



**SEMEN QUALITY, SEX HORMONE AND ANTIOXIDANT
STATUS OF MALE RABBITS AS INFLUENCED BY TWO FORMS
OF ONION**

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Received: 10/01/2019

Accepted: 27/01/2019

ABSTRACT: This work mainly aims to evaluate the effect of using two forms of onion (*Allium cepa* L.) (powder or juice) separately on semen quality, serum sex hormone and antioxidant profile of bucks. Thirty V-Line bucks (9 months old) were treated for 8 weeks and divided into five groups. Groups 1st, 2nd and 3rd were supplemented with 0, 400, 800 mg of onion powder/kg diet, respectively, while, groups 4th and 5th were given orally 1.5 and 3ml fresh onion juice /kg BW /day, respectively. Semen quality such as libido time, volume, sperm count, sperm motility, fructose and acrosome reaction with some blood sex hormones (FSH, LH, Testosterone) and serum antioxidant status (T. antioxidant capacity, malonaldehyde, superoxide dismutase) were evaluated. Onion powder or juice treatments at any dose displayed a significant shortened the time of libido with increased ejaculated volume, individual motility, sperm concentration, total sperm output, live sperm, total motility and acrosome reaction as compared to the untreated group. Serum sex hormones such as Luteinizing H (LH), Follicle Stimulating H (FSH) and testosterone were significantly ($P<0.05$) boosted compared to the control. Results revealed that serum antioxidant profile significantly improved by all onion treatments. In conclusion, using two forms of onion (powder or juice) separately for male rabbits can improve their reproductive and antioxidant status

Key word: rabbit – onion – sex hormones – semen quality – antioxidant

INTRODUCTION

Onions (*Allium cepa* L.) occupied the second place in the greatest production worldwide crop following tomatoes (Grzelak-Błaszczyk et al., 2018; Abdelrahman et al., 2016). Many researches shown that onions own many therapeutic activities by many cultures. Onions are usually recognized for their high content of phytochemicals, such as carotenoids, copanens, flavanols. Quercetin 4'-glucoside and quercetin 3,4'-diglucoside are the main onion flavonols (Grzelak-Błaszczyk et al., 2018). Besides, onions are generally used in regulate blood cholesterol, reduced risk of cardiovascular diseases and many pharmacological actions such as anti-inflammatory, antiseptic, antibiotic, antitumor, antifungal, antioxidant immunomodulatory and anti-allergic (Hui et al., 2018).

Onions are widely known as a source to quercetin (strong antioxidant) belongs to flavonoids group (Terao & Piskula, 1998), kaempferol (Dorant et al., 1994) that scavenge free radicals with inhibits lipid peroxidation and pigments such as anthocyanins (Fitzpatrick et al., 1993). Flavonoids in onions is more bioavailable than any other sources like apples and tea (de Vries et al., 1998). The onion antioxidant substance (quercetin) has antitoxic effect on sperm cell and testicular alterations (Adedara et al., 2017 and Aldemir et al., 2014) beside protective effect against oxidation (Moretti et al., 2012; Ben Abdallah et al., 2011).

The purpose of this research was to evaluate the beneficial power of two form of onion (powder or juice) on semen characteristics, sex hormones and serum blood antioxidant status of buck rabbits.

2. MATERIALS AND METHODS

2.1. Source of onion:

Onion powder was a purchased from American garden product-New York, USA. White fresh onions were obtained from local market in Alexandria, Egypt, in April to June 2017. White onion juice was prepared by a fruit juice machine before oral administration of treated bucks and set in pyrogen-free bottles according to (khaki et al., 2009&2012).

2.2. Location:

The research was carried out at rabbits Lab. in the Department of Animal and Fish Production, Agriculture Faculty, Saba Bash, Alexandria University, Egypt, during spring season (April to June 2017).

