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FERTILITY OF RABBIT BUCKS ORALLY ADMINISTERED WITH SOME BEE PRODUCTS, UNDER EGYPTIAN SUMMER CONDITIONS

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The present study was designed to investigate the efficacy of royal jelly (RJ) and propolis (P) on semen quality and fertility of New Zealand White (NZW) rabbit bucks, under Egyptian summer conditions. Thirty NZW rabbit bucks and 60 hybrid nonparous females were used in the present study. Rabbit bucks were randomly divided to 3 groups (10 bucks per group), bucks were administered orally with 0.5 mL of a solution/ kg body weight (BW), once weekly for 6 weeks; which contained: 1) water for control (C-group), 2) 400 mg royal jelly (RJ) dissolved in 0.25 mL water + 0.25 mL bee honey for (RJ-group), 3) 10 mg propolis (P) was suspended in 0.5 mL water for (P-group). The current study demonstrated that NZW rabbit bucks received either royal jelly or propolis showed significant (P<0.05) increase of reaction time, ejaculate volume, percentage of sperm progressive motility, sperm-cell concentration and seminal plasma fructose concentration compared to control group. On the other hand, percentages of dead and abnormal spermatozoa increased significantly (P<0.05) for control group compared to the other two groups. Blood plasma concentration of testosterone increased significantly (P<0.05) for both royal jelly and propolis treated bucks compared to Control. Otherwise, plasma concentrations of cholesterol, aspartate amino transferase (AST) and alanine amino transferase (ALT) were reduced significantly (P<0.05) for royal jell and propolis groups compared to control group. Rabbit bucks treated with either royal jelly or propolis showed better fertility (higher conception rate and litter size) than control bucks.

***Conclusively**, the results of the present study showed that royal jelly or propolis could be used beneficially to improve semen quality and fertility of rabbit bucks under Egyptian summer conditions. This improvement was also mirrored on better liver functions as observed with lower concentrations of AST and ALT.*

Keywords: Fertility, propolis, rabbit buck, royal jelly, semen, summer.

Ambient temperature is the major constrained factor controlling animal productivity under subtropical conditions (Hashim *et al*., 2013). In Egypt, summer and early autumn seasons (from May to September) are characterized by high temperature combined with elevated humidity (Attia *et al.*, 2011). These conditions are not relevant to the thermal neutral zone of the rabbits, whereas the comfortable zone temperature is around 21ºC and that their productive and reproductive performance could be impaired, which in turn prevents the continuation of breeding season for about five consecutive months (García-Tomás *et al*., 2008). Under such environmental conditions, several physiological and reproductive disorders are induced ascribing to disturbances in blood metabolites, oxidative status, enzymatic reactions and hormonal secretions (Alvariño, 2000; Marai *et al*., 2002; García-Tomás *et al*., 2008). During summer season in Egypt, rabbit bucks produce low quality semen (El-Sherbiny, 1987); in order to avoid this hypo-reproductive activity of male rabbits at summer, a GnRH analogue was used. El-Sherbiny (1994) injected NZW rabbit bucks with 2 µg GnRH agonist three times weekly for 2 weeks improved semen quality and fertility of these bucks compared to control group.

At last years, there are international interests concerning application of natural sources in animal production field (Hashim *et al*., 2013).

Royal jelly (RJ) is a secretion product of the cephalic glands of nurse bees that has been used for centuries for its extraordinary properties and health effects (Pavel *et al*., 2011; Mărghitas, 2008). There still remains much to reveal about Royal jelly biochemistry and biological activity in future research. On the other hand, the chemistry and bioactive compounds of RJ are not sufficiently known. On the other hand, RJ has various health benefits because of components like B-complex vitamins such as vitamins B_5 and B_6. The overall composition of RJ is 67% water, 12.5% crude protein and 11% simple sugars, also including a relatively high amount (5%) of fatty acids. It also contains many trace minerals, some enzymes, antibacterial and antibiotic components, and trace amounts of vitamin C. However, vitamins A, D, E and K are completely absent from RJ (Graham, 1992). RJ has immune-modulatory activities (Gasic *et al*., 2007).

