Plant Archives Volume 20 No. 2, 2020 pp. 6233-6236 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF ADDING THE DIFFERENT CONCENTRATION OF LYCOPENE POWDER ON COOLED LOCAL AWASSI RAM DILUTED SEMEN

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Abstract

The objective of this study was to evaluate the effect of adding different concentrations of Lycopene powder on cooled local Awassi ram diluted semen. We hypothesized that the semen characteristics would be decreased following adding different concentrations of lycopene powder. Semen samples were collected from local Awassi ram. Two samples were pooled and diluted with egg-yolk extender (1:10) then divided randomly into five groups: four treatments (T1, T2, T3, T4) and control (C). Lycopene powder was added to the treated samples as following: (T1=0.1, T2=0.05, T3=0.01 and T4=0.001 gm). Groups were cooled into 4ºC. Samples were evaluated immediately after treatment time 0 hour and after 24, 48, 72 and 95 hours. Mass activity (%) and individual motility (%) and dead sperms were evaluated for all samples. For accuracy, the experiment was repeated six times. The percentage of mass activity and individual motility were decreased significantly in T1 and T2 compared to T3, T4 and control groups. Also, dead sperm was increased significantly in T1 and T2 compared to T3, T4 and control groups. All results showed a significant effect in Awassi ram diluted semen following treatment with 0.01 gm concentration of lycopene after 72 hours. In conclusion, the lycopene additive to the local Awassi diluted semen enhanced the sheep semen quality by decreasing the free radicals that formed with prolonged storage. It might decrease the oxidative stress and preserve the semen life with a prolonged period.

Key words: lycopene, Awassi ram, semen.

Introduction

The animal resources consider one of the most important sources of agriculture production in Iraq. Sheep is representing a major axis in animal production. However, local Awassi sheep productivity is low (Al-Azzawi *et al.,* 1979). Artificial insemination (AI) is one of the techniques that used in sheep to increase its productivity (Abecia *et al.,* 2012) and conserves the genetic characteristic of the herd (Gibbons, Fernandez, Bruno-Galarraga, Spinelli and Cueto, 2019). In sheep, fresh diluted semen is used in AI to extend the number of inseminations per ram and store it for a long period (Naim *et al.,* 2009). But, the longevity of the process between semen collection and insemination resulted in developing free radicals (John Aitken, 1995) that develop oxidative stress (Gibb and Aitken, 2016). This might result in a decrease in semen quality. Free radicals formed because of semen activity which leads to compromise the sperm viability (Gosalvez *et al.,* 2017). During storage, oxidative stress developed which might cause sperm DNA damages (Homa *et al.,*, 2019). Several rich antioxidant plants were used with semen dilution to reduce the impact of antioxidants such as Liquorice (Mahdi, 2010), black seed (Haseena, Aithal, Das and Saheb, 2015), ginger (Al-Malaly *et al.,* 2013), Thymus (Al-Zubaidy *et al.,* 2013), etc. lycopene is one of the rich natural resources of antioxidants (Tvrda, Mackovich, Greifova, Hashim and Lukac, 2017). It gives the reddish-pinkish color that found in different types of fruits and vegetables such as watermelon, grapefruit and tomato (Naviglio, Pizzolongo, Ferrara, Aragòn and Santini, 2008). It has been used in many species as a supplement to the semen extenders to reduce the effect of oxidative stress and free radicals. This will increase the stored semen characteristics by compromise the effectiveness of the free radicals such as bulls (Tvrda *et al.*, 2017), ram (Al-Sarray *et al.,* 2019) and Turkey (Rosato, Centoducati, Santacroce and Iaffaldano, 2012). Although the effect of lycopene was previously studied on semen ram however we do not know the effect of lycopene in local Awassi semen.

Therefore, we conducted this study to evaluate the effect of adding different concentrations of lycopene on local Awassi semen.

Materials and Methods

This study was conducted at the Animal Production Department, College of Agriculture, Al-Qasim Green University. Samples were collected from a local Awassi ram weight 65Kg and aged 4 years. Semen was collected by an artificial vagina for six weeks, one per week. After collection, semen was diluted with Tris-egg yolk extender (1:10). Samples were divided randomly into five groups: four treatments (T1, T2, T3 and T4) and control (C). Lycopene was added to the treated samples as following: (T1=0.1, T2=0.05, T3=0.01 and T4=0.001 gm) and cooled into 4ºC. Samples were evaluated immediately after treatment time 0 hour and after 24, 48, 72 and 95 hours. Mass activity and individual motility were evaluated for all samples according to the method described by (Walton, A., 1933). Dead sperms were estimated according to the method described by (Swanson and Bearden, 1951) by using Eosin and Nigrosine stain. For more accuracy, the experiment was repeated for six times

