IMPACT OF LYCOPENE OR FOLIC ACID TREATMENT ON SEMEN QUALITY, BLOOD CONSTITUENTS AND FERTILITY OF RABBIT BUCKS

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(Received 13/6/2017, accepted 1/8/2017)

SUMMARY

Effect of lycopene or folic acid supplementation in drinking water as enhancing factors on reproductive performance of NWZ rabbit bucks was investigated in this study. Total of 30 sexually adult bucks were homogeneously divided into 3 experimental groups (10 bucks/each). Bucks in the 1st, 2nd and 3rd group daily received drinking water supplemented with 0, 500 mg lycopene and 500 mg folic acid/l, respectively, for 4 weeks as a treatment period. Semen was collected twice/week for another six consecutive weeks. Blood constituents and semen quality were evaluated. At the end of the semen collection period, 90 NWZ does were divided into 6 groups (15/each). The 1st three groups were naturally mated with bucks treated with 0, lycopene or folic acid, while other three groups were artificially mated with pooled semen of each treated buck group. Results showed that lycopene or folic acid insignificantly increased final body weight and increased (P<0.05) water intake of bucks during treatment period. At the end of treatment period, both treatments increased (P<0.05) hemoglobin concentration, hematocrit value, count of red blood cells and platelets, serum total proteins, albumin, globulin, glucose and high density lipoproteins concentrations, while decreased (P<0.05) white blood cells count, and concentration of serum urea, creatinine, total lipids, triglycerides, total cholesterol and low density lipoproteins concentrations. Concentrations of total antioxidant and testosterone increased (P<0.05), while malondialdehyde concentration decreased (P<0.05) in treatment groups as compared to control. Both treatments improved (P<0.05) volume, quality and total sperm output, initial semen fructose concentration, conception and kindling rates, and litter size at birth of NZW does naturally or artificially mated by treated bucks, while decreased (P<0.05) semen pH value. Although both treatments had superiority in comparing with control, most blood constituents and semen traits were better (P<0.05) for lycopene than folic acid treatment. In conclusion, treatment of rabbit bucks with lycopene at a level of 500 mg/l drinking water or as oral administration of 105 mg/buck for 4 weeks prior to natural mating or semen collection could be useful as a strong antioxidant and could have interesting applications in rabbit farms.

Keywords*: Rabbit bucks, lycopene, folic acid, semen, fertility, oxidative capacity.*

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INTRODUCTION

Successful fertilization requires good quality semen containing functional spermatozoa with a normal membrane status (Flesch and Gadella, 2000). The wide use of artificial insemination (AI) in commercial farms is an important tool for improving the reproduction of rabbit bucks (Rriad *et al.,* 2016), reducing the number of bucks used genetically for breeding programs (Vasicek *et al.,* 2014), and controlling disease and decreasing the cost of insemination (Laurinčík *et al.,* 2008) as compared to natural mating. Therefore, proper protocols to improve spermatozoa characteristics are needed, including several factors affecting seminal traits (Boiti *et al.,* 2005).

It is very essential to identify the factors or conditions affecting normal sperm functions. In this respect, many environmental and physiological factors have been implicated in the poor sperm functions and infertility (Gul Baykalir *et al.,* 2016). It is well known that lipid composition is an important structural and functional component of sperm cells. In rabbits, spermatozoa contain lower amounts of n-6 poly-unsaturated fatty acids (PUFA) series and higher amounts of n-3 PUFA as compared to other mammals (Mangiagalli *et al.,* 2012). Presence of PUFA in plasma membrane of sparmatozoa are a target of reactive oxygen species (ROS) action leading to lipid peroxidation, in term of malondialdehyde (MDA) production and losing sperm motility (Opuwari and Henkel, 2016) as a result of DNA fragmentation and poor fertilization rates (La Vignera *et al.,* 2013; Opuwari and Henkel, 2016).