Many investigators studied the effect of royal jelly on male fertility. On rabbits, Elnagar (2010) concluded that RJ administration to heat stressed

male rabbits can counteract their "summer infertility" and improve their physiological status and also for growing rabbits (Elnagar *et al.*, 2010). Hassan (2009) found that, royal jelly is a beneficial treatment of male rats especially on sperm count and livability. Yang *et al.* (2012), concluded that high-dose of oral royal jelly administration for 4 weeks adversely affected the reproductive system of pubescent male rats, but the unfavorable effects are alleviated to some extent by cessation of administration.

Royal jelly is safe and effective in the treatment of male infertility; after three months of treatment infertile men with RJ increased significantly sperm active motility and sperm concentration (Al-Sanafi *et al.*, 2007).

Propolis has been known as natural resinous substance collected by bees from parts of plants, buds and exudates (Zhou *et al*., 2008). About 300 components, mainly phenolic compounds, have been identified. Most of these isolated compounds belong to three main groups, flavonoids and phenolic acids and esters (Simoes *et al*., 2004). Propolis has several therapeutic properties including antioxidant, antimicrobial, antiparasitic, antiviral, anti-inflammatory and antitumoral properties (Kumazawa *et al*., 2004; Paulino *et al.*, 2008). Inclusion of propolis in male rabbit's diets during the hot season could be used effectively to mitigate negative impacts of elevated temperature on semen quality of NZW rabbit bucks (Hashim *et al.*, 2013).

Further experimentation (*in vitro*, in animal's research) and validation would be needed to prove any useful benefit and action mechanism of native bee honey and RJ and isolated compounds as well.

Therefore, this study was designed to examine the efficiency of royal jelly and propolis as natural honey bee products to mitigate summer stress effects on semen characteristics and fertility of adult rabbit bucks during summer season under the Egyptian conditions.

MATERIALS AND METHODS

The present study was conducted during summer season (from June to August 2014) at the Intensive Rabbit Production Unit, Faculty of Agriculture, Ain Shams University, Cairo, Egypt and a Private Rabbit Farm, Kalioubia, Egypt.

Averages of air temperature and percentages of relative humidity were shown in Table 1. The temperature-humidity index (THI) was calculated using the equation modified by Marai *et al*. (2005): THI = db^{o}C – [(0.31 – 0.31 RH) (db^{o}C - 14.4)], where db^{o}C = dry bulb temperature in ^{o}C and RH = relative humidity percentage. The THI values obtained were classified as

follows: <27.8 = Absence of hest stress, 28.9 to <30.0 = Severe heat stress and >30.0 = Over severe heat stress. Data in Table 1 show that among summer months (June, July and August), THI values reflected over severe heat stress (>30.0).

Table 1. Maximum, minimum and average air temperature (ºC) and percentages of relative humidity (%) during the experimental period.

Month	Air temperature (ºC)			Relative humidity (%)			THI
	Max.	Min.	Mean	Max.	Min.	Mean	
June	43	26.4	34.7	77.4	28.2	52.8	31.71
July	39	25.5	32.25	85.1	36.5	60.82	30.09
August	38	27.2	32.6	85.6	35.3	60.45	30.36

***Experimental animals and groups**:*

A total number of thirty New Zealand White (NZW) rabbit bucks, twelve months old, with mean body weight 3.4 ± 0.09 kg were used in the present study. For testing the fertility of rabbit bucks, 60 hybrid nonparous females aged 4.5 – 5 months old, with average body weight 2.85 ± 0.16 kg at a private rabbitry, Kalioubia governorate, Egypt, were used in the experiment (20 females per each experimental group). All experimental animals were individually kept in galvanized wire cages in a naturally ventilated building and fed a commercial concentrate pelleted diet according to their reproductive condition recommended by NRC (1977); clean fresh water was available all the time.

Bucks were randomly divided into three groups (10 rabbit bucks per group). Each rabbit buck received 0.5 mL of a solution/ kg body weight (BW), once weekly for 6 weeks; which contained: 1) water for control (C-group), 2) 400 mg royal jelly (RJ) dissolved in 0.25 mL water + 0.25 mL bee honey for (RJ-group), 3) 10 mg propolis (P) was suspended in 0.5 mL water for (P-group).

Blood collection:

Blood samples were collected with heparinized tubes from the ear vein of each buck on weeks 0, 3, 6 and 9 after starting of oral administration. Blood plasma were separated by centrifugation at 700 x G for 20 min and stored at -20ºC until analysis as described by Hashim *et al*. (2013).