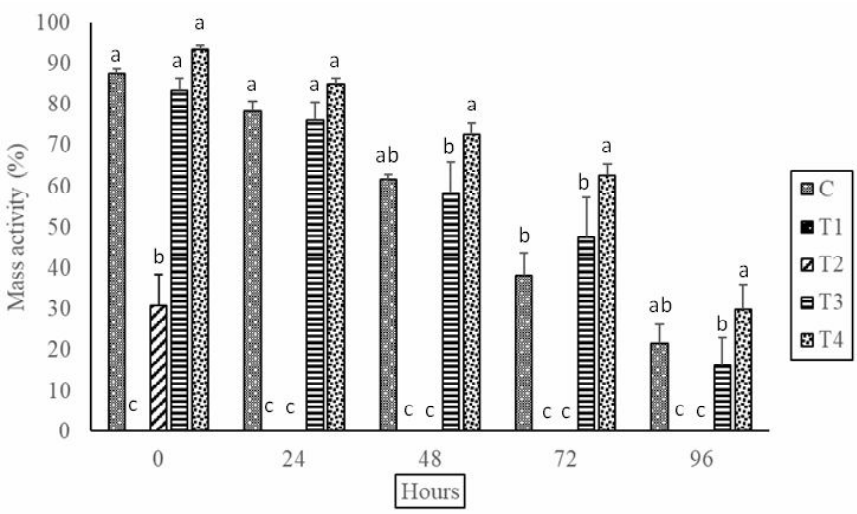

Fig. 1: Effect of adding different lycopene concentrations on mean (± SEM) mass activity on local Awassi ram semen. Semen samples were estimated immediately before treatment (0 hr) and after (24, 48, 36 and 96 hr) in treated (T1, T2, T3 and T4) and control group (C). Values with deferent superscripts ^{abc} are significantly different ($p<0.0001$).

Statistical Analysis

All parameters were reported as mean ± standard error (SEM). The general leaner model was used for statistical analysis by using the SAS program (SAS, 2012). Duncan's Multiple Range Test was used to compare means and to examine the effect of time, treatment and interaction between time and treatment on semen characteristics (Duncan, 1955).

Results and Discussion

In the current study, the statistical analysis showed a significant effect of lycopene concentration on local Awassi ram diluted semen in all the studied parameters. The mass activity significantly differed between Time, Treatment and interaction between Time and Treatment ($p< 0.0001$, $p< 0.0001$ and $p< 0.0001$), respectively (Fig. 1). In addition to that, Individual motility also differed significantly between Time, Treatment and interaction between Time and Treatment ($p< 0.0001$, $p< 0.0001$ and $p< 0.0001$), respectively (Fig. 2). It is important to mention that the lycopene concentration (T1=0.1 and T2=0.05 gm) was detrimental to the diluted semen since the mass activity and individual motility was (0%) immediately following treatment. But the concentration (T4=0.001 gm) had the highest mass activity and individual motility (72 hours) following treatment with lycopene. These results were supported by (Al-Sarray *et al.,* 2019) how reported that adding 0.3% of lycopene to the tris extender preserved the semen quality until 72 hours. It is important to mention that in Al-Sarray and coworkers

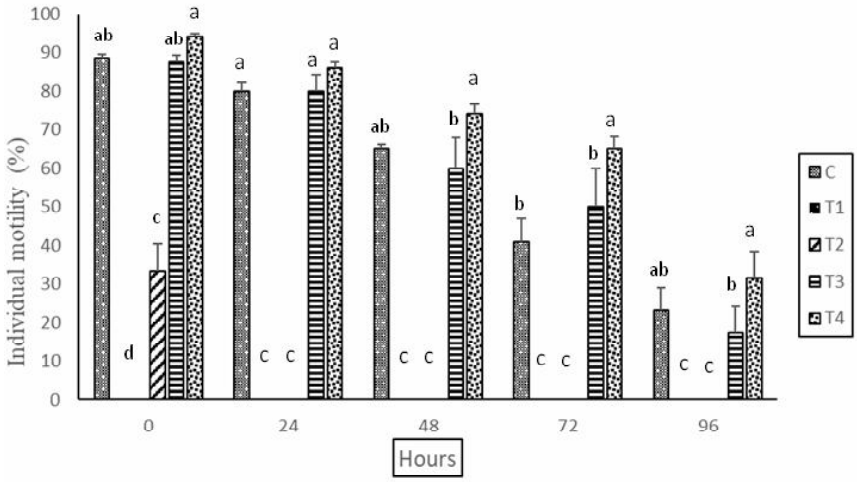

Fig. 2: Effect of adding different lycopene concentrations on mean (± SEM) individual motility on local Awassi ram semen. Semen samples were estimated immediately before treatment (0 hr) and after (24, 48, 36 and 96 hr) in treated (T1, T2, T3 and T4) and control group (C). Values with deferent superscripts ^{abc} are significantly different ($p<0.0001$).