Several natural antioxidants can protect DNA and other molecules from cell damage induced by oxidation, improving sperm quality and increasing reproductive efficiency of male (Yang *et al.,* 2006). Some natural antioxidants such as lycopene (Mangiagalli *et al.,* 2010) or folic acid (Tolba *et al.,* 2015) are important components of antioxidant defense and protect the plasma membrane against lipid peroxidation, and play an important role in amino acid and DNA metabolism.

Lycopene is a bioactive carotenoids synthesized by plant (red fruits and vegetables) or by microorganisms, but not by animals (Pozzo *et al.,* 2013; Durairajanayagam *et al.,* 2014). It regarded as one of the most potent singlet oxygen quenchers in the carotenoid family, because it is twice as effective as β-carotene and up to 10 times more effective than α-tocopherol (Palozza *et al.,* 2012). It is a highly unsaturated straight chain hydrocarbon, which has a powerful natural antioxidant (Tvrdá *et al.,* 2016), anti-inflammatory (Jae *et al.,* 2017), antimicrobial and immunomodulatory (Omodamiro and Amechi, 2013) properties, being the major carotenoid in tomato fruit (80–90% of the total pigment contents in ripe tomatoes) (Tvrdá *et al.,* 2016).

Folic acid is known as vitamin B9 and recognized as a family of cofactors that participate in one-carbon metabolism and cellular pathways like purine, thymidylate, and methionine biosynthesis (Kamel, 2012). It is a coenzyme in the body directly acting on, exhibit antioxidant effects, red blood cells production and DNA synthesis (Stanger, 2002), amino acid and DNA metabolism (Tolba *et al.,* 2015), spermatogenesis (Kamen, 1997), and general normal male fertility (Hussein *et al.,* 2012).

Therefore, the objective of this study was to investigate effect of lycopene or folic acid supplementation in drinking water as enhancing factors on reproductive performance of rabbit bucks.

MATERIALS AND METHODS

The present study was carried out at a private rabbit farm, located in Tanikh village, Nabroh city, Dakahlia governorate, Egypt, during the period from October 2016 to February 2017.

Total of 30 sexually mature NWZ rabbit bucks weighing 2.520±6.54 kg LBW and 5 months of age as semen donors as well as 90 receptive multiparous NWZ rabbit does were used in this study for fertility trails. Throughout the experimental period, all animals were individually housed in stainless steel cages supplied with feeders and nipples, and fed *ad libitum* on a commercial complete pelleted diet.

Rabbit bucks were homogeneously divided into 3 experimental groups (10 bucks in each). Bucks in the 1st group received drinking water without any supplementation (control group), while those in the 2nd and 3rd groups daily received drinking water supplemented with 500 mg lycopene or 500 mg folic acid/l, respectively) for 4 wks (15 October to 11 November 2016) as a treatment period.

Semen was collected twice weekly for 6 weeks (12 November to 23 December 2016) as a semen collection period (120 ejaculates, 10 bucks x 6 wks) using an artificial vagina and a teaser doe. Immediately after collection, ejaculates were kept at 37°C in water bath for semen evaluation.

Semen volume of each ejaculate was recorded with or without gel mass. Mass motility (MSM, score 0-5), semen pH value, individual motility (ISM), dead (DS), abnormality (SA) and acrosomal damage (AD) percentages, and sperm cell concentration (SCC) of spermatozoa were determined in each ejaculate. However, motility index (MI), total sperm output (TSO) were calculated as the following:

$$MI = MM\ (score) \times ISM\% \qquad TSO = EV\ (ml)\ without\ gel \times SCC/ml.$$

Initial fructose concentration was determined immediately after collection in row semen according to Mann (1948).

