

EFFECT OF LYCOPENE SUPPLEMENTATION ON SEMEN QUALITY AND REPRODUCTIVE PERFORMANCE IN RABBIT

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ABSTRACT: A total of 18 hybrid strain Martini male rabbits were randomly divided into 3 experimental groups. The animals in the first and second groups drank water with 0.1 and 0.5 g/L of lycopene addition (0.1 and 0.5 groups, respectively), while water without any supplement was administered to the control group rabbits (0 group) for 8 wk. Semen was collected from 18 bucks (6 animals/group) for 5 consecutive weeks. Ejaculate volume was determined by graduated test tube and sperm concentration was calculated by Neubauer chamber. Sperm motility was evaluated subjectively by a phase contrast microscope and forward progressive motility (FPM) scored 1-4 (low-high). Sperm viability was assessed by nigrosin/eosin (N/E) staining procedure. At the end of the semen evaluation period, 20 females/group were inseminated on day 11 *post partum* with a pool of semen collected from each experimental group. Data showed that the highest level of lycopene (0.5 group) resulted in a significantly greater volume of ejaculate and total number of sperm than in the control group (0.98 vs. 0.78 mL and 364 vs. 227; $P<0.05$), while sperm concentration was not affected. Lycopene addition did not significantly affect the sperm quality of fresh semen, but influenced the semen traits during storage (24 h at 5°C). In particular, the motility, FPM and viability of the 0.5 group were significantly higher ($P<0.05$) than those found in the 0 and 0.1 groups. No significant differences were found in reproductive performance. This study shows the positive effects of lycopene supplementation on semen production and refrigerated semen characteristics. Lycopene supplementation of drinking water could have interesting applications in field conditions, although further research into the role of this antioxidant in animal production is needed.

Key Words: lycopene, antioxidants, semen quality, rabbit bucks.

INTRODUCTION

Artificial insemination (AI) is widely used on rabbit farms and is essential for “cycled production”, which has radically improved animal management in intensive rabbit farming systems. In this production system, evaluation of buck semen has particular importance in the choice of breeders, as occurred in other livestock species.

In rabbit production, AI is usually performed with refrigerated semen (Roca *et al.*, 2000; López-Gatius *et al.*, 2005; Gogol and Wierzchoś-Hilczler, 2009). Many studies on additives for rabbit semen extenders have been done to improve the quality of stored spermatozoa (López and Alvaríno, 2000; Roca *et al.*, 2000; Nagy *et al.*, 2002). Lipid composition is acknowledged as an important structural and functional component of spermatic cells. Rabbit sperm contains high amounts of n-6 PUFA series and lower amounts of n-3 PUFA; this is a peculiarity of the rabbit within mammals, in which n-3 PUFA prevail (Gliozzi *et al.*, 2009). The presence of unsaturated fatty acids increases the susceptibility of spermatozoa to peroxidation, which contributes to

Correspondence: V. Cesari, valentina.cesari@unimi.it. Received March 2012-Accepted June 2012
<http://dx.doi.org/10.4995/wrs.2012.1150>

a negative effect on semen quality (de Lamirande *et al.*, 1997; Bansal and Bilaspuri, 2011). According to some authors (Mournaki *et al.*, 2010; Castellini *et al.*, 1999, 2003; Castellini, 2008), the 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). In particular, the role of antioxidants is to contrast the spermatid cell membrane lipid peroxidation and sperm DNA fragmentation caused by reactive oxygen species, responsible for male infertility in animals and man (Baker *et al.*, 1996; Comhaire *et al.*, 2000; Aitken *et al.*, 2003; Greco *et al.*, 2005).

Furthermore, spontaneous peroxidation occurs during *in vitro* storage. For this reason, the addition of antioxidants is important for the efficacy of extenders (Maldjian *et al.*, 1998; Funahashi and Sano, 2005; Akhter *et al.*, 2007; Michael *et al.*, 2009). Many studies have been carried out on carotenoids, considered an important group of natural antioxidants (Bast *et al.*, 1998; Møller *et al.*, 2000; Schabath *et al.*, 2004; Rao and Rao, 2007). In the last decade, lycopene, a carotenoid present in tomatoes (*Lycopersicon esculentum*) and several ripe fruits and vegetables, such as watermelon, pink grapefruit and carrots (Nguyen and Schwartz, 1999; Perkins-Veazie *et al.*, 2003; Tadmor *et al.*, 2005), has been considered in some studies related with human and animal health (Bhuvaneshwari and Nagini, 2005; Rao *et al.*, 2006; Avci and Durak, 2007; Bhom, 2007) and reproductive physiology (Martino *et al.*, 2006; Goyal *et al.*, 2007; Mangiagalli *et al.*, 2007; Turk *et al.*, 2007; Mendiola *et al.*, 2010). In rooster, in particular, Mangiagalli *et al.* (2010) found positive effects of lycopene addition on fertility and qualitative characteristics of semen.