Biochemical analysis and testosterone concentration:

Blood plasma concentrations of aspartate amino transferase (AST), alanine amino transferase (ALT) and cholesterol were determined using colorimetric method by commercial kits obtained from Biodiagnostic, Dokki, Giza, Egypt. Plasma testosterone concentration was measured using solid-phase enzyme immuonoassay (Elisa) kits for rabbits obtained from Chemux Bioscience, INC., San Francisco, USA. The lower limit of assay detection

was 0.1 ng / mL and the upper limit was 18.0 ng / mL. The intra-and inter-assay coefficients of variation (CV) were 9.6 and 6.1 %, respectively.

Semen collection and evaluation:

Semen was collected from each buck once weekly using an artificial vagina. The parameters examined semen quality of rabbit bucks were: reaction time (seconds) using a stop-watch; pH values using pH comparative paper ranging from 6.0 – 8.1 (Whatman pH indicator papers; Whatman Limited Maidstone, England); Ejaculate volume (mL) using calibrated collecting tubes; sperm progressive motility %; percentage of dead spermatozoa assessed by nigrosin-eosin stain technique; percentage of abnormal spermatozoa using 0.5% alcoholic eosin; sperm-cell concentration ($\times 10^6$/ mL) estimated haemocytometrically and initial fructose concentration (mg / 100 mL semen) using the method described by Mann (1964). Semen was evaluated as described by El-Sherbiny (1987) and Madhuri *et al*. (2012).

Artificial insemination:

Semen from each experimental group was pooled together and diluted with Tris-citric-glucose contained 20% egg yolk, as described by El-Sherbiny (2013). Females chosen for insemination were thought to be sexually receptive (had red color of vulva lips). In order to induce ovulation, females were injected intramuscularly with 0.25 ml receptal (GnRH analogue, 1.05 µg of busereline acetate; intervet, Cairo, Egypt). Then, each doe was inseminated artificially with 0.5 ml diluted semen (containing approximately 30 x 10^6 sperms) just after GnRH injection. Pregnancy was detected by trans-abdominal palpation 14 days post-insemination to determine conception rate%. Litter size was determined for each doe directly after kindling.

Statistical analysis:

Data of percentages of progressive motility, dead and abnormal spermatozoa of NZW rabbit semen were analyzed using repeated measurements analysis. Litter size data were analyzed using One Way ANOVA. Whereas, for pregnancy rate trait, Catmod procedure and Chi-square test for homogeneity of variance were performed. All statistical analysis for the different traits was realized using SAS program (SAS, 2011). Differences among experimental groups were tested by Duncan's Multiple Range test (Duncan, 1955).

Repeated measurements analysis was according to the following model:

$$y_{ijk} = \mu + trt_i + an_k\,(trt)_{i} + time_j + (trt * time)_{ij} + e_{ijk}$$

Where: μ is the overall mean, y_{ijk} is the observation of the studied trait of k^{th} animal of i^{th} trt in j^{th} time, trt_i is the effect of i^{th} trt (i = 1, 2, 3), $an_k\,(trt)_i$ is k^{th} animal within i^{th} treatment (the first error), $time_j$ is the effect of j^{th} time (j = 1,

2, 3, 4, 5 and 6 for semen quality and j = 1, 2, 3 and 4 for blood plasma biochemical analysis), $(trt*time)_{ij}$ is the effect of the interaction between trt and time, e_{ijk} is the individual error.

One way ANOVA was according to the following model:

$$Y_{ij} = \mu + t_i + e_{ij}$$

Where: μ is the overall mean, Y_{ij} is the observation of the studied trait of j^{th} animal of i^{th} treatment, t_i is the fixed effect of treatment (i = 1, 2 and 3), e_{ij} is the individual error.

RESULTS AND DISCUSSION

Effects of royal jelly and propolis on semen characteristics:

Effect of royal jelly and propolis on semen characteristics of NZW rabbit bucks during summer are presented in Table 2 and Figure 1. Bucks treated with royal jelly and propolis had higher sexual activity reflected on lower reaction time (RT) compared with bucks in the control group ($P<0.05$). Both treatments increased significantly ($P<0.05$) the overall mean of pH, ejaculate volume, sperm progressive motility, sperm-cell concentration and seminal fructose concentration. On the other hand, both treatments decreased percentages of dead and abnormal spermatozoa. The present results on the effect of RJ and P administration on libido and semen characteristics of rabbit bucks during summer are in line with the findings of Elnagar (2010) and Hashim *et al*. (2013).