Live body weight of rabbit bucks and water intake were recorded during the treatment period (4 wks). Blood was collected into clean test tubes at the end of semen collection (end of the experiment) period from ear vein of each buck. Each blood sample was divided into two portions; the 1st was to determine the hematological parameters (hemoglobin concentration, hematocrit value, and count of red blood cells (RBCs), white blood cells (WBCs) and platelets using blood hematology analyzer (HB 7021). The 2nd blood portion was left to clot (2-3 h), then blood serum was separated by centrifugation at 3500 rpm for 15 min and stored at -20 ^{o}C for later analyses of concentration of some biochemicals (total proteins, albumin, glucose, total lipids, urea, creatinine, triglycerides, total cholesterol, total antioxidants, MDA) and testosterone in blood serum. Biochemicals concentration was determined by colorimetric enzymatic methods using commercial kits purchased from Egyptian company for biotechnology (Obour city

industrial area Cairo, Egypt). Total antioxidant capacity was according to Erel (2004) and MDA was assayed in the serum according to Conti *et al.* (1991) using commercially available kits (Bio-Diagnostic Research, Giza, Egypt). Serum testosterone concentration of bucks was determined using RIA Kits (Immunotech, A Coulter Co., France) according to the manufacturer information. Globulin concentration was calculated by subtracting albumin from total protein concentration.

At the end of semen collection period, ninety receptive multiparous rabbit does were divided into 6 groups (n=15 in each); the 1st three groups were naturally mated with bucks treated with 0, lycopene or folic acid, while other three groups were artificially mated with pooled semen (0.5 ml straw) of each buck group (0, lycopene or folic acid) diluted at a rate of 1:5 with glucose yolk citrate diluent and 50 µg/ml gentamycin. Artificially mated does were given an intramuscular injection with 0.25 ml/doe GnRH (Receptal, intervet equivalent B.V. Boxmeer Holland) to induce ovulation. The artificial mating technique was applied according to Eschborn (1985) using especial devises disposable a plastic curved pipette (Imporvet, S.A., Barcelona, Spain). Does were mated by introducing about 0.5 ml fresh diluted semen into at least 12 cm after passing pelvic brim to ensure appropriate delivery of semen to the vagina (Rriad *et al.,* 2016). Pregnancy diagnosis of mated rabbit does was performed by abdominal palpation 10-12 days post-mating to determine conception rate (%). While, kindling rate and total litter size/doe were recorded at birth.

Data were analyzed by one-way ANOVA using GLM procedures of SAS (2000). Duncan's Multiple Range Test was set at P<0.05 to determine the significant differences among means according to Duncan (1955).

RESULTS AND DISCUSSION

Body weight and water intake:

Lycopene or folic acid administrated at a level of 500 mg/L in drinking water did not affect significantly the final body weight of rabbit bucks, while significantly (P<0.05) increased water intake of both treatment groups in comparison with control one. It is worthy noting that water intake reflect slight difference in the amount of each treatment (Table 1).

Although dietary additives of folic acid significantly increased live body weight of rabbit bucks at a level of 2 mg/kg diet (Kamel, 2012) or quail laying hen at a level of 10 mg/kg diet (Tolba *et al.,* 2015) during hot climate stress, the obtained results are in agreement with those reported by Mangiagalli *et al.* (2012), who found that lycopene administration at different levels (0.01 or 0.5 g/l) in drinking water of rabbits had no significant effect on final body weight, while water intake significantly increased in treated compared with control groups. In broilers, Pozzo *et al.* (2013) reported that body weight was not affected by lycopene administration. The confliction in these results may be attributed to dose or method of treatment and/or species of treated animals.

Table (1): Effect of lycopene or folic acid treatment on live body weight and water intake of rabbit bucks during the experimental period.

Parameter	Control group	Treatment group		±SEM
		Lycopene	Folic acid	
Initial live body weight (g)	2513.0	2502.0	2520.0	6.238
Final live body weight (g)	2748.0	2759.3	2761.0	6.543
Chang in live body weight (%)	9.35	10.28	9.56	-
Daily water intake (ml/buck)	168.20[b]	209.90[a]	201.18[a]	7.992
Daily treatment (mg/buck)	-	105	101	-

[a and b]: Means denoted within the same row with different superscripts are significantly different at P<0.05.

In addition using different types of antioxidants had insignificant effect on final body weight, in terms of ginger as dietary supplement (El-Saieh, 2014), propolis as oral administration (Gabr, 2013) and ascorbic acid, vitamin E or their combination in drinking water (Yousef *et al.,* 2003) of rabbits as well as ginger addition in diets of birds (Al-Moramadhi, 2010) and propolis in fish (*Rainbow Trout*) diets (Kashkooli *et al.,* 2011).

