

## **EFFECT OF GREEN TEA AS DIETARY SUPPLEMENTS (*Camellia sinensis*) ON SEMEN QUALITY AND TESTOSTERONE PROFILE IN RABBITS.**

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### **ABSTRACT**

This study aimed to investigate effect of green tea level as a dietary additive on semen quality and testosterone concentration of adult APRI line rabbit bucks. A total of 12 adult bucks were allotted into 4 equal groups fed complete feed diet (17.4% CP and 2257 Kcal metabolizable energy on dry matter basis), without additive (T1) or supplemented with 2, 4 and 6 g green tea/kg diet (T2, T3 and T4 respectively). The experimental period was 13 weeks, 4 weeks as a preliminary period and 9 weeks as main period of semen collection. Semen was evaluated for volume (SV), pH value, and percentages of motility (SMP), livability (SLP) and abnormality (SAP) of spermatozoa as well as sperm cell concentration (SCC). Sperm count as total output (TSO) and total motile (TMO), total live (TLO) and total normal (TNO) was calculated. Testosterone concentration in blood plasma was determined pre-treatment, and mid and end of the collection period. Results showed that bucks in T4 treated with 6 g green tea/kg diet showed the highest ( $P<0.05$ ) SV, pH value, SMP, SCC, TSO, TMO, TLO, TNO and the lowest ( $P<0.05$ ) SAP. However, SLP and testosterone concentration were not affected by treatment.

In conclusion, green tea demonstrates significant improvement in the antioxidant status, as shown by the increased antioxidant enzyme activity and GSH levels. Green tea could serve as a supportive treatment in the nutritional management to improve semen quality of rabbit bucks, particularly at a level of 6 g/kg diet.

Keywords: Rabbit, green tea, semen, sperm output, testosterone.

### **INTRODUCTION**

Artificial insemination (AI) is widely used on rabbit farms. It is essential for "cycled production" which has radically improved animal management in intensive rabbit farming system. In this production system, evaluation of buck semen has a particular importance in the choice of breeders, as occurred in other livestock species (Mangiagalli *et al.*, 2012). Semen quality is the guarantee of successful insemination in breeding rabbits. There are many endogenous and exogenous factors affecting reproduction of rabbit bucks.

Tea is among the most highly consumed beverages worldwide. It is produced from the leaves of the plant *Camellia sinensis* (Costa *et al.*, 2002). Tea is consumed in different parts of the world as green, black, or oolong tea (Kenji Sato *et al.*, 2010). Green tea is produced by drying and steaming the fresh leaves and no fermentation or oxidation occurs (Zuo *et al.*, 2002). Green tea is one of the most commonly consumed beverages worldwide (Figueiroa *et al.*, 2009) and is considered to have beneficial effects on health due to his high content in polyphenols, e.g. epigallocatechin-3-gallate

(EGCG), which is known to possess anti-oxidative properties (Soussi *et al.*, 2006; Trevisanato and Kim 2000). Polyphenols found in green tea show 20 times more powerful antioxidant activity than vitamin C (Craig, 1999; Elhalwag *et al.*, 2008). Administration of antioxidants such as vitamin E, selenium, vitamin C, and carotenoids may reduce the oxidative stress and improve sperm motility (Agarwal *et al.*, 2004; Castellini, 2008; Mournaki *et al.*, 2010).

Green tea has recently become a subject of investigation in connection with the prevention of various diseases and also its effects on reproduction (Jiřina Kročková and Anton Kováčik, 2013). Researchers from the University of Calabria reported that EGCG at low doses improved measures of sperm quality including motility (De Amecis *et al.*, 2012). In this respect, Kenji Sato *et al.* (2010) suggested the protective effects of dietary intake of green tea extracts may be attributed to the polyphenolic antioxidants in green tea. Polyphenolic compounds which are often abundant in beverages derived from plant origin, such as herbal teas and teas, may contribute to the inhibitory effect of diets on oxidative stress.

It is important to investigate the protective properties of green tea on testicular function in term of semen and testosterone production. Therefore, the present study was designed to investigate the efficacy of different dietary levels of green tea as a source of water-soluble antioxidants on semen quality and testosterone concentration of APRI rabbit bucks.

## **MATERIALS AND METHODS**

The present study was carried out at the International Livestock Management Training Center (ILMTC), Sakha, Kfrelsheikh governorate, belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture, Egypt during the period from October to December 2013.

