International Journal of Biological Sciences and Applications
2014; 1(1): 19-23
Published online April 10, 2014 (http://www.aascit.org/journal/ijbsa)

Keywords
Tomato Juice,
Extender,
Semen Quality,
Liquid Storage,
Chicken Semen

Received: March 05, 2014
Revised: March 27, 2014
Accepted: March 28, 2014

Impact of extender supplementation with tomato juice on semen quality of chicken semen during liquid storage

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Citation
H. J. Al-Daraji. Impact of Extender Supplementation with Tomato Juice on Semen Quality of Chicken Semen During Liquid Storage. *International Journal of Biological Sciences and Applications.* Vol. 1, No. 1, 2014, pp. 19-23

Abstract
The aim of this experiment was to determine the effects of tomato juice inclusion in semen extender on sperm characteristics during *in vitro* storage of chickens' semen. Sixty white Leghorn roosters were used in this study. They were divided into six treatments; undiluted control, fresh semen (C1); diluted control (C2); and treatments T1, T2, T3 and T4 using diluted semen with inclusion of 2, 4, 6 and 8 ml of tomato juice per 100 ml of extender, respectively. Mass activity, individual motility, dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities percentages were determined twice a week for 8 wks period after *in vitro* storage time of 0, 12, 24 and 36 hr of chickens' semen in refrigerator. Results showed that adding tomato juice up to 8 ml per 100 ml of semen extender significantly ($P<0.05$) increased mass activity and individual motility percentages and decreased the percentages of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities at all storage times when compared with control. No significant changes were observed in all parameters and storage times when tomato juice was added to the extender up to 6 ml per 100 ml of extender (T1, T2 and T3). Increasing the addition level of tomato juice from 6 to 8 ml per 100 ml to the extender (T4) significantly led to additional improvement in all parameters studied *in vitro* storage times. Improvement sperm characteristics during storage of chickens' semen due to tomato juice addition to the extender support published concepts about the positive effects of tomato juice as an antioxidant-rich product.

1. Introduction

Optimization of the management of roosters includes the need for efficient methods of semen storage. However, the current methods of semen storage are only effective for short periods of time (up to 12 hr) and need to be improved (32). Improvements in the methods of liquid storage of spermatozoa are limited by the lack of basic knowledge of the biochemical mechanisms regulating spermatozoa functions *in vivo* and *in vitro*. Lipids are known to have a major impact on the structure and function of spermatozoa both *in vivo* and *in vitro* (13; 22; 24; 28). In birds, lipids are believed to have a significant role in *in vitro* storage in the female uterovaginal glands (6; 7). A decrease in the lipid content of chicken spermatozoa has been shown to occur after 48 hr of *in vitro* storage (8). It has been reported that a high consumption of tomato lowers

plasma lipid peroxidation (27), and improves the antioxidant defense of low density lipoprotein (LDL) against attack by singlet oxygen (26). Also, epidemiological studies suggested that antioxidant capacity is improved by the consumption of tomato products, thereby decreasing the risk of the development of diseases relative to oxidative stress (11; 19; 21; 27). Tomatoes contain different compounds (e.g. carotenoids, vit. C, flavonoids) that may be responsible for the antioxidant properties. Although tomatoes contain an array of phytochemicals, most of the attention has been focused on lycopene, the main carotenoid in tomato products possesses the greatest quenching ability of singlet oxygen among the various carotenoids (14), and is effective in protecting blood lymphocytes from NO_2 radical damage (9).

Lycopene, the red pigment in tomatoes, is a natural pigment synthesized by plants and microorganisms (1). Antioxidants nutrients, including lycopene and other carotenoids, neutralize the adverse effects of free radicals. Hence the balance between free radicals and antioxidants is important for maintaining healthy body systems (18). Lycopene is one of the most potent antioxidants (14; 25), with a singlet-oxygen-quenching ability ten times higher than that of α-tocopherol (14). Dietary supplementation with α-tocopherol, the major lipid-soluble antioxidant presents in cell membranes, has been demonstrated to reduce the susceptibility to lipid peroxidation and improved the characteristics and fertility of semen (10; 20; 23). The findings mentioned above may be led to expect that the use of lycopene sources may achieve at least the same results as related with semen quality characteristics. Thus, the objective of this work was to study the effect of the addition of tomato juice, as a lycopene-rich source, to semen extenders at different levels on some characteristics of chickens' semen stored for different times. To our knowledge, this is the first work describing the effects of the addition of different levels of tomato juice to semen extenders on semen quality traits in chicken.