Blood parameters:

Hematological parameters:

Treatment of rabbit bucks with lycopene or folic acid significantly (P<0.05) increased hemoglobin (Hb) concentration, hematocrit value, count of RBCs and platelets, while significantly (P<0.05) decreased count of WBCs as compared to control bucks, being the best for lycopene than folic acid treatments (Table 2).

In accordance with the present results, Sharma and Vijaya (2015) indicated that administration of lycopene (20 mg/kg LBW) for 15 days enhanced hematopoiesis including RBCs, Hb concentration and packed cell volume in mice. Also, administration of lycopene (10, 20 and 40 mg/kg) to diabetic rats significantly (P<0.05) elevated Hb concentration and RBCs count (Daniel, 2015).

Natural antioxidant treatments improved hematological parameters (RBCs count and Hb concentration) of rabbit does treated with green tea extract (El-Ratel *et al.,* 2017) and rabbit bucks treated with propolis (Hashem *et al.,* 2013).

Table (2): Effect of lycopene or folic acid treatment on some hematological parameters in blood of rabbit bucks.

Parameter	Control group	Treatment group		±SEM
		Lycopene	Folic acid	
Hemoglobin (mg/dl)	9.26^{c}	11.29^{a}	11.05^{b}	0.049
Hematocrit (%)	41.50^{b}	47.75^{a}	44.50^{ab}	1.164
RBCs (x 10^{6}/mm^{3})	4.72^{b}	5.88^{a}	5.73^{a}	0.062
WBCs (x 10^{3}/mm^{3})	7.47^{a}	6.20^{b}	6.29^{b}	0.063
Platelets (x 10^{3}/mm^{3})	206.00^{c}	254.75^{a}	240.00^{b}	3.467

$^{a,b\ and\ c}$: Means denoted within the same row with different superscripts are significantly different at P<0.05.

The observed improvement in hematological parameters following lycopene or folic acid treatments in this study might be related to the strong antioxidant effect of these treatments on hematopoietic cells. Hematopoietic cells appear to be particularly vulnerable in the presence of unchecked accumulation of ROS, because deficiencies in several ROS scavengers result in either anemia that is severe or even lethal in some cases and/or malignancies of hematopoietic tissues (Kong *et al.,* 2004). Also, Palmieri *et al.* (2001) implicated the role of ROS in the mechanism of damage of RBCs in diabetic patients.

Biochemical parameters:

Treatment of rabbit bucks with lycopene or folic acid significantly (P<0.05) increased biochemical blood parameters in terms of total proteins, albumin, globulin, glucose and HDL concentrations, while significantly (P<0.05) decreased concentration of urea, creatinine, total lipids, triglycerides, total cholesterol and LDL in blood serum of rabbit bucks as compared to control bucks. Rate of change in all the previous parameters was more pronounced with lycopene than with folic acid treatments (Table 3).

These findings indicated beneficial effects of both treatments as antioxidants on protein metabolism. In harmony with the results of lycopene, several authors found remarkable impact of lycopene as a dietary supplementation of 1% tomato powder on concentration of total proteins, albumin and globulin in blood plasma of rabbits (Asal, 2013), fish (Ibrahim and Banaee, 2014) or in male rat (Elkomy and Hassan, 2005). Concerning the positive effect of folic acid, Kamel (2012) reported that administration of folic acid increased total proteins and albumin concentration in seminal plasma of rabbit bucks.

Carbohydrate metabolism also improved with both treatments as antioxidants. In this respect, antioxidants supplementation significantly increased plasma glucose level of rabbit treated with royal jelly (Elnagar *et al.,* 2010; El-Hanoun *et al.,* 2014) or propolis (Gabr, 2013). Also, reduction in protein metabolites (urea and creatinine) indicating an improved kidney function as affected by both treatments was proved by many authors in blood of heat stressed rabbit bucks treated with royal jelly (El- Elnagar, 2010; El-Hanoun *et al.,* 2014) or in seminal plasma of Japanese quail males treated with folic acid (Tolba *et al.*, 2015).