The aim of the present work was to evaluate the effect of lycopene supplementation via drinking water on fresh and stored semen characteristics and on reproductive performance of the buck.

MATERIALS AND METHODS

Animals and treatments

The trial was conducted on 25 hybrid Martini male rabbits kept at the experimental rabbit farm site in the Department of Animal Science. The 26 wk old rabbits were individually housed in cages in a controlled room (16L:8D; 18-20°C) and fed a commercial breeder standard diet *ad libitum* (162 g crude protein and 10.7 MJ digestible energy per kg).

After a suitable period of adaptation to semen collection using an artificial vagina, the bucks were randomly assigned to 3 groups, which received 3 different level of addition of Lycopene 10% WS (Roche Vitamins, Base, Switzerland) in drinking water. Lycopene 10% WS composition was: sucrose 330 mg/g, maize starch 250 mg/g, fish gelatine 200 mg/g, lycopene 100 mg/g, maize oil 55 mg/g, ascorbyl palmitate 50 mg/g and DL-alpha-tocopherol 15 mg/g.

The animals in the first and the second experimental group received drinking water supplemented with 0.1 and 0.5 g/L of lycopene (0.1 and 0.5 groups, respectively), while water without any supplementation was administered to the control group rabbits (0 group).

Water consumption was determined 4 times a week and the daily intake of lycopene/buck was calculated. Moreover, feed consumption was registered twice a week and body weight of each animal was recorded every week.

Semen collection and evaluation

After 8 wk of adaptation to experimental treatments, semen collection and evaluation was done on 18 bucks (6 animals per treatment) randomly assigned to the experimental groups. Bucks were 36 wk old at the beginning of the experiment and their mean body weight was 4 858±160 g.

The semen was collected twice a week for 5 consecutive weeks by the same handler in all groups according to IRRG Guidelines standard procedure (2005).

Soon after collection (time 0), ejaculates were diluted 1:2 in tris buffer (Tris 3.029 g, citric acid 1.676 g, D-glucose 1.250 g, streptomycin 100 mg, penicillin 10 mg, distilled water 100 mL; pH=7.1) and an aliquot was stored for 24 h (time 24) at 5°C. Semen quality parameters were measured in each ejaculate at time 0 and 24. Ejaculate volume was determined by graduated test tube and sperm concentration by Neubauer hemocytometry. The total sperm number per ejaculate (TSN) was calculated (volume×concentration). Motility, viability and sperm morphological anomalies were evaluated after incubation at 37°C in water bath for 10 min.

Sperm motility was subjectively evaluated by a phase contrast microscope (magnification 20×) fitted with heating plate at 37°C (3 replicates/ejaculate were done). In particular, 10 µL of extended semen (1/2-1/5) were placed on Makler chamber (Makler, 1980) and the proportion of motile cells on the total observed cells (at least 200 cells) was recorded. Forward progressive motility (FPM) was subjectively scored 1 to 4 (1=no forward movement; 2=slow linear movement; 3=good linear movement; 4=rapid linear forward progression). Sperm viability and morphological anomalies were assessed by nigrosin/eosin (N/E) staining procedure according to Bakst and Cecil (1997). About 500 spermatozoa in each smear were counted by microscopy (magnification 100×) and the proportion of viable spermatozoa was calculated on the total number of cells.

Fertility trial

At the end of the semen evaluation period, 60 multiparous rabbit does (20 females/group) were housed in wire-net flat deck cages. The animals were kept under a constant 16/8 h light/dark cycle in a building with a temperature of 20°C and fed a commercial diet *ad libitum*. The rabbit does were inseminated with about 10 million motile spermatozoa per doe on day 11 *post partum* with a pooled semen sample collected from each experimental group. Fertility rate (number of kindlings/number of insemination×100), total newborn, live born and litter weight at kindling were recorded.

Statistical analysis

Data recorded during the trial were analysed using a General Linear Model procedure (SAS 9.1, 2001). The lycopene drinking water treatment was considered as the only source of variation on the sperm traits and reproductive performance.