2. Materials and Methods

The experiment was carried out to investigate the effect of adding tomato juice to extender on semen quality and liquid storage of chickens' semen. Sixty White Leghorn roosters, 24 wks old, were used. A study of the sperm was performed twice a week for a period of 8 weeks. Semen was collected by abdominal massage method according to Burrows and Quinn (12). Care was taken to avoid any contamination of semen with the cloacal products and particularly with the transparent fluid excreted from the lymph folds of the cloaca during ejaculation. The semen extender referred to as Al-Daraji 2 extender (AD_2E) (4), consist of potassium citrate, 0.64 g; sodium glutamate, 8.67 g ; sodium acetate, 4.3 g ; magnesium chloride , 0.34 g ; potassium diphosphate, 12.7 g ; potassium monophosphate , 12.7 g ; fructose , 5 g ; TES , 1.95 g ; vitamin A , 4 mg ; vitamin C , 16 mg and vitamin E , 8 mg . Both AD2E and tomato juice (Tj) were used in this experiment. The experimental design was completely randomized for the experiment with six treatments: undiluted control, fresh semen (C1); diluted control, AD2E-diluted semen (C2); and treatments T1, T2, T3 and T4 using AD2E-diluted semen with 2, 4, 6 and 8 ml of tomato juice per 100 ml, respectively. All parameters studied were estimated after storage periods of 0, 12, 24 and 36 hours in refrigerator. The method of measuring the mass activity and individual motility tests has been described by Sexton (30). Percentages of dead spermatozoa were evaluated by using fast-green-eosin B stain (5). Abnormal spermatozoa were distinguished by using a Gentian Violet-eosin stain (2). Acrosomal abnormalities test was carried out according to Al-Daraji (3).

The experiment was conducted using a completely random design. Data were analyzed using analysis of variance (ANOVA) (29). A significant difference was used at 0.05 probability level and differences among treatments were tested using the Duncan's procedure (17).

3. Results and Discussion

Effects of tomato juice addition to the semen extender on mass activity and individual motility percentages are presented in Tables 1 and 2, respectively. The use of AD2E significantly increased mass activity and individual motility percentages at all *in vitro* storage times studied (C2 *versus* C1). Also, adding tomato juice up to 8 ml per 100 ml of the AD2E led to a significant additional increase in mass activity and individual motility percentages at all *in vitro* storage times studied (T1, T2, T3 and T4 *versus* C2). No significant changes were observed in mass activity and individual motility percentages along with increasing tomato juice addition to the extender from 2 to 6 ml per 100 ml of the extender (T1, T2 and T3). While, increasing the addition level of tomato juice from 6 to 8 ml per 100 ml of AD2E significantly led to an additional increase in mass activity and individual motility percentages at all *in vitro* storage times (T4 *versus* T1, T2 and T3).

Dead and abnormal spermatozoa and acrosomal abnormalities percentages are shown in Tables 3, 4 and 5, respectively. Adding tomato juice to the extender (AD2E) even at low level in this experiment (2 ml / 100 ml of extender,T1), resulted in a significant decreases in the percentages of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities at all *in vitro* storage times (T1, T2, T3 and T4 *versus* C2). Also, no significant differences were found for percentages of dead spermatozoa,abnormal spermatozoa and acrosomal abnormalities among AD2E-diluted samples supplemented with 2, 4 and 6 ml of tomato juice per 100 ml of AD2E (T1, T2 and T3, respectively) at all *in vitro* storage times studied. Furthermore, as tomato juice addition increased from 6 to 8 ml per 100 ml of extender (T4), dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities

percentages decreased significantly (P<0.05) when compared with other treatments in this study.