Table (3): Effect of lycopene or folic acid treatment on concentration of some biochemicals in blood serum of rabbit bucks.

Biochemical parameter	Control group	Treatment group		±SEM
		Lycopene	Folic acid	
Total proteins (g/dl)	6.13^{c}	6.94^{a}	6.76^{b}	0.025
Albumin (g/dl)	3.29^{c}	3.53^{a}	3.42^{b}	0.024
Globulin (g/dl)	2.84^{b}	3.41^{a}	3.34^{a}	0.029
Glucose (mg/dl)	107.50^{b}	116.25^{a}	113.50^{ab}	2.228
Urea (mg/dl)	34.75^{a}	30.53^{b}	31.55^{ab}	1.039
Creatinine (mg/dl)	1.33^{a}	1.19^{b}	1.22^{b}	0.011
Total lipids (mg/dl)	256.00^{a}	143.50^{c}	173.75^{b}	5.103
Triglycerides (mg/dl)	75.50^{a}	54.75^{c}	63.25^{b}	1.875
Total cholesterol (mg/dl)	87.50^{a}	73.75^{b}	79.50^{ab}	2.618
Low density lipoproteins, LDL (mg/dl)	114.75^{a}	86.75^{c}	91.75^{b}	0.975
High density lipoproteins, HDL (mg/dl)	61.00^{c}	71.75^{a}	66.50^{b}	0.878

$^{a,b\ and\ c}$: Means denoted within the same row with different superscripts are significantly different at P<0.05.

In accordance with the positive effect of both treatments on reducing lipid profile in rabbit bucks, similar results were reported by Zeweil *et al.* (2016) in growing rabbit treated with 100 or 200 mg lycopene in the diet, by Jouzi *et al.* (2015) in broiler cockerels treated with tomato pulp, by Silaste *et al.* (2007) in human fed lycopene or tomato products and by Sahin *et al.* (2006) in Japanese quail treated with lycopene.

These results emphasized on the antioxidant role of lycopene, in term of maintaining the normal values of biochemical parameters during experimental hepatitis and restoring the normal liver function via its protective effect due to its antioxidant defense mechanism (Asal, 2013). Also, Napolitano *et al.* (2007) suggested that lycopene may reduce the macrophage foam cell formation induced by modified LDL, by decreasing lipid synthesis and down regulating the activity and expression of scavenger receptor activity. Therefore, lycopene could reduce cholesterol synthesis and improve the cardiovascular system (Sun *et al.,* 2014).

Oxidative capacity and testosterone concentration:

Treatment of rabbit bucks with lycopene or folic acid group significantly (P<0.05) increased concentration of total antioxidant (TA) and significantly (P<0.05) decreased Malondialdehyde (MDA) concentration as compared to control, being insignificantly higher in for lycopene than for folic acid treatment (Table 4).

Male reproductive system is negatively affected by oxidative stress by lipid peroxidation induction and damage of DNA, leading to apoptosis. Therefore, a reduction in problems related to oxidative stress may improve oxidative stress the biomarkers (Durairajanayagam *et al.,* 2014). Tomato contained phytochemicals, which have antioxidant properties and combination with lycopene may contribute to protect against peroxidation (Alshatwi *et al.,* 2010). In addition, folic acid has antioxidant activity interactions with enzyme endothelial nitric oxide synthesis and affects co-factor bioavailability of nitric oxide (Stanger, 2002). During heat stress, feeding folic acid improved antioxidant status of rabbit bucks (Kamel, 2012) and folate treatment was associated with increasing antioxidative capacity (Stanger, 2002).

As proved in the present study, Sahin *et al.* (2006) showed that serum MDA levels decreased in Japanese quail fed diet supplemented with lycopene and vitamin E compared with the control. Also, dietary lycopene as a carotenoid compound protected lipid, protein and DNA from oxidation (Rao and Agarwal, 1999). An inverse association between MDA and antioxidant vitamins has been mentioned by Halliwell and Gutteridge (1989). In chicken treated with lycopene, a reduction in MDA production with a protection role of lycopene against depletion of glutathione during viral-induced acute oxidant stress (Leal *et al*., 1999).