### **Animals:**

A total of 12 mature rabbit bucks of APRI line (3-3.5 kg live body weight and aged 9-12 mo) raised at Sakha experimental rabbit farm, belonging to APRI, were divided into four similar groups, 3 bucks in each. Bucks in the 1<sup>st</sup> group (T1) were fed complete fed diet (17.4% CP and 2257 Kcal metabolizable energy on DM basis) without any supplements (control). While, those in the 2<sup>nd</sup> (T2), 3<sup>rd</sup> (T3) and 4<sup>th</sup> (T4) treatment groups were fed the same diet supplemented with 2, 4 and 6 g green tea/kg diet.

Bucks were individually housed in wire cages supplied with nipple drinkers and fed *ad libitum* on the experimental diets for 13 weeks, 4 weeks as a preliminary period and 9 weeks as a main period of semen collection.

### **Semen collection:**

Semen was collected twice weekly from bucks in all groups using artificial vagina for rabbits. Semen was collected before feeding at 8.00 a.m. Gel plug was removed immediately after ejaculate collection and semen was kept at 35-37 °C in water bath, then the collected semen was taken immediately to the laboratory.

**Semen evaluation:**

Immediately after collection, semen pH value was determined by pH meter pen. Semen volume without gel fraction) and gel volume were measured by graduated collection test tube.

Percentage of mass motility, livability and abnormality of spermatozoa was determined. Sperm motility percentage was assessed using research microscope with warmed stage (37°C) according to Amman and Hammerstedt (1980). Sperm livability percentage was determined using eosin and nigrosin mixture stain according to Hackett and Macpherson (1965). Live spermatozoa (unstained ones) and dead spermatozoa (stained ones) were counted in field of a total of 200 spermatozoa, then percentage of live spermatozoa was calculated. Sperm abnormalities percentage was determined during the examination of live/dead sperm percentage at a high power magnification (400x), according to the classification adopted by Blom (1983). Sperm cell concentration (SCC) was evaluated by Neubauer hemocytometer.

Total sperm output (TSO) as well as count of motile sperm (MSO), live sperm (LSO) and normal sperm (NSO) per ejaculate were calculated as the following:

$$TSO/ejaculate = ejaculate\ volume\ (ml) \times SCC\ (sperm/ml)$$

$$MSO/ejaculate = TSO/ejaculate \times sperm\ motility\ (\%)$$

$$LSO/ejaculate = TSO/ejaculate \times live\ sperm\ (\%)$$

$$NSO = TSO/ejaculate \times sperm\ normality\ (\%)$$

$$where: Sperm\ normality = sperm\ abnormality\ (\%) - 100$$

**Blood sampling:**

Blood samples were taken from the ear vein of each buck in all experimental groups into heparinized tubes. The samples were centrifuged at 3000 rpm for 15 minutes and blood plasma was separated and frozen at -20 °C until assaying the testosterone concentration with a double antibody radioimmunoassay (Diagnostic Products Corporation Kits). Blood samples were taken pre-treatment, and mid and end of the collection period.

**Statistical analysis:**

Data were analyzed by two-way analysis of variance (ANOVA) using the general linear model procedure according to SAS (2004). Values were considered significant only at  $P < 0.05$ . The significant differences among groups or sampling times were tested using Duncan's multiple range test (Duncan, 1955).

The percentage values were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages. Data were expressed as mean  $\pm$  standard error.

## RESULTS

**Semen characteristics:**

Data in Table (1) show that effect of treatment was significant on all semen characteristics studied. Volume of semen and gel was significantly

( $P < 0.05$ ) higher only in T4 than in T1 (control). However, semen pH value significantly ( $P < 0.05$ ) increased in all treatments (T2-T4) as compared to T1. Generally, values of ejaculate volume, semen gel volume and pH values were the highest for bucks treated with 6 g of green tea/kg diet (T4) as compared to other treatments and control.

As affected by collection week, only pH values of semen showed significantly ( $P < 0.05$ ) fluctuated trend of changes throughout the collection weeks, being significantly ( $P < 0.05$ ) the lowest after 3 weeks and the highest after 9 weeks of semen collection (Table 1).

Analysis of variance revealed that the effect of interaction between treatment and collection week was not significant on semen characteristics studied (Table 1).

According to this study, dietary addition of green tea (T4) had beneficial effects on semen volume and regulation of semen pH value toward neutrality by increasing gel volume in semen of rabbit bucks.