2.2 .Animals

Thirty V-Line bucks, age 9 months with similarly body weight mean (3.33 ± 0.12 kg), were used in the current experiment. The males were randomly spread to five treatments of 6 rabbits each. The males were caged individually in wire ground cages of 45 x 36 x 36 cm under normal daylight (16 h). Groups 1st, 2nd and 3rd were supplemented with 0, 400, 800 mg of onion powder/kg diet, respectively, while, groups 4th and 5th were given orally 1.5 and 3ml fresh onion juice /kg BW /day, respectively. The feed and clean water were available *ad libitum*. The basal diet was formulated according to NCR 1977.

2.4 .Semen quality:

The ejaculate samples from each male were weekly collected during the experimental trial. Artificial vagina used to collect semen with exposing a matured female as a teaser. Handling and collection of semen carried out according to (IRRG, 2005). Libido was measured as a period between the introductions of female in the male cage until ejaculation.

rabbit – onion – sex hormones – semen quality – antioxidant

After that, the ejaculate volume (ml) was measured. pH value was determined by using pH paper (Merck KgaA,64271 Darmstadt, Germany). Sperm cell concentration was evaluated as described by Smith and Mayer (1955). Total sperm output was calculated by multiplying ejaculate volume by semen cell concentration. Semen mass motility was given a score (0 to 3) according to Moule (1965). Individual sperm activity was measured (Kamar, 1960). Seminal initial fructose was evaluated instantly after ejaculate collection as described by (Mann, 1948). Live spermatozoa percentage was measured by an eosin-aniline blue staining mixture (Shaffer and Almquist, 1948). Packed sperm volume was measured by using Micro-AID® microhematocrit tubes and microhematocrit-centrifuge which centrifuged for 5 min at 4000 rpm. To determine acrosome reaction a dried semen smears stained with naphthol yellow S and erythrosine B. Appearance or nonappearance of the acrosomal cover in 200 sperms were noted and classified in to intact and nonintact acrosome sperms. Total motile sperm= motile sperm (%) × total sperm output (10⁶/ejaculate).

Antioxidant and hormone status:

Blood sample in each buck of treatment group was taken from the ear vein by a sterile syringe. 5ml of the blood sample was put into a sterile vacutainer tube without an anticoagulant for serum antioxidant and hormone analysis. Total antioxidant capacity, MAD and SOD levels in blood serum were evaluated by commercial kits (Bio Merieux, France) as stated by the manufacturer outlined. Serum FSH, LH and testosterone hormones concentrations were measured by Radio Immuno assay (RIA) using the

kits supplied by Diagnostic Products Corporation, USA.

Statistical analysis

All Data results were analyzed with one-way analysis of variance (ANOVA) using SPSS 11.0 statistical software (SPSS, Inc., Chicago, Il, 2007). Significant between means were noticed by new Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Semen quality:

Table 1 show the impact of use two forms of onion (powder or juice) separately for semen quality. Data show that non-significant change was observed in mass activity by different treatments. Libido and semen pH of onion treatments were significantly (P<0.001) decreased. In contrast, ejaculate volume; individual motility; sperm cell concentration and percentage of live sperm of onion groups significantly (P<0.001) improved that reflect on increased each of total sperm output; PSV and total motile sperm. Similar pattern of results was observed in recent publication where they examined the effect of nutritional quercetin (main active ingredient of onion) on rabbit semen quality throughout summer months (Naseer et al. 2018). Improved ejaculate volume upon onion treatment may be due to the ROS scavenge character of onion in the sex accessory gland. Furthermore, the enhancement of motility, sperm concentration and live sperm reflect the antioxidant power of onion because keep ROS low improved motility through increasing axonemal protein phosphorylation and spermatozoa immobilization (de Lamirande et al., 1995) that display of glucose-6-phosphate-dehydrogenase in sperm to maintain the glucose level for motility (Liu et al., 2015). Improving

concentration and motility of sperm related by increased the serum LH and FSH levels as show in Fig 1. FSH acts on Sertoli cells in the seminiferous tubules to imitate spermatogenesis. Alike to the other semen characteristic, acrosome reaction is also significantly ($P < 0.001$) increased by using two forms of onion (powder or juice) as compared with control group. Higher amount of polyunsaturated fatty acids and insufficient antioxidant of spermatozoa make it sensitive to the ROS injury; so, sperm membrane beside the acrosome or tail is saved by seminal plasma antioxidants (Talwar and Hayatnagarkar, 2015) to maintenance sperm membrane against high ROS could be taken by onion treatments.