Increasing testosterone concentration in rabbit bucks treated with both treatments in our study is in agreement with some investigators, who found that dietary supplementation with tomato powder (Asal, 2013) or folic acid (Yousef *et al.,* 2006; Kamel, 2012) significantly increased testosterone concentration in blood plasma as compared to control rabbits. Also, natural antioxidant nutrients such as doum, *Hyphaene Thebaica* (Ghazal *et al.,* 2016) or propolis (Hashem *et al.,* 2013) significantly increased plasma testosterone levels in rabbit bucks.

The noticed improvement in antioxidant capacity and testosterone concentration was mainly due to good antioxidant activity (Tvrdá *et al.,* 2016) and antimicrobial activity, especially against bacteria (Omodamiro and Amechi, 2013) of lycopene.

Generally, antioxidants contain some chemicals, being naturally toxic to bacteria, and have antimicrobial activity, especially against multidrug resistant bacteria (Tasdelen *et al.,* 2009) along with antimutagenic, anticarcinogenic and anti-inflammatory properties (Tvrdá *et al.,* 2016; Jae *et al.,* 2017).

Table (4): Effect of lycopene or folic acid treatment on oxidative capacity and testosterone concentration in blood serum of rabbit bucks.

Oxidative capacity	Control group	Treatment group		±SEM
		Lycopene	Folic acid	
Total antioxidants (mmol/l)	0.49[b]	0.61[a]	0.58[a]	0.012
Malondialdehyde (nmol/ml)	14.99[a]	11.89[c]	13.30[b]	0.070
Testosterone (ng/ml)	1.845[c]	2.54[a]	2.15[b]	0.035

[a and b]: Means denoted within the same row with different superscripts are significantly different at P<0.05.

Semen production and sperm output:

Lycopene or folic acid administration significantly improved (P<0.05) all semen physical characteristics, in terms of increasing ejaculate volume (with or without gel), mass sperm motility (MSM), percentages of individual motility (ISM), motility index (MI), dead (DS), abnormality (SA) and acrosomal damage (AD) of spermatozoa, sperm cell concentration (SCC) and total sperm output (TSO) as compared to control. On the other hand, semen pH value significantly (P<0.05) decreased, while concentration of initial semen fructose significantly (P<0.05) increased for lycopene and folic acid treatments comparing with control group. It is of interest to show that most semen traits were significantly (P<0.05) better for lycopene than folic acid (Table 5).

A potential positive effect of lycopene at a level of 0.5 g/l of drinking water was established on semen ejaculate volume without gel and total sperm count in rabbit bucks (Mangiagalli *et al*., 2012), semen quality in broiler chickens (Mangiagalli *et al.,* 2010) and human (Goyal *et al.,* 2007). Also, Saemi *et al.* (2012) showed that dietary inclusion of dried tomato pomace up to 30% increased (P<0.05) SCC, and decreased percentage of SA in roosters. Furthermore, lycopene administration resulted (P<0.001) in a maintenance of the spermatozoa motion parameters in bovine (Tvrdá *et al.,* 2016).

Table (5): Effect of lycopene or folic acid treatment on characteristics, sperm output and concentration of fructose in semen of rabbit bucks.

Semen characteristics	Control group	Treatment group		±SEM
		Lycopene	Folic acid	
Ejaculate volume without gel (ml)	0.64[c]	0.94[a]	0.81[b]	0.019
Semen gel volume (ml)	0.45[c]	0.53[a]	0.49[b]	0.016
Semen pH value	7.21[a]	7.05[b]	7.12[b]	0.0242
Mass motility (Score 1-5)	3.10[b]	4.30[a]	4.00[a]	0.231
Individual sperm motility (%)	60.50[c]	76.50[a]	71.82[b]	0.821
Motility index	187.55[b]	328.95[a]	287.28[a]	16.26
Dead sperm (%)	30.90[a]	18.80[c]	21.36[b]	0.616
Sperm abnormality (%)	16.50[a]	14.36[b]	12.80[c]	0.537
Acrosomal damage (%)	22.40[a]	14.30[c]	16.82[b]	0.434
Sperm cell concentration (x106/ml)	348.00[c]	492.80[a]	468.64[b]	5.361
Total sperm output (x106/ejaculate)	222.72[c]	463.23[a]	379.59[b]	4.787
Initial semen fructose (mg/dl)	72.50[c]	89.25[a]	76.75[b]	1.253

[a,b and c]: Means denoted within the same row with different superscripts are significantly different at P<0.05.