**Table (1): Semen characteristics of APRI rabbit bucks as affected by green tea level, collection week and their interaction.**

Item	Semen characteristics		
	Semen volume (ml)	Semen gel volume (%)	Semen pH value
<b>Effect of treatment (green tea level, g/kg diet):</b>			
0 (T1)	0.50±0.03 <sup>b</sup>	0.42±0.09 <sup>b</sup>	7.08±0.05 <sup>c</sup>
2 (T2)	0.62±0.05 <sup>ab</sup>	0.69±0.13 <sup>ab</sup>	7.22±0.06 <sup>b</sup>
4 (T3)	0.53±0.03 <sup>b</sup>	0.41±0.12 <sup>b</sup>	7.09±0.06 <sup>c</sup>
6 (T4)	0.74±0.06 <sup>a</sup>	1.05±0.22 <sup>a</sup>	7.60±0.07 <sup>a</sup>
<b>Effect of collection week:</b>			
1	0.64±0.13	0.78±0.27	7.23±0.08 <sup>cd</sup>
2	0.68±0.09	0.81±0.30	7.13±0.09 <sup>d</sup>
3	0.49±0.06	0.64±0.26	6.83±0.08 <sup>e</sup>
4	0.57±0.08	0.71±0.28	7.38±0.06 <sup>ab</sup>
5	0.65±0.05	0.29±0.15	7.40±0.10 <sup>bc</sup>
6	0.59±0.08	0.59±0.14	7.45±0.11 <sup>ab</sup>
7	0.56±0.05	0.72±0.19	7.48±0.10 <sup>ab</sup>
8	0.66±0.08	0.42±0.17	7.44±0.08 <sup>ab</sup>
9	0.53±0.04	0.83±0.28	7.59±0.08 <sup>a</sup>
<b>P-value:</b>			
Treatment	0.0062 <sup>**</sup>	0.0073 <sup>**</sup>	0.0001 <sup>***</sup>
Week	0.6680	0.6616	0.0001 <sup>***</sup>
Interaction	0.9428	0.2152	0.0883

a, b.....e: Means denoted within the same column for each effect with different superscripts are significantly different at  $P < 0.05$ .

<sup>\*\*</sup> Significant at  $P < 0.01$ . <sup>\*\*\*</sup> Significant at  $P < 0.001$ .

**Sperm characteristics:**

Results in Table (2) show that effect of treatment was significant on percentage of sperm motility and abnormality as well as sperm cell

concentration. However, percentage of sperm livability was not affected by treatment. All levels of green tea significantly ( $P<0.05$ ) improved sperm characteristics studied in term of increasing motility and concentration of spermatozoa and decreasing sperm abnormality. Meanwhile, sperm livability slightly increased though not significant in treatment groups (T2-T4) as compared to control one (T1). In general, rabbit bucks treated with 6 g of green tea/kg diet (T4) significantly ( $P<0.05$ ) showed the best results, reflecting impact of green tea on semen quality of rabbit bucks.

As affected by collection week, all sperm characteristics significantly ( $P<0.05$ ) improved by advancing collection week, being the best after 9 weeks of collection (Table 2).

Analysis of variance revealed that the effect of interaction between treatment and collection week was not significant on all sperm characteristics, except for sperm motility (Table 1). The significant effect of this interaction reflected in the highest sperm motility in semen of bucks in T4 at all collection weeks (Fig. 1).

**Table (2): Sperm characteristics of APRI rabbit bucks as affected by green tea level, collection week and their interaction.**