Results of the present study showed that the impaired physical semen characteristics of rabbit bucks observed during summer, can be enhanced by both RJ and P administration. Comparison between groups showed that RJ and P-treated bucks had higher libido, semen quality and initial concentration of fructose. This enhancement in RJ and P-treated bucks could be associated with higher concentration of testosterone recorded due to RJ and P supplementation (Table 2), particularly libido of bucks, ejaculate volume and seminal plasma fructose which are testosterone dependent process (Nishiyama (1955); Fujihara *et al*., 1983; Hafez and Hafez, 2000; Hashim *et al*., 2013). Hafez and Hafez (2000) also concluded that fructose synthesis by the accessory sex glands was dependent on the secretion of testosterone hormone by testes Leydig cells. The enhancement observed in sperm motility is consistent with the findings of Karacal and Aral (2008) who reported higher sperm motility when male mice were treated with RJ. The significant increase in sperm-cell concentration can be explained by the findings of Kohguchi *et al.* (2004) who demonstrated that golden hamster treated with RJ showed more intensive spermatogenesis than the control group. Similar observations were recorded by Karacal and Aral (2008) who reported higher sperm concentration when male mice were treated with RJ. Testosterone is

Table 2. Overall mean (±SE) of semen characteristics, testosterone, cholesterol, AST, ALT and fertility of NZW rabbit bucks treated with RJ and P under Egyptian summer conditions.

Items	Control group	Royal jelly group	Propolis group
Reaction time (seconds)	9.1 ± 0.04^{A}	4.7 ± 0.04^{B}	5.0 ± 0.04^{B}
pH	6.8 ± 0.02^{B}	6.9 ± 0.02^{A}	6.9 ± 0.02^{A}
Ejaculate volume (mL)	0.38 ± 0.02^{C}	0.55 ± 0.02^{B}	0.66 ± 0.02^{A}
Sperm progressive motility %	75.0 ± 0.01^{B}	88.0 ± 0.01^{A}	88.0 ± 0.01^{A}
Sperm concentration / ml ($\times 10^6$)	192.3 ± 2.60^{B}	274.4 ± 2.60^{A}	271.2 ± 2.60^{A}
Dead sperms %	16.5 ± 0.30^{A}	10.3 ± 0.30^{B}	6.7 ± 0.30^{C}
Abnormal sperms %	10.1 ± 0.28^{A}	6.7 ± 0.28^{B}	6.4 ± 0.28^{B}
Seminal plasma fructose (mg/100 mL)	264.4 ± 1.94^{C}	311.5 ± 1.94^{A}	295.6 ± 1.94^{B}
Testosterone (ng / ml)	1.33 ± 0.02^{C}	2.28 ± 0.02^{A}	1.98 ± 0.02^{B}
Cholesterol (mg / dL)	115.8 ± 0.30^{A}	105.0 ± 0.30^{B}	105.2 ± 0.30^{B}
AST (IU / L)	30.7 ± 0.21^{A}	26.8 ± 0.21^{B}	27.2 ± 0.21^{B}
ALT (IU / L)	15.6 ± 0.05^{A}	14.0 ± 0.05^{B}	13.7 ± 0.05^{C}
Conception rate % (/ 20 doe)	60 ± 0.1^{NS}	80 ± 0.1^{NS}	85 ± 0.1^{NS}
Litter size at birth (No. Kids/ doe)	4.3 ± 0.20^{B}	8.3 ± 0.17^{A}	8.0 ± 0.17^{A}

Overall means within a row with different superscript letters differ significantly ($P < 0.05$)
NS= Non significant.

essential for spermatogenesis from spermatogonium to spermatid (West and Taylor, 1997). In the same way, ElKelawy and Aboulnaga (1995) reported that ejaculate volume, sperm motility and sperm concentration were significantly increased when male rabbits treated with testosterone.