High level of onion juice and both levels of onion powder significantly resulted in increase in fructose concentration by 8.49; 33.94; 22.92 and 20.27% compared with control bucks, respectively, the improvement of the sperm motility might be recognized to the stimulate fructose production in ejaculation (Zeweil et al., 2013; Yousef et al., 2003)

Sex hormones:

Hormonal profile can be used as good indicator for semen quality. So, the effects of use two forms of onion (powder or juice) separately for blood sex hormone concentration (testosterone, FSH and LH) are presented in Fig. 1.

Two forms of onion (1.5 ml; 3ml OJ/kg BW) and (400mg; 800mg OP/kg diet) significantly ($P < 0.05$) increase concentration of each of testosterone by 11.07; 13.84; 15.87; 5.54, FSH 9.61; 11.79; 10.92; 14.41 and LH 11.35; 17.03; 18.38; 34.59%, respectively, as compared with control bucks. The highest value of testosterone was found in 400mg OP/kg diet group, whereas, the highest

concentration of FSH and LH was found in 800mg OP/kg diet group. Results of the present study suggested that powder form of onion have significance benefits on sex hormones. Date of the previse research suggested that onion has significant benefits on both LH and FSH (Ghalehkandi et al., 2012). In other study by Khaki et al., 2009 found that serum testosterone increased in groups received fresh onion juice (1 g/rat) for 3 weeks.

Antioxidant status:

The effects of use two forms of onion (powder or juice) separately for serum antioxidant profile are presented in Fig. 2. Two level of each onion juice or onion powder treatments significantly increased ($P < 0.01$) TAC by 21.84; 18.45; 22.33; 23.30 and SOD 53.33; 38.33; 27.78; 16.67%, respectively, with a significantly decreased in MAD by 5.24; 8.06; 21.77; 29.43%, respectively, when compare with control. Grzelak-Błaszczyk et al., (2018) cleared that supplementation with quercetin increased the serum TAC of high fat diet rat and improved the serum lipid profile

Conclusively, the results of discussed study recommend that two forms of onion (juice or powder) have shown beneficial effects in rabbit semen parameters, sex hormones and antioxidant status and suggest using the onion powder (400 or 800 mg/kg diet) rather than onion juice

Table (1): Effect of onion powder (OP) and onion juice (OJ) on semen characteristics of V-Line bucks (means \pm SEM)

	Treatment groups					SEM	P-Value
	Control	400 mg	800 mg	1.5 ml	3 ml		
Libido (sec.)	14.44 ^a	11.86 ^c	10.64 ^d	13.20 ^b	11.79 ^c	0.25	0.0001
Ejaculate volume(ml)	0.62 ^c	0.77 ^b	0.81 ^a	0.80 ^a	0.77 ^b	0.01	0.0001
Hydrogen ion(pH)	7.55 ^a	7.08 ^b	7.13 ^b	6.92 ^b	7.11 ^b	0.06	0.0001
Mass activity (1-3)	2.31	2.64	2.73	2.56	2.78	0.06	0.13
Individual motility	65.31 ^c	73.57 ^b	76.86 ^a	71.74 ^b	72.89 ^b	1.09	0.0001
Sperm Concentration (10 ⁶ /ml)	328.22 ^d	413.41 ^b	443.61 ^a	395.57 ^c	434.83 ^a	7.79	0.0001
Total sperm output ($\times 10^6$ /ejaculate)	207.49 ^d	315.77 ^c	359.10 ^a	315.76 ^c	333.37 ^b	9.80	0.0001
Total motile output($\times 10^6$ /ejaculate)	135.62 ^d	232.36 ^c	275.53 ^a	227.04 ^c	243.04 ^b	8.77	0.0001
Live sperm %	69.61 ^d	74.46 ^c	83.60 ^a	79.66 ^b	79.86 ^b	1.56	0.0001
PSV ¹ %	16.59 ^c	21.01 ^b	23.12 ^a	22.64 ^a	23.28 ^a	0.52	0.0001
Acrosome reaction %	69.01 ^c	75.68 ^b	80.85 ^a	75.78 ^b	77.39 ^b	1.19	0.0001
Initial Fructose(mm/dl)	265.40 ^c	315.17 ^b	319.20 ^b	278.17 ^c	355.50 ^a	6.55	0.0001