Item	Sperm characteristics			Concentration (X 10 <sup>6</sup> / ml)
	Motility (%)	Livability (%)	Abnormality (%)	
<b>Effect of green tea level (gm/ kg diet):</b>				
0	66.1±2.22 <sup>d</sup>	80.7±0.80	17.22±1.03 <sup>a</sup>	146.9±1.97 <sup>d</sup>
2	70.4±2.98 <sup>c</sup>	80.9±1.35	13.96±0.79 <sup>bc</sup>	165.6±1.49 <sup>c</sup>
4	76.9±2.17 <sup>b</sup>	80.7±1.71	16.48±1.11 <sup>ab</sup>	183.3±1.71 <sup>b</sup>
6	82.8±1.45 <sup>a</sup>	82.9±2.31	13.59±1.15 <sup>c</sup>	200.4±1.67 <sup>a</sup>
<b>Effect of collection week:</b>				
1	47.9±4.82 <sup>e</sup>	66.3±4.39 <sup>b</sup>	18.08±2.01 <sup>a</sup>	178.8±7.76 <sup>ab</sup>
2	74.6±2.78 <sup>c</sup>	84.7±1.63 <sup>a</sup>	13.25±1.42 <sup>bc</sup>	164.2±4.80 <sup>c</sup>
3	68.3±2.16 <sup>d</sup>	83.3±1.44 <sup>a</sup>	14.67±1.32 <sup>ab</sup>	172.8±5.87 <sup>b</sup>
4	79.6±2.26 <sup>ab</sup>	80.5±0.76 <sup>a</sup>	15.00±1.59 <sup>ab</sup>	176.7±6.27 <sup>ab</sup>
5	75.8±2.53 <sup>bc</sup>	82.3±1.57 <sup>a</sup>	16.75±1.47 <sup>ab</sup>	178.8±7.76 <sup>ab</sup>
6	80.4±1.89 <sup>ab</sup>	82.4±0.81 <sup>a</sup>	16.50±1.50 <sup>ab</sup>	164.2±4.80 <sup>c</sup>
7	77.5±1.79 <sup>bc</sup>	83.8±1.27 <sup>a</sup>	15.58±1.74 <sup>ab</sup>	172.8±5.87 <sup>b</sup>
8	79.6±2.08 <sup>bc</sup>	82.0±1.19 <sup>a</sup>	17.83±0.85 <sup>a</sup>	176.7±6.27 <sup>ab</sup>
9	82.5±1.44 <sup>a</sup>	86.4±1.33 <sup>a</sup>	10.17±1.29 <sup>c</sup>	181.6±6.68 <sup>a</sup>
<b>P-value:</b>				
Treatment	0.0001 <sup>***</sup>	0.5453	0.0125 <sup>*</sup>	0.0001 <sup>***</sup>
Week	0.0001 <sup>***</sup>	0.0001 <sup>***</sup>	0.0035 <sup>**</sup>	0.0001 <sup>***</sup>
Interaction	0.0371 <sup>*</sup>	0.4622	0.1457	0.3008

a, b.....e: Means denoted within the same column for each effect with different superscripts are significantly different at  $P<0.05$ .

\* Significant at  $P<0.05$ . \*\* Significant at  $P<0.01$ . \*\*\* Significant at  $P<0.001$ .

These results indicated that treatment of rabbit bucks with green tea (T4) had beneficial effects on motility, abnormality and concentration of rabbit spermatozoa. This improvement was observed by advancing collection month.

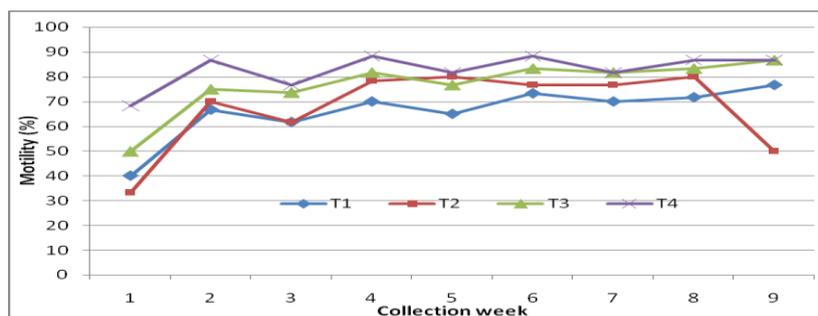


Fig. (1): Sperm motility percentages in semen of bucks at different collection weeks.

**Total sperm output:**

Results in Table (3) show that sperm output in terms of total count, total motile, total live and total normal per ejaculate was affected significantly ( $P < 0.05$ ) by treatment, being higher in bucks of treatment groups than in the control group.

It is of interest to note that all sperm counts did not increase significantly by increasing green tea level from 2 to 4 g/kg (T2 and T3, respectively). However, increasing green tea level from 4 to 6 g/kg (T4) resulted in further significant ( $P < 0.05$ ) increase in comparing with T2 and T3.

Table (3): Total sperm output of APRI rabbit bucks as affected by green tea level, collection week and their interaction.