In addition, RJ treatment was capable of significantly reducing abnormal and dead sperm percentages. That comes in line with the findings of Karacal and Aral (2008) who reported lower abnormal sperm concentrations when male mice were treated with RJ, where the percentage of abnormal spermatozoa in the control group was higher than those of treated groups. Hassan (2009) concluded that royal jelly is a beneficial treatment of male adult rats, especially on sperm count and the percentage of live sperms. Improvement in buck reproductive status as a result of RJ treatment can possibly be attributed to different amino acids and/or vitamins (Graham, 1992), as discussed by Nizza *et al.* (2000). RJ was shown to contain testosterone and have steroid hormone-type activities (Hidaka *et al*., 2006) may be also the reason for its beneficial effects on rabbits' reproductive status. On rabbits, Elnagar (2010) concluded that RJ administration to heat stressed male rabbits can counteract their "summer infertility"; while Hashim *et al*. (2013) observed that inclusion of propolis in male rabbits' diets during the hot season could be used effectively to mitigate negative impacts of elevated temperature on semen quality.

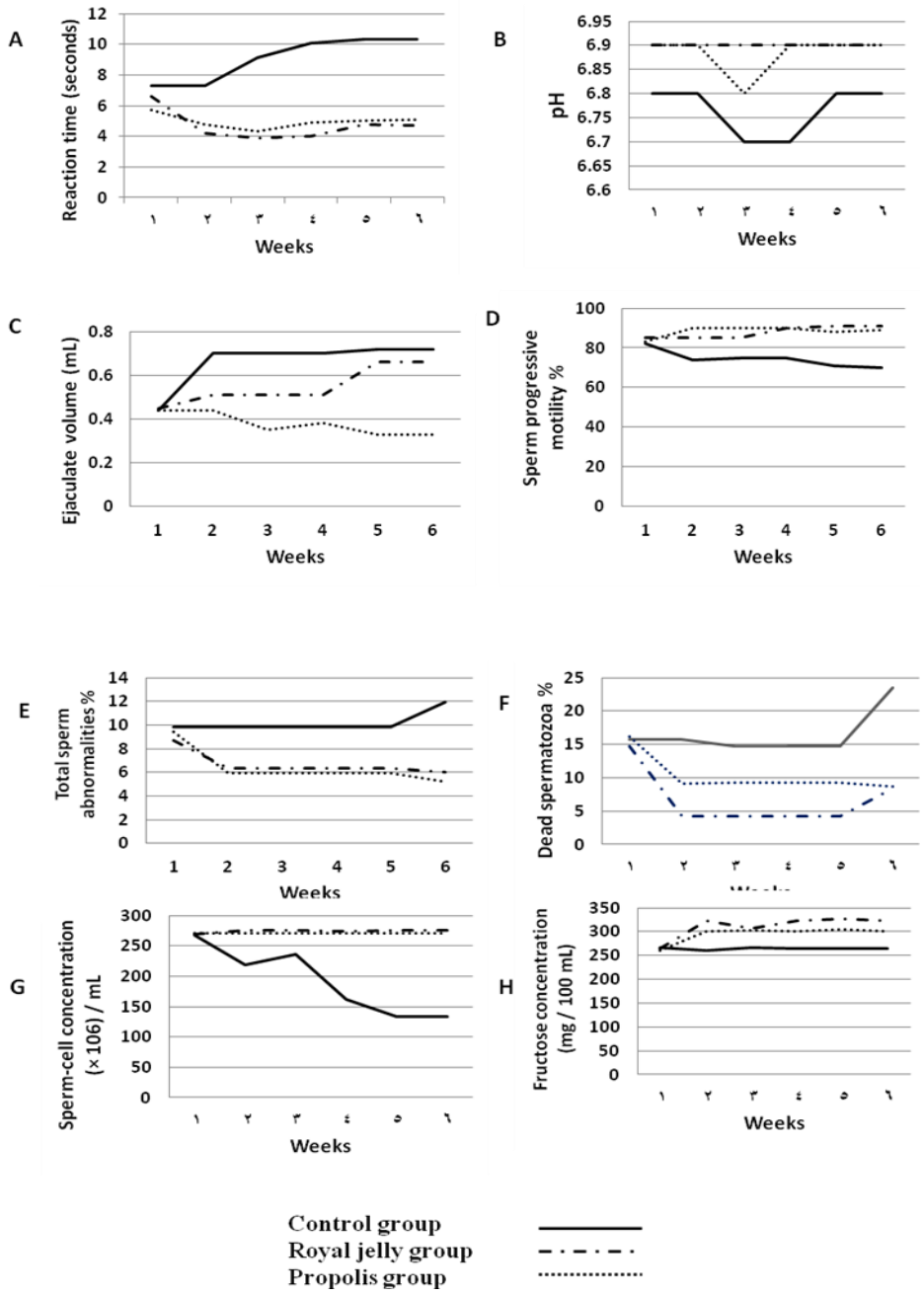

Figure 1: Semen characterestics of NZW rabbit bucks orally administered with royal jelly and propolis.