With increasing in vitro storage time, mass activity and individual motility percentages of fresh semen reached to 0 % accompanied with reaching of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities percentages to approximately 100 % after 72 hr of *in vitro* storage period. While, it was found that supplementation of the semen extender with tomato juice can prolong the period required to reaching these percentages. These results are in agreement with previous reports by Sexton (31) who found that semen quality and fertility are generally decreased when semen is stored for 24 hr *in vitro*, and Douard *et al* (16) who found that the motility, viability and morphological integrity of spermatozoa decreased during storage.

It was observed that the phospholipids content of turkey spermatozoa is severely affected by *in vitro* storage and the evolution of phospholipids is parallel to the decrease in semen quality (16). This could originate from the endogenous metabolism of the fatty acids of the membrane phospholipids and induce membrane destabilization (16). The semen of birds showed a tendency to form high concentrations of the products of lipid peroxidation during *in vitro* storage and this was associated with a partial or complete loss of fertilizing ability (33). Addition of antioxidants to the semen extender increase semen quality (15).

Table 1. *Effect of diluents supplementation with different levels of tomato juice on mass activity of chicken semen for different in vitro storage times. (Mean ± SE).*

Treatments		Storage times (hours)			
		0	12	24	36
C1	Fresh	80.6 ± 4.0 [d]	36.0 ± 2.7 [d]	16.2 ± 1.0 [d]	0.0 ± 0.0 [d]
C2	AD2E	86.2 ± 2.6 [c]	81.7 ± 3.9 [c]	74.6 ± 2.8 [c]	61.9 ± 1.7 [c]
T1	AD2E + Tj (2 ml / 100ml)	90.0 ± 1.7 [b]	85.1 ± 2.0 [b]	79.1 ± 3.0 [b]	68.2 ± 1.9 [b]
T2	AD2E + Tj (4 ml / 100ml)	91.2 ± 3.6 [b]	86.0 ± 1.7 [b]	80.2 ± 1.8 [b]	69.1 ± 3.3 [b]
T3	AD2E + Tj (6 ml / 100ml)	92.9 ± 2.9 [b]	86.9 ± 3.1 [b]	80.9 ± 2.2 [b]	69.9 ± 1.4 [b]
T4	AD2E + Tj (8 ml / 100ml)	98.2 ± 3.0 [a]	92.2 ± 2.8 [a]	85.6 ± 1.7 [a]	81.0 ± 2.9 [a]

[a-d] Means in a column with no common superscript differ significantly (P< 0.05).
- AD2E , Al-Daraji 2 extender ; Tj , tomato juice.

Table 2. *Effect of diluents supplementation with different levels of tomato juice on individual motility of chicken semen for different in vitro storage times. (Mean ± SE).*

Treatments		Storage times (hours)			
		0	12	24	36
C1	Fresh	81.3 ± 1.7 [d]	37.9 ± 4.0 [d]	18.3 ± 1.0 [d]	0.0 ± 0.0 [d]
C2	AD2E	89.6 ± 2.2 [c]	84.1 ± 1.7 [c]	77.0 ± 2.4 [c]	64.3 ± 1.8 [c]
T1	AD2E + Tj (2 ml / 100ml)	92.0 ± 3.7 [b]	87.9 ± 2.8 [b]	81.1 ± 1.3 [b]	70.0 ± 3.3 [b]
T2	AD2E + Tj (4 ml / 100ml)	94.7 ± 2.4 [b]	89.1 ± 3.0 [b]	83.8 ± 2.4 [b]	71.1 ± 1.7 [b]
T3	AD2E + Tj (6 ml / 100ml)	94.8 ± 1.9 [b]	89.3 ± 1.7 [b]	83.9 ± 1.9 [b]	72.9 ± 2.6 [b]
T4	AD2E + Tj (8 ml / 100ml)	99.9 ± 2.3 [a]	95.8 ± 4.1 [a]	89.0 ± 2.3 [a]	86.0 ± 1.3 [a]

[a-d] Means in a column with no common superscript differ significantly (P< 0.05).
- AD2E , Al-Daraji 2 extender ; Tj , tomato juice.