In general, different lycopene supplementations have shown promising results in alleviating male infertility of animals by decreasing lipid peroxidation and damage of DNA and by improving TSO and DS.

The observed improvement in physical semen characteristics of bucks treated with folic acid was reported by Kamel (2012) and Yousef *et al*. (2006) on rabbit bucks, Japanese quail males (Tolba *et al*., 2015). In this line, Audet *et al.* (2004) recorded a positive correlation between folic acid concentration in seminal plasma and sperm production in young boars. Potential enhancement in physical semen characteristics of males treated with folic acid, perhaps attributed to that folic acid might be vital to proper sperm development because it is for the production of DNA (Wallock *et al*., 2001).

Fertility study:

Fertility rate of NZW does naturally or artificially mated by bucks from treated by lycopene or folic acid groups in terms of conception and kindling rates as well as litter size at birth were improved as compared to control group, but the differences were significant (P<0.001) only for litter size at birth (Table 6).

Similar results were reported regarding the reproductive performance of rabbit does artificially mated by fresh semen from rabbit bucks treated with lycopene (Mangiagalli *et al.*, 2012) or *red algae* (Ali and Mervat, 2013). Also, hatchability, fertility, and embryo mortality rates of laying hens inseminated with fresh semen from cockerels treated with lycopene (Mangiagalli *et al.*, 2010). In addition, semen of rabbit bucks supplemented with folic acid improved kindling rate and litter size at birth of rabbit does compared with control does (Kamel, 2012).

Table (6): Reproductive performance of rabbit does naturally or artificially mated by bucks treated with lycopene or folic acid.

Item	Control group	Treatment group		±SEM
		Lycopene	Folic acid	
Naturally mated rabbit does:				
Conception rate	(15/11) 73.33	(15/14) 93.33	(15/13) 86.67	9.430
Kindling rate	(11/8) 72.73	(14/ 12) 85.71	(13/10) 76.92	12.694
Litter size at birth/doe	6.38[b]	8.33[a]	8.200[a]	0.289
Artificially mated rabbit does:				
Conception rate	(15/9) 60.00	(15/13) 86.67	(15/12) 80.00	11.10
Kindling rate	(9/6) 66.67	(13/12) 92.31	(12/10) 83.33	12.826
Litter size at birth/doe	6.50[b]	8.58[a]	8.40[a]	0.259

[a and b]: Means denoted within the same row with different superscripts are significantly different at P<0.05.

These results indicated that improving semen quality of rabbit bucks treated with lycopene or folic acid as antioxidants is linking to improve reproductive efficiency of rabbit does. These improvements may indicate a decrease of the oxidative stress, and subsequently oxidative damage, which enhances the normal fertilizing ability of spermatozoa (Calogero *et al.*, 2017). Also, kindling rate and total litter size of female were significantly influenced by semen quality of male (Lavaraa *et al.*, 2005).

CONCLUSION

Conclusively, supplementing lycopene or folic acid in drinking water of rabbit bucks caused significant improvement in semen quality and fertilizing ability of rabbit buck spermatozoa, as well as fertility traits of rabbit does naturally or artificially mated by treated bucks. In particular, treatment of rabbit bucks with lycopene at a level of 500 mg/l drinking water or as oral administration of 105 mg/buck for 4 wks prior to natural mating or semen collection could be useful as a strong antioxidant and could have interesting applications in rabbit farming.