Effects of royal jelly and propolis on plasma biochemical parameters and testosterone concentration:

Data in Table 2 and Figure 2, showed that supplementation of RJ and P increased significantly ($P<0.05$) plasma testosterone concentration compared with that recorded in the control group. On the other hand, blood plasma concentrations of cholesterol, AST and ALT decreased significantly ($P<0.05$) in RJ and P groups compared to control group. The effect of RJ on testosterone comes in agreement with the findings of Kohguchi *et al*. (2004) who demonstrated that feeding golden hamster with diet contained RJ showed higher testosterone than control group. The previous results are partly in agreements with the findings of Elnagar (2010) and Hashim *et al*. (2013). Hassan (2009) repoted that, royal jelly is a beneficial treatment of male adult rats, especially on testosterone level and subsequent seminal characteristics.

Royal jelly treatments reduced plasma cholesterol concentration of rabbit bucks during summer supporting the findings of XinNan *et al*. (1995) who found that treating rats with experimentally induced hyperlipaemia with 700 mg RJ/kg reduced serum cholesterol levels. Likewise, Al-Mufarrej and El-Sarag (1997) reported that treating chickens with 200 mg RJ reduced blood cholesterol. During heat stress levels of liver enzymes (ALT and AST) tend to rise suggesting some liver damage in mammals and birds (QingHua and Genlin, 2007; Faisal *et al*., 2008). In the present study, either royal jelly or propolis treatment caused a reduction in both enzymes, revealed an improvement in liver function. These effects are in agreement with the finding of Elnagar (2010) and Hashim *et al*. (2013).

Effects of royal jelly and propolis on rabbit bucks fertility:

Table 2 and Figure 3 represent the effect of RJ and P on conception rated % (CR) and litter size at birth (LS) for rabbit does inseminated artificially with semen of the three experimental groups. Chi-square test showed that, there were non-significant differences among experimental groups. Values of CR were 60, 80 and 85% for control (C), RJ and P groups, respectively. On the other hand, there were significant differences ($P<0.05$) among experimental groups; values of CR were 4.3, 8.3 and 8.0 litters / doe at birth for C, RJ and P groups, respectively. In the present study, RJ administered orally with 0.25 mL of bee honey, while, P administered, suspended with water. Elnagar (2010) and Elnagar *et al*. (2010) gave rabbits RJ with water orally, while, Hashim *et al.* (2013) supplemented rabbit's diet with propolis powder; and indicated that semen properties were enhanced in propolis treated-group, which had better libido (lower reaction time) and