RESULTS AND DISCUSSION

As expected, the higher antioxidant intake (0.14 g/d per animal), calculated from water intake, was registered in the 0.5 group, while each buck of the 0.1 group assumed 0.03 g of the antioxidant per day.

Throughout the experimental period, lycopene administration at different levels did not affect feed intake and body weight of male rabbits (Table 1). In agreement with our results, Yousef *et al.* (2003) found that supplementation of other antioxidant as ascorbic acid, vitamin E and their combination in drinking water did not affect body weight gain. In contrast, in the present study water intake was influenced by the experimental treatment; in particular, the supplementation of lycopene (0.1 and 0.5 group) seems to increase the palatability of water, causing a significantly higher water intake in these groups in comparison to the 0 group ($P<0.001$).

Table 1: Effect of lycopene supplementation on live weight, water intake and feed ingestion of male rabbits during the experimental period.

	Lycopene supplementation (g/L)			SEM ¹	P-value
	0	0.1	0.5		
No. of observation	6	6	6		
Initial live weight (LW, g)	4 689	4 742	4 658	109	0.857
Final live weight (g)	4 790	4 825	4 781	92.1	0.937
Water intake (mL/d per animal)	258 ^b	325 ^a	310 ^a	6.63	0.001
Dry matter intake (g/kg LW)	65.5	62.9	64.4	2.16	0.212

¹ SEM: standard error of means.

^{a,b}: Means not sharing letter in the same row are significant different at $P<0.05$.

The results for quantitative semen traits are presented in Tables 2 and 3. The highest level of lycopene (0.5 group) resulted in an ejaculate volume and TSN significantly higher ($P<0.05$) than those found in the control group, whereas values registered in the 0.1 group were not significantly different compared to those recorded in the 0 and 0.5 groups. However, the significant increases in ejaculate volume and TSN were not matched by a higher semen concentration (Table 2). On the contrary, Mangiagalli *et al.* (2010) found a significant positive effect of lycopene supplementation on sperm concentration in fowl. In male rabbits given drinking water supplemented with vitamin E, Yousef *et al.* (2003) found a significant improvement in sperm volume and concentration. In contrast, Castellini *et al.* (2001, 2003) found no positive effect on the same sperm parameters in rabbits fed a diet supplemented with vitamin E and C.

In our study, the lycopene addition had no significant effect on motility rate and forward progressive motility in fresh semen (time 0). The same results were found in fowl by Mangiagalli *et al.* (2010). Eid *et al.* (2006) in male domestic fowl and Eskenazi *et al.* (2005) in man reported that a higher antioxidant intake was associated with a greater motility and sperm numbers.

Lycopene supplementation, instead, had a significant effect on sperm quality characteristics in semen samples stored for 24 h at 5°C. In particular, the values of motility, FPM and viability recorded in the 0.5 group, which received the highest concentration of lycopene, were significantly higher than those registered in the other experimental groups ($P<0.05$). The supplementation of lycopene, therefore, had a positive effect in partially preventing the decrease of semen quality during storage and such an effect was dose dependent.

Similar positive results of the effect of lycopene on spermatozoa survival were found by Mangiagalli *et al.* (2007) in fowl. These authors reported that lycopene addition to a commercial extender significantly increased sperm viability after *in vitro* storage, as a result of a protective

Table 2: Effect of lycopene supplementation on semen production of bucks.

	Lycopene supplementation (g/L)			SEM ¹	P-value
	0	0.1	0.5		
No. of observation	6	6	6		
Volume (mL)	0.78 ^b	0.84 ^{ab}	0.98 ^a	0.05	0.031
Concentration (10 ⁶ ×spz/mL)	278	329	359	28.7	0.138
Total spermatozoa number	227 ^b	285 ^{ab}	364 ^a	31.9	0.013

¹ SEM: standard error of means.

^{a,b}: Means not sharing letter in the same row are significant different at $P<0.05$.

Table 3: Effect of lycopene supplementation on the kinetic characteristics and viability of semen.

	Lycopene supplementation (g/L)			SEM ¹	P-value
	0	0.1	0.5		
No. of observation	6	6	6		
0 h					
Motility (%)	54.2	60.4	57.8	2.30	0.170
Forward progressive motility	1.96	2.20	2.14	0.09	0.131
Viability (%)	70.7	74.3	74.8	1.85	0.231
24 h					
Motility (%)	20.6 ^b	26.8 ^b	37.0 ^a	3.22	0.003
Forward progressive motility	0.70 ^b	0.80 ^b	1.14 ^a	0.11	0.016
Viability (%)	46.3 ^b	48.7 ^b	56.5 ^a	2.35	0.010

¹ SEM: standard error of means.