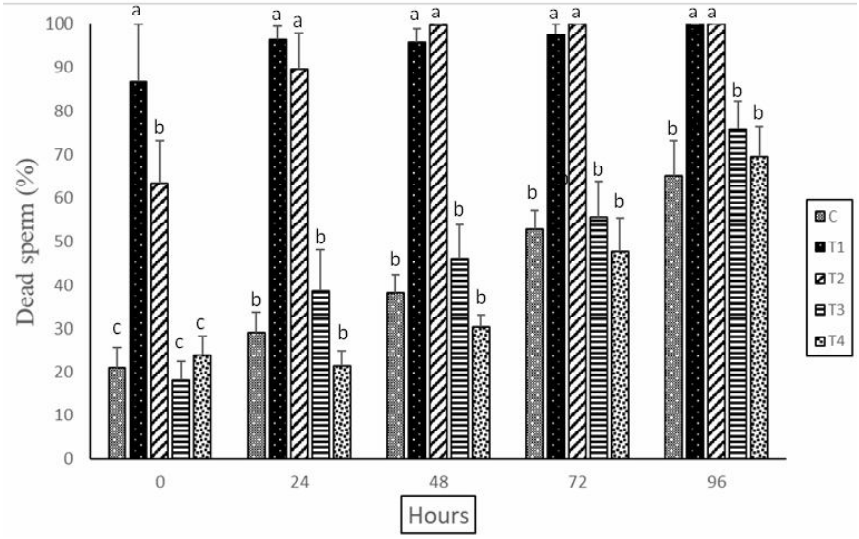

Fig. 3: Effect of adding different lycopene concentrations on mean (± SEM) dead sperm on local Awassi ram semen. Semen samples were estimated immediately before treatment (0 hr) and after (24, 48, 36 and 96 hr) in treated (T1, T2, T3 and T4) and control group (C). Values with deferent superscripts ^{abc} are significantly different ($p<0.0001$).

results did not report the concentration of the lycopene while in our study we emphasized that. Also, our results showed significant differences in dead sperms in Treatment ($p<0.0001$), Time ($p<0.0001$) and interaction between Time and Treatment ($p<0.007$) compared to control group (Fig. 3). It is important to mention that the treatment with lycopene in T1 and T2 had the highest dead sperm compared to T3, T4 and C. This finding was supported by (Al-Sarray *et al.,* 2019) how reported that the dead sperm increased with increased the concentration of lycopene. In our study, the positive effect of lycopene is improving the semen quality in mass and individual motility in addition to decrease the percentage of dead sperm. These results might be related to the role of lycopene as an antioxidant capability. Lycopene is an antioxidant that reduces the consequence of free radicals formed in semen (Durairajanayagam, Agarwal, Ong and Prashast, 2014). The sperm cell wall components consist of fatty acids which expose to oxidation more than other cell bodies because of the electrons in the unconjugated double bonds in the fatty acids (Zini *et al.,* 2010). The electron's reactions formed the lipid peroxides (Agarwal *et al.,* 2012) which formed free radicals. Previous research reported that oxidative stress is the main cause of reducing semen viability via either phosphorylation of the axonemal protein or DNA damages and then male infertility (Niederberger, 2012). Several rich antioxidant plants were used with semen dilution to reduce the impact of antioxidants such as Liquorice (Mahdi, 2010), black seed (Haseena *et al.*, 2015), ginger (Al-Malaly *et al.,* 2013), Thymus (Al-Zubaidy *et al.,* 2013), etc. lycopene is one of the rich natural resources of antioxidants (Tvrda *et al.*, 2017). It protects the sperm from the oxidative stress by either neutralizing and reacting with the free radicals or prevent the formation of it. As a result, the lycopene will protect it and then increase the semen viability (Al-Sarray *et al.,* 2019). In conclusion, adding 0.01 or 0.001gm lycopene to the semen extender enhanced the viability of the Awassi ram in Iraq after three days of additives. This result might be useful to the artificial insemination in sheep.

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