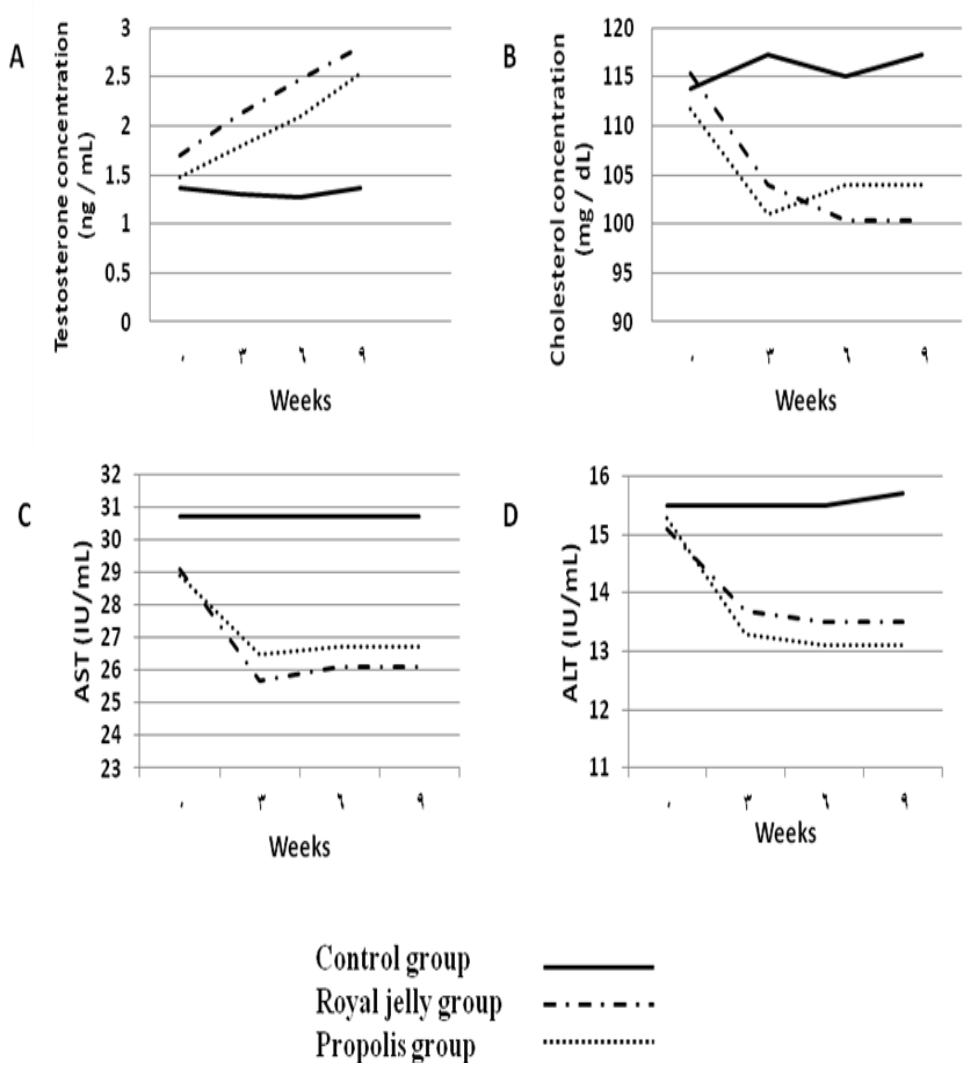

Figure 2: Plasma testosterone, cholesterol, AST and ALT of NZW rabbit bucks orally administered with royal jelly and propolis.

higher sperm concentration and viability; these enhancements were parallel to increased plasma concentrations in propolis treated group.

Additionally, the overall mean concentrations of seminal initial fructose were significantly increased in propolis-treated group.

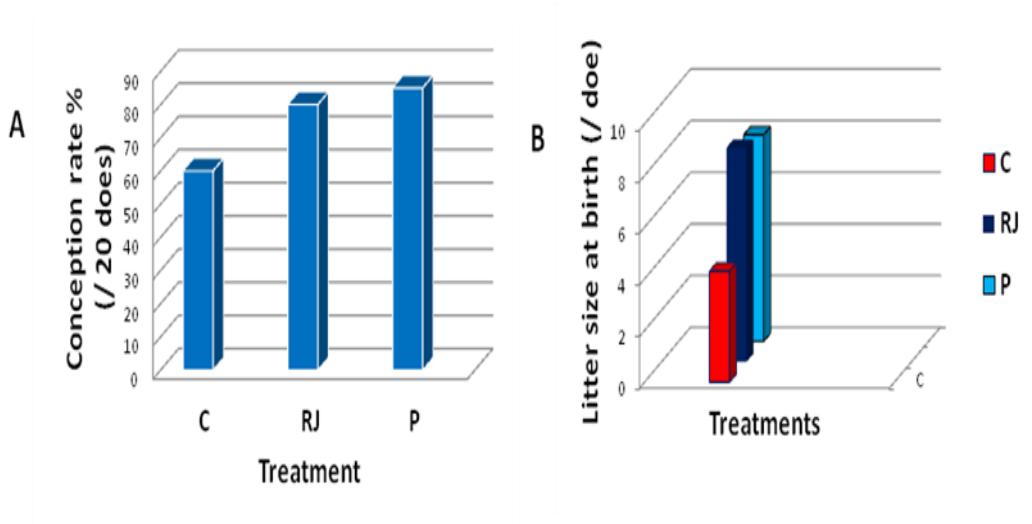

Figure 3: Fertility of NZW rabbit bucks orally administered with royal jelly and propolis.

Also, honey may had an effect on the fertility of RJ-treated rabbit bucks as mentioned by Sayazana *et al*. (2011), who reported that Gelam honey has the potential to increase the fertility of male rats by increasing sperm count and number of sperm with normal morphology.