^{a,b}: Means not sharing letter in the same row are significant different at $P < 0.05$.

effect of this antioxidant against cell damage during storage. Higher sperm motility was also found after caffeine addition (10 mM/L) in rabbit semen stored for 24 h at 18°C (Lopez and Alvarino, 2000). Better semen characteristics after 24 h storage at 4°C were reported in bucks fed diet supplemented with vitamin E and C (Castellini *et al.*, 2001). Semen quality parameters measured before and after 24 h storage are reported in the Table 3. After storage, a general decrease in the quality of semen, regardless of the experimental treatment, was observed. Similar results have been reported previously in rabbit (Castellini *et al.*, 2003) and fowl (Mangiagalli *et al.*, 2007) semen after liquid storage.

Although in our study semen susceptibility to oxidation was not determined, the positive effect of lycopene addition on lipid peroxidation could be hypothesised, because peroxidation and sperm viability are strictly linked and, in our study, this parameter was significantly higher in animals treated with the highest level of the antioxidant.

No severe sperm morphological anomalies were noted in any experimental groups at time 0 and 24.

The positive effects of lycopene supplementation found in our trial are in agreement with several studies reported in the literature on the antioxidant property of lycopene and its biological role on semen quality in animals and man. Turk *et al.* (2007) reported that lycopene administration in rats treated with cyclosporine A (CsA) significantly increased sperm concentration and motility in comparison to CsA-treated group, confirming the role of lycopene as a potential protector of structural and functional damages. Atessahin *et al.* (2006) found that lycopene addition significantly improved sperm quality in rats treated with cisplatin due to its protective role on oxidative stress. In man, Zini *et al.* (2010) studied the antioxidant properties of lycopene on sperm DNA integrity; the preincubation of spermatozoa with this antioxidant, in fact, caused a significantly lower DNA damage of spermatozoa incubated with hydrogen peroxide. This could explain the results found in the fowl research, in which the positive effect of lycopene was on the viability but not on all kinetic parameters. In contrast, in the rabbit semen after storage the lycopene treatment group showed the best semen quality for all considered parameters. This result suggests that the lycopene could have a protective action that is higher in the semen of rabbits than in those of other species.

Table 4: Effect of lycopene supplementation on reproductive performance of rabbit does.

	Lycopene supplementation (g/L)			SEM ¹	P-value
	0	0.1	0.5		
No. of observation	20	20	20		
Live weight at insemination (g)	4 501	4 352	4 417	93.3	0.510
Fertility rate ² (%)	78.9	73.7	77.8	10.2	0.926
Litter size					
No. Born alive	7.73	9.29	8.50	0.75	0.362
No. Born dead	2.56	1.57	1.00	1.13	0.524
Litter weight at birth (g)	444	537	479	42.9	0.299
Average weight of kits born alive (g)	58.6	58.0	57.9	2.18	0.972
Average weight of kits born dead (g)	41.0	50.4	43.7	5.63	0.638

¹ SEM: standard error of means.

²Fertility rate as No. of kindlings/No. of inseminations×100.

Results of the fertility trial are presented in Table 4. Lycopene addition did not significantly affect reproductive performance of multiparous rabbit does inseminated with fresh semen. Similar results were reported in laying hens inseminated with fresh semen from cockerels that received lycopene in drinking water (Mangiagalli *et al.*, 2010) and in rabbit does inseminated with fresh and stored semen from rabbits fed alfa-tocopheryl acetate (Castellini *et al.*, 2007). Likewise, Zaniboni *et al.* (2006) reported that reproductive performance features (hatchability rate, fertility, and embryo mortality) of female breeder turkeys were not affected after artificial insemination of semen enriched in vitamin E.

CONCLUSIONS

The results show that in rabbit bucks the addition of lycopene to drinking water increases the production of semen, volume and total number of spermatozoa, and improves sperm kinetic characteristics and viability during semen storage at 5°C. Therefore, lycopene could be used as an antioxidant in alternative to other molecules, such as vitamins E and C, widely studied and used in animal production. In field conditions, where the environment is not perfectly controlled, the lycopene supplementation via drinking water could provide antioxidant protection to contrast the oxidative stress. Lycopene supplementation could have interesting applications in rabbit farming, so further research into the role of this antioxidant is suggested.

Acknowledgements: The authors wish to thank DSM Nutritional Products, Segrate, Milano, supplier of lycopene additive.

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