Item	Sperm output ( $\times 10^6$ ) per ejaculate			
	Total count	Total motile	Total live	Total normal
<b>Effect of green tea level (gm/ kg diet):</b>				
0	73.2 $\pm$ 4.70 <sup>c</sup>	48.2 $\pm$ 3.51 <sup>c</sup>	58.8 $\pm$ 3.63 <sup>c</sup>	60.6 $\pm$ 4.08 <sup>c</sup>
2	102.8 $\pm$ 8.60 <sup>b</sup>	72.7 $\pm$ 6.83 <sup>b</sup>	83.1 $\pm$ 7.06 <sup>b</sup>	88.1 $\pm$ 7.19 <sup>b</sup>
4	97.7 $\pm$ 6.19 <sup>b</sup>	75.4 $\pm$ 5.43 <sup>b</sup>	79.3 $\pm$ 5.49 <sup>b</sup>	81.6 $\pm$ 5.14 <sup>b</sup>
6	147.3 $\pm$ 13.01 <sup>a</sup>	121.2 $\pm$ 9.64 <sup>a</sup>	117.4 $\pm$ 8.24 <sup>a</sup>	126.6 $\pm$ 11.08 <sup>a</sup>
<b>Effect of collection week:</b>				
1	117.0 $\pm$ 26.79	60.2 $\pm$ 17.82	70.3 $\pm$ 11.90	96.8 $\pm$ 23.11
2	114.1 $\pm$ 16.13	86.1 $\pm$ 13.67	97.3 $\pm$ 14.55	100.2 $\pm$ 15.02
3	84.6 $\pm$ 9.67	58.2 $\pm$ 7.23	70.4 $\pm$ 8.14	72.1 $\pm$ 8.34
4	102.4 $\pm$ 15.71	83.4 $\pm$ 14.25	82.1 $\pm$ 12.50	87.5 $\pm$ 13.62
5	118.3 $\pm$ 12.21	92.1 $\pm$ 11.72	97.6 $\pm$ 10.39	98.8 $\pm$ 10.56
6	98.6 $\pm$ 14.98	80.8 $\pm$ 13.35	81.0 $\pm$ 12.17	82.3 $\pm$ 12.48
7	97.3 $\pm$ 10.24	76.3 $\pm$ 9.09	81.2 $\pm$ 8.40	81.0 $\pm$ 7.26
8	119.2 $\pm$ 15.72	97.8 $\pm$ 14.42	98.7 $\pm$ 13.79	98.2 $\pm$ 13.27
9	95.9 $\pm$ 6.78	79.4 $\pm$ 6.37	83.0 $\pm$ 6.24	86.3 $\pm$ 6.58
<b>P-value:</b>				
Treatment	0.0001***	0.0001***	0.0001***	0.0001***
Week	0.6272	0.1229	0.3001	0.7087
Interaction	0.9605	0.8666	0.9472	0.9621

a, b and c: Means denoted within the same column for each effect with different superscripts are significantly different at  $P < 0.05$ . \*\*\* Significant at  $P < 0.001$ .

Data in Table (3) show that the effect of collection week or its interaction with treatment was not significant on all counts of spermatozoa.

**Testosterone concentration:**

Data in Table (4) revealed that testosterone concentration in blood plasma of bucks was not affected significantly by treatment, collection week or their interaction. Such trend may be associated with wide variation in testosterone concentration within each group.

**Table (4): Concentration of testosterone in blood plasma of APRI rabbit bucks as affected by green tea level, sampling time and their interaction.**

Item	Testosterone concentration (ng/ml)	
	Mean± SE	Range
<b>Effect of green tea level (gm/ kg diet):</b>		
0	6.77±1.16	0.36-13
2	5.41±0.97	0.7-9.01
4	5.47±1.21	0.13-9.86
6	5.08±1.11	0.64-11.71
<b>Effect of sampling time:</b>		
Pre-treatment	4.87±1.09	0.07-11.71
Mid-collection period	6.54±1.03	1.53-13
End of collection period	5.63±0.67	1.71-9.84
<b>P-value:</b>		
Treatment	0.7014	-
Week	0.4638	-
Interaction	0.2644	-

## DISCUSSION

Oxidative stress plays a prominent role in patho-physiology of reproductive malfunction and infertility in animals and humans (Levy *et al.*, 1999; Feldman *et al.*, 2000). Green tea is complex and includes many proteins (15-20% dry weight) of which enzymes constitute an important fraction. Also included are amino acids such as teanine or 5-N-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine and lysine (Cabrera *et al.*, 2006). Green tea can be considered an important dietary source of polyphenols particularly flavonoids (Cherubini *et al.*, 1999), i.e. catechins, which can exist in two different configurations, with the “epi” configuration being the more common (Cherubini *et al.*, 1999), and it is an important dietary source of antioxidant nutrients (Mckay and Blumberg, 2002; Kim *et al.*, 2003).