Conclusively, it can be concluded that administration of royal jelly and propolis orally to rabbit bucks during summer improved their reproductive performance as observed with higher testosterone concentration, libido, ejaculate volume, sperm progressive motility, sperm concentration, seminal fructose, conception rate and litter size; also by decreasing abnormal and dead sperm concentration. This improvement was also mirrored on better liver functions as observed with lower concentrations of AST and ALT. The results of the present study showed that royal jelly or propolis could be used beneficially to improve semen quality and fertility of rabbit bucks under Egyptian summer conditions.

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خصوبة ذكور الأرانب بعد تجريعها ببعض منتجات نحل العسل تحت ظروف الصيف فى مصر

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اجريت هذه الدراسة لبحث فعالية غذاء الملكات و البروبوليس على خصائص السائل المنوى و الخصوبة لذكور الأرانب النيوزيلندى الأبيض خلال الصيف. تم استخدام ٣٠ ذكر نيوزيلندى أبيض ناضج و ٦٠ أنثى بكر خليط فى هذه الدراسة. تم تقسيم ذكور الأرانب أرنب على ٣ مجموعات عشوائيا (١٠ ذكور لكل مجموعة)، تم تجريع هذه الذكور ٠,٥ ملليلتر محلول / كجم من وزن الجسم، مرة واحدة أسبوعيا لمدة ٦ أسابيع، و إحتوى هذا المحلول على:

١) ماء للمجموعة الضابطة،
٢) ٤٠٠ مجم غذاء ملكات مذاب فى ٠,٢٥ مل عسل نحل + ٠,٢٥ مل ماء للمجموعة المعاملة بغذاء الملكات،
٣) ١٠ مجم بروبوليس معلقة فى ٠,٥ مل ماء للمجموعة المعاملة بالبروبوليس.

أوضحت هذه الدراسة أن ذكور الأرانب النيوزيلندى الأبيض المعاملة سواء بغذاء الملكات أو البروبوليس قد أظهرت زيادة معنوية (عند مستوى إحتمال 0.05) للزمن اللازم للقذف، و حجم القذفة، و نسبة الحيوانات المنوية المتحركة حركة تقدمية، و تركيز الحيوانات المنوية، و تركيز الفركتوز فى بلازما السائل المنوى مقارنة بالمجموعة الضابطة. على الجانب الآخر، فإن النسب المئوية للحيوانات المنوية الميتة و الشاذة قد زادت معنويا (عند مستوى إحتمال 0.05) فى المجموعة الضابطة مقارنة بالمجموعتين الأخرتين. تركيز هرمون التستيستيرون فى بلازما الدم للذكور المعاملة بالغذاء الملكى و البروبوليس قد زاد معنويا (عند مستوى إحتمال 0.05) مقارنة بالمجموعة الضابطة. من ناحية أخرى، فإن تركيزات الكلوسترول و AST و ALT قد انخفضت معنويا (عند مستوى إحتمال 0.05) للمجموعتين المعاملتين بغذاء الملكات و البروبوليس مقارنة بالمجموعة الضابطة. الأرانب المعاملة سواء بغذاء الملكات أو البروبوليس أظهرت خصوبة أفضل (نسبة حمل عالية و عدد خلفة للأم عالى) عن المجموعة الضابطة.

التوصية: تجريع ذكور الأرانب عن طريق الفم بغذاء الملكات و البروبوليس تحت الظروف البيئية فى مصر خلال شهور الصيف قد حسن من كفائتها التناسلية و الذى ظهر من خلال زيادة فى تركيز التستستيرون و الرغبة الجنسية و حجم القذفة و الحركة التقدمية للحيوانات المنوية و تركيز الحيوانات المنوية و تركيز الفركتوز و نسبة الإناث الحوامل و عدد الخلفة للأم، كذلك بإنخفاض نسبتى الحيوانات المنوية الميتة و الشاذة. هذا التحسن كان منعكسا على وظائف جيدة للكبد و ظهر ذلك من انخفاض تركيزات كل من AST و ALT.

أظهرت نتائج هذه الدراسة أن غذاء الملكات أو البروبوليس مفيد فى إستخدامه لتحسين خصائص السائل المنوى و الخصوبة لذكور الأرانب خلال موسم الصيف فى مصر.