Different letters (a-d) within a row denote significant differences between treatments ($p \leq 0.05$). ¹ PSV= Packed sperm volume.

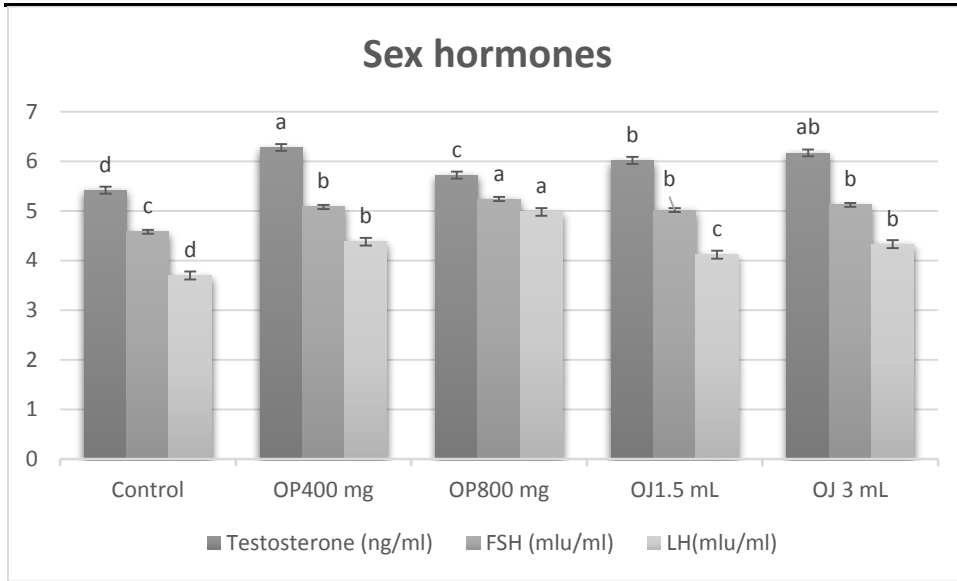


Fig. (1): Effect of onion powder (OP) and onion juice on serum sex hormones of V-Line bucks (Means ± SEM)

Different letters (a-d) within a same column color denote significant differences between treatments ($p \leq 0.05$).

