breed tolerated heat stress more than NZW under Egyptian summer climate. On the other hand, addition of ascorbic acid (Vitamin C) at a dose of 20 mg/kg BW/day to rabbit bucks exposed to heat stress reduced the pronounced adverse effects of heat stress on most of the physiological parameters and semen quality traits. This treatment could be used as a routine treatment in rabbit commercial production farms during summer season.

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تفاعلات الجسم الفسيولوجية و صفات السائل المنوي لذكور الأرانب المتأثرة بالنوع و إضافة فيتامين ج خلال ظروف الصيف المصري

حسن عبد الغفار خليل، وليد حسين كشك، رابعة أحمد ثروت، محمد أحمد يس، مصطفى عبد الستار أيوب
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أجريت هذه التجربة في مزرعة الأرانب التجريبية بكلية الزراعة جامعة قناة السويس، محافظة الإسماعيلية. استخدم في هذه الدراسة ٢٤ ذكر بالغ (١٢) من سلالة النيوزيلاندى الأبيض و (١٢) من سلالة الكاليفورنيا بهدف دراسة تأثير استخدام فيتامين ج (٢٠ ملليجرام/كجم وزن حي/ذكر/يوم لمدة ٤٥ يوم) في تقليل تأثيرات الحرارة العالية للمساكن وتحسين صفات السائل المنوي و بعض القياسات الفسيولوجية على ذكور الأرانب خلال فصل الصيف. أظهرت النتائج أن لحرارة الصيف تأثيرا ضارا على الأداء الفسيولوجي لسلالتي الأرانب المستخدمة. كانت معظم المقاييس المدروسة مختلفة معنويا (٠.٠٥) بسبب اختلاف السلالة. ولقد كانت سلالة الكاليفورنيا أقل معنويا (٠.٠٥) في قيمة درجة حرارة المستقيم و أعلى معنويا (٠.٠٥) في قيمة عدد كرات الدم البيضاء عن النيوزيلندي الأبيض. أظهرت سلالة الكاليفورنيا انخفاضا معنويا (٠.٠٥) في زمن جمع السائل المنوي و درجة حموضة السائل المنوي عن النيوزيلاندى. كما كان حجم القذفة المنوية ، نسبة الحيوانات المنوية الحية و المخرج من الحيوانات المنوية أعلى (٠.٠٥) في الكاليفورنيا عن النيوزيلاندى. بغض النظر عن السلالة أظهر تناول فيتامين ج تحسنا ملحوظا (٠.٠٥) في معظم المعايير المدروسة خلال ظروف فصل الصيف. أتضح ذلك عند إضافة فيتامين ج للأرانب حيث حدث انخفاض (٠.٠٥) في درجة حرارة المستقيم و معدل النبض و زيادة معنوية (٠.٠٥) في قيم الهيموجلوبين و الهيماتوكريت وعدد كرات الدم البيضاء مقارنة بمجموعة الكنترول القياسية خلال فصل الصيف. بالإضافة لذلك فأن إضافة فيتامين ج قللت معنويا (٠.٠٥) زمن قذف السائل المنوي ودرجة حموضة السائل المنوي وزودت حجم السائل المنوي وحيوية الحيوانات المنوية مقارنة بالمجموعة القياسية.كما زادت معنويا (٠.٠٥) قيم نسب الحيوانات المنوية الحية و تركيز الحيوانات المنوية في القذفة و المخرج من الحيوانات المنوية و قللت (٠.٠٥) نسب الحيوانات المنوية الشاذة و ذات كروسوم غير طبيعي مقارنة بالمجموعة الكنترول.
والخلاصة أن هذه الدراسة أظهرت تفوق سلالة الكاليفورنيا معنوياً علي سلالة النيوزيلاندى الأبيض في معظم الصفات الفسيولوجية المدروسة تحت الاجهاد الحرارى فى فصل الصيف. كما اظهرت النتائج أن اضافة فيتامين ج بمعدل ٢٠ ملليجرام/كجم وزن حى/ذكر/يوم كان لة تأثيراً ايجابياً ومعنوياً على معظم الصفات المدروسة خلال فصل الصيف.