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تأثير المعاملة بـالليكوبين أوحامض الفوليك على جودة السائل المنوى، مكونات الدم والخصوبة لذكورالأرانب

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تهدف هذه الدراسة إلى تقييم تأثير إضافة الليكوبين أو حامض الفوليك فى ماء الشرب كعوامل محسنة للأداء التناسلى لذكورالأرانب النيوزيلاندى البيضاء. إستخدم فى هذه الدراسة 30 ذكر ناضج جنسيا تم تقسيمهم الى 3 مجموعات (10 ذكور/مجموعة). المجموعة الأولى (كنترول) بينما عوملت المجموعة الثانية والثالثة يوميا ولمدة 4 اسابيع (مدة المعاملة) بـــ 500 ملجرام ليكوبين/لتر و 500 ملجرام حامض الفوليك/لتر ماء الشرب، على التوالى. تم جمع السائل المنوى مرتين أسبوعيا لمدة 6 أسابيع أخرى متتالية (مدة جمع السائل المنوى). تم فحص وتقييم جودة السائل المنوى ومكونات الدم. بعد إنتهاء مدة جمع السائل المنوى، تم إستخدام 90 أم نيوزيلاندى بيضاء قسمت إلى 6 مجاميع (15/مجموعة). تم تلقيح الأمهات فى الثلاث مجاميع الأولى طبيعيا ولقحت أمهات الثلاث مجاميع الأخرى صناعيا بالذكور المعاملة بـ (صفر، ليكوبين وحمض الفوليك)، على التوالى. أظهرت النتائج عدم وجود زيادة معنوية بالمعاملة بالليكوبين أو حامض الفوليك على وزن الجسم الحى مع وجود زيادة معنوية ($P<0.05$) فى كمية الماء المستهلكة يوميا لكل ذكر أثناء مدة المعاملة. أدت المعاملة بالليكوبين أو حامض الفوليك إلى زيادة معنوية ($P<0.05$) فى تركيز هيموجلوبين الدم والهيماتوكريت، عدد كرات الدم الحمراء والصفائح الدموية وتركيز البروتينات الكلية، الألبيومين، الجلوبيولين، الجلوكوز والليبوبروتينات عالية الكثافة فى سيرم الدم، بينما لوحظ إنخفاض معنوى ($P<0.05$) فى عدد كرات الدم البيضاء وتركيز اليوريا، الكرياتينين، الليبيدات الكلية، الدهون الثلاثية، الكولسيترول الكلى والليبوبروتينات منخفضة الكثافة فى سيرم الدم. لوحظ زيادة معنوية ($P<0.05$) فى تركيزات مضادات الأكسدة الكلية وهرمون التستسترون مع إنخفاض معنوى ($P<0.05$) فى تركيز المالونداياالدهيد فى المجاميع المعاملة بالليكوبين أو حمض الفوليك مقارنة بالكنترول. أدت المعاملة بالليكوبين أو حمض الفوليك إلى تحسن معنوى ($P<0.05$) فى حجم، جودة والناتج الكلى للحيوانات المنوية وكذلك تركيز فركتوزالسائل المنوى ومعدل الإخصاب، الولادة وعدد الخلفات عند الولادة لأمهات الأرانب الملقحة طبيعيا أو صناعيا بالذكور المعاملة مقارنة بالكنترول، بينما لوحظ إنخفاض معنوي ($P<0.05$) فى درجة تركيز أيون الهيدروجين فى السائل المنوى للذكور المعاملة مقارنة بالكنترول. بالرغم من أن كلتا المعاملتين أظهرت تفوقا ملحوظا مقارنة بالكنترول، كانت معظم خصائص السائل المنوى ومكونات الدم أفضل للذكور المعاملة بالليكوبين مقارنة بحامض الفوليك.

نستخلص من هذه الدراسة أن معاملة ذكور الأرانب بـــ 500 ملجرام/لتر من الليكوبين فى ماء الشرب أو التجريع بـ105 ملجرام/ذكر لمدة 4 أسابيع قبل التلقيح الطبيعى أو جمع السائل المنوى قد يكون مفيدا كمضاد أكسدة قوي يمكن تطبيقه فى مزارع تربية الأرانب.