The beneficial health effects of green tea in human and animal models with respect to their antioxidants, especially flavonoids, in protecting against various diseases have been reported. A shift in pro-oxidant and oxidant status could lead to reactive oxygen species (ROS), but a change in antioxidant activities is frequently used as an important indicator of ROS and the antioxidant defense status determines the extent to which oxidative

damage occurs in the sperm (Sikka, 2001; Ong *et al.*, 2002). The protective role of antioxidants, more specifically flavonoids, have been demonstrated in several studies (Ola-Mudathir *et al.*, 2008; Khan and Ahmed, 2009; Suresh *et al.*, 2009).

The generation of ROS by sperm is a normal physiological process, however a shift between ROS production and scavenging activity is deleterious to sperm and it has been shown to be associated with male infertility (Sharma and Agarwal, 1996). According to Halliwell and Gutteridge (1999), mechanisms of antioxidant action can include, 1) suppressing ROS formation either by inhibition of enzymes or chelating trace elements involved in free radical production; 2) scavenging reactive oxygen species; and 3) upregulating and protecting antioxidant defenses.

According to the present results in this study, green tea supplements improved semen volume of bucks in treatment groups, but the present ejaculate volume in all treatment and control groups are within the normal range of semen volume in rabbits as reported by Abdel-Khalek *et al.* (2005) on NZW bucks and Mangiagalli *et al.* (2012) on hybrid Martini male rabbits. However, the obtained semen pH value was higher than that reported by Abdel-Khalek *et al.* (2005) on NZW rabbits and lower than that reported by Ongun *et al.* (2010) on semen of Californian rabbits. Therefore the recorded beneficial effects was on increasing gel volume. In this respect, some authors indicated a negative relationship between pH values of semen and sperm cell concentration in large animals (Hafez and Hafez, 2002) and rabbits (Abdel-Khalek *et al.*, 2005). However, the present study revealed a relationship between pH values and each of volume of ejaculate and semen gel volume. Increasing ejaculate volume was associated with increased semen gel volume, leading to increasing in pH values toward neutrality in semen of rabbit bucks of treatment groups.

In mammals, the epididymis is known to play a major role in the final development of motility, fertilizing ability and sperm storage. Sperm concentration can increase during epididymal transit with a simultaneous increase in sperm metabolism and the possibility of ROS threatens the survival of these male gametes (Dacheux *et al.*, 2003). The observed improvement in the present study in sperm characteristics, including motility, abnormality and concentration may be attributed to the prevention of excessive generation of free radicals produced by sperm by means of the antioxidant properties of green tea. In this respect, Purdy *et al.* (2004) demonstrated that flavonoids caused an increase sperm motility. These findings supported the obtained results concerning improvement in sperm motility in association with marked increase in sperm cell concentration and reducing sperm abnormality without pronounced effect on sperm livability (which was at acceptable percentages, above 80%) as affected by green tea treatment.

In addition, it is possible that the consumption of green tea may have a reducing or lowering capacity on ROS thereby improving fertility (Roth *et al.*, 2002). Green tea supplement showed a protection against ROS due to their varying degrees of tendency to reduce the levels of lipid peroxidation (LPO) and ROS, and enhance the levels of the antioxidant enzymes,

superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH). Increasing SOD in rat sperm and an increasing trend in CAT activity trend in testicular tissue may suggest that green tea could have varying protective synergistic effect on enzyme induction and removal of hydroxyl radicals and subsequent removal of the product produced by the activities of SOD (Awoniyi, 2010). Also, Ramiro-Puig *et al.* (2007) reported increased activities of certain antioxidant enzymes following flavonoids supplementation in Wistar rats. The present results support the findings that flavonoids in green tea with antioxidant properties and its resultant may enhance catalase activities, and could be important in protecting against induced-oxidative stress.