FSH= Follicle stimulation hormone; LH= Luteinizing hormone

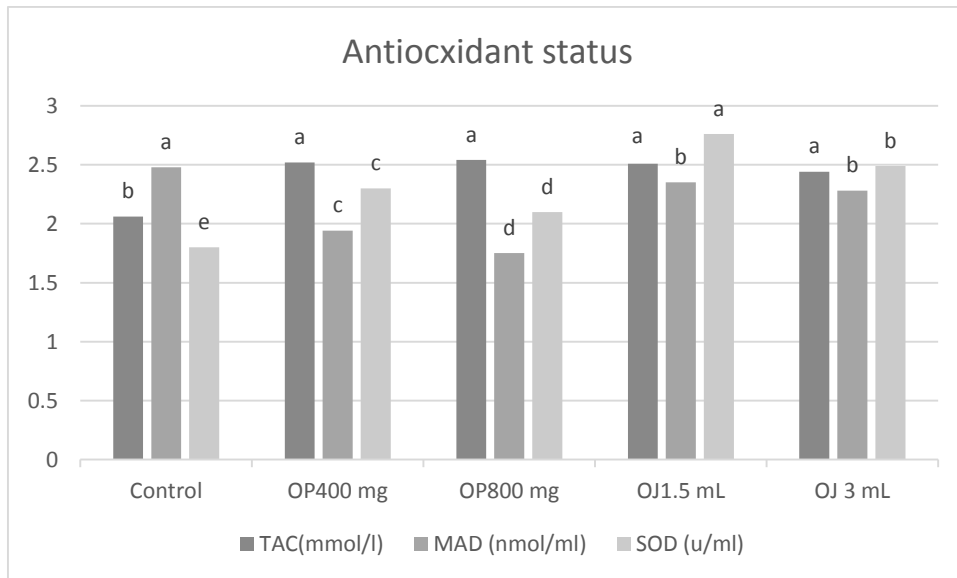


Fig. (2): Effect of onion powder (OP) and onion juice (OJ) on serum antioxidant profile of V-Line bucks (Means ± SEM)

Different letters (a-e) within a same column color denote significant differences between treatments ($P \leq 0.05$).

TAC= Total antioxidant capacity; MAD= Malonaldehyde; SOD= Superoxide dismutase.

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الملخص العربي

جودة السائل المنوي وتركيز هرمونات الجنسية ومضادات الأكسدة لذكور الأرانب متأثره بصورتين من البصل

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يهدف هذا البحث إلى تقييم تأثير استخدام صورتين من البصل (المسحوق أو العصير) بشكل منفصل على جودة السائل المنوي وتركيز هرمون الجنس في الدم و مضادات الأكسدة لذكور الأرانب. تم معاملة ثلاثون ذكر من الأرانب V-Line (عمر 9 أشهر) لمدة 8 أسابيع وتم تقسمها إلى خمس مجموعات تجريبية. حيث غذيت المجموعات التجريبية الأولى والثانية والثالثة على 0 ، 400 ، 800 ملجم من مسحوق البصل / كجم من العلف، على التوالي ، بينما جرعت المجموعتان الرابعة والخامسة ب 1.5 و 3 مل عصير بصل طازج / كجم وزن جسم / يومياً، على التوالي. تم تقييم نوعية السائل المنوي مثل وقت الرغبة الجنسية، حجم القذف، تركيز الحيوانات المنوية، حركة الجماعية والفرديّة للحيوانات المنوية، تركيز الفركتوز والتفاعل الاكروسومي للحيوان المنوي مع تقدير مستوى بعض هرمونات الجنسية في الدم (LH ، FSH ، التيستوستيرون) وتركيز مضادات الأكسدة في الدم (مجموع مضادات الأكسدة، malonaldehyde ، ديسموتاز superoxide). اوضحت النتائج ان جميع معاملات البصل (مسحوق أو العصير) قللت معنوياً من وقت الرغبة الجنسية مع زيادة حجم القذف، الحركة الفردي، وتركيز الحيوانات المنوية، وإجمالي عدد الحيوانات المنوية للقذف، ونسبة الحيوانات المنوية الحية، والحركة الجماعية و التفاعل الاكروسومي للحيوان المنوي مقارنة مع مجموعة الكنترول. مع زيادة مستوى هرمون الجنس معنوي (LH ، FSH و هرمون التستوستيرون إلى حد كبير مقارنة مع مجموعة الشاهد (الكنترول). وتحسن بشكل معنوي تركيز مضادات الأكسدة بالدم في جميع معاملات البصل.

التوصية: نستخلص من نتائج البحث ان استخدام مسحوق او عصير البصل للذكور الارانب حسن معنوياً الحالة التناسلية وصفات السائل المنوي ومستوى مضادات الأكسدة بدم ذكور الارانب.