Free radical-induced oxidative damage to spermatozoa is a condition that has gained favourable attention for its role in inducing poor sperm function and infertility (Russo *et al.*, 2006). Glutathione is a powerful intracellular antioxidant and plays a prominent role in stabilizing various enzymes and could also be regarded as a good marker for tissue antioxidant capacity (Van Acker *et al.*, 2000; Wang and Jiao, 2000). Glutathione protects cells against ROS and other types of damage, which may originate from compounds of endogenous and exogenous sources. It was reported that plants rich in flavonoids as green tea increased GSH level in the epididymal sperm (Türk *et al.*, 2008) or in testis (Khan and Ahmed, 2009) of rats. The substantial increase in the GSH level in the sperm and testicular tissue of rats that consumed green tea may suggest a decreased oxidative stress or an increased antioxidant capacity in the cell, thereby lowering the risk of oxidative damage (Awoniyi, 2010).

In spite the insignificant effect of treatment on testosterone concentration, bucks in treatment groups tended to have lower testosterone concentration than in the control bucks, indicating adverse effect of green tea on testosterone concentration. In this respect, Al-Harrby *et al.* (2010) found that aqueous extract of green tea had a negative effect on androgen levels, which produced in testes especially testosterone. Generally, the present concentration of testosterone in blood plasma of APRI bucks in this study is higher than that reported in NZW bucks by several authors (Tharwat, 1990; Abd El Elmoty, 1991; Abdel-Khalek *et al.*, 2005).

Testosterone is the major secretory hormone of the mature testis and spermatogenesis is controlled directly by testosterone concentration. Therefore, improving semen volume, sperm characteristics and outputs without increasing testosterone concentration may reveal pronounced effect of green tea on accessory sex glands and testicular tissues (spermatocytes) within the somniferous tubules of the testis as well as on epididymal spermatozoa (Awoniyi, 2010).

## **CONCLUSION**

The current findings suggested that green tea have significant value in improving the antioxidant status as shown by the increased antioxidant enzymes activity and GSH levels and could serve as a supportive treatment in the nutritional management to improve semen quality of rabbit bucks, in particular at a level of 6 g/kg diet.

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### **تأثير الشاي الأخضر كأضافه غذائيه علي جودة السائل المنوي ومستوي التيسيتسترون في الأرانب**

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يهدف هذا البحث إلي دراسة تأثير إضافة الشاي الأخضر إلي عليقة ذكور الأرانب الأبري البالغة علي جودة السائل المنوي ومستوي التيسيتسترون. استخدم في هذه الدراسة ١٢ ذكر أرنب أبري بالغ قسمت إلي أربع مجاميع متساوية في الوزن والعدد. وتم تغذية ذكور الأرانب علي علائق متكاملة غذائيا تحتوي علي ١٧,٤% بروتين خام و٢٢٥٧ كليوكالوري طاقة ممثلة. وكانت العلائق مختلفة في محتواها من مسحوق أوراق الشاي الأخضر علي النحو التالي: المجموعة الأولى مجموعة مقارنة (بدون أي إضافات) بينما المجموعة الثانية والثالثة والرابعة تحتوي علي ٢،٤ و ٦ جم/كجم عليقه من الشاي الأخضر، علي الترتيب واستمرت الفترة التجريبية ١٣ أسبوع ، أربع اسابيع فترة تمهيدية و٩ اسابيع لجمع السائل المنوي. تم تقييم السائل المنوي من حيث حجم السائل المنوي والجل و درجم الحموضة والنسبة المويه للحيوانات المنوية المتحركة والحية والشواذ وكذلك تركيز الحيوانات المنوية كما تم تقدير تركيز التيسيتسترون في الدم قبل المعامله ووسط ونهاية فترة الجمع.

**كانت النتائج المتحصل عليها كالتالي:**

أظهرت المجموعة الرابعة زيادة معنوية (علي مستوي ٥%) في كل من حجم القنفة وقيم رقم الحموضة ونسبة الحيوانات المنوية المتحركة وتركيز الحيوانات المنوية وإجمالي الحيوانات المنوية الكليه والمتحركة والحيه و الطبيعية بينما انخفض نسبة الحيوانات المنوية الشاذة. أما نسبة الحيوانات المنويه الحيه وتركز التيسيتسترون فلم تتأثر بالمعاملات. يتضح من هذه الدراسة أن إضافة الشاي الأخضر له أهمية معنوية علي زيادة نشاط الإنزيمات المضادة للأكسدة. وان إضافة الشاي الأخضر بمعدل ٦ جم لكل كجم من عليقة ذكور الأرانب أدت إلي تحسين صفات السائل المنوي.

### **قام بتحكيم البحث**

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