Table 3. *Effect of diluents supplementation with different levels of tomato juice on dead spermatozoa of chicken semen for different in vitro storage times. (Mean ± SE).*

Treatments		Storage times (hours)			
		0	12	24	36
C1	Fresh	27.8 ± 1.0 [a]	66.8 ± 1.9 [a]	89.9 ± 3.3 [a]	100.0 ± 0.0 [a]
C2	AD2E	22.0 ± 2.3 [b]	34.3 ± 2.7 [b]	42.1 ± 1.8 [b]	52.9 ± 1.3 [b]
T1	AD2E + Tj (2 ml / 100ml)	16.0 ± 1.7 [c]	29.6 ± 1.8 [c]	32.0 ± 2.2 [c]	42.2 ± 2.8 [c]
T2	AD2E + Tj (4 ml / 100ml)	15.2 ± 1.3 [c]	28.5 ± 2.0 [c]	30.1 ± 1.7 [c]	40.3 ± 1.7 [c]
T3	AD2E + Tj (6 ml / 100ml)	14.1 ± 1.0 [c]	26.1 ± 3.1 [c]	29.3 ± 2.0 [c]	39.0 ± 2.1 [c]
T4	AD2E + Tj (8 ml / 100ml)	6.2 ± 1.3 [d]	10.3 ± 2.4 [d]	17.0 ± 1.3 [d]	26.9 ± 1.3 [d]

[a-d] Means in a column with no common superscript differ significantly (P< 0.05).
- AD2E , Al-Daraji 2 extender ; Tj , tomato juice.

Table 4. *Effect of diluents supplementation with different levels of tomato juice on abnormal spermatozoa of chicken semen for different in vitro storage times. (Mean ± SE).*

Treatments		Storage times (hours) 0	12	24	36
C1	Fresh	25.7 ± 1.0 [a]	61.8 ± 3.0 [a]	93.9 ± 4.2 [a]	98.1 ± 4.8 [a]
C2	AD2E	12.0 ± 1.7 [b]	24.1 ± 2.1 [b]	39.7 ± 2.0 [b]	56.0 ± 3.3 [b]
T1	AD2E + Tj (2 ml / 100ml)	8.1 ± 1.1 [c]	19.2 ± 1.3 [c]	27.9 ± 1.3 [c]	42.0 ± 1.7 [c]
T2	AD2E + Tj (4 ml / 100ml)	6.0 ± 1.3 [c]	18.8 ± 1.0 [c]	25.1 ± 0.9 [c]	41.1 ± 2.2 [c]
T3	AD2E + Tj (6 ml / 100ml)	6.6 ± 1.0 [c]	17.3 ± 1.7 [c]	24.0 ± 1.3 [c]	39.8 ± 1.7 [c]
T4	AD2E + Tj (4 ml / 100ml)	1.0 ± 0.9 [d]	6.9 ± 1.1 [d]	13.0 ± 2.6 [d]	22.0 ± 2.1 [d]

[a-d] Means in a column with no common superscript differ significantly ($P< 0.05$).
- AD2E , Al-Daraji 2 extender ; Tj , tomato juice.

Table 5. *Effect of diluents supplementation with different levels of tomato juice on acrosomal abnormalities of chicken semen for different in vitro storage times. (Mean ± SE).*

Treatments		Storage times (hours) 0	12	24	36
C1	Fresh	23.9 ± 1.0 [a]	73.2 ± 1.3 [a]	90.8 ± 3.0 [a]	97.3 ± 4.8 [a]
C2	AD2E	16.0 ± 1.3 [b]	26.0 ± 1.2 [b]	43.2 ± 1.7 [b]	54.0 ± 1.3 [b]
T1	AD2E + Tj (2 ml / 100ml)	11.1 ± 1.6 [c]	18.9 ± 2.0 [c]	29.3 ± 2.0 [c]	43.1 ± 1.1 [c]
T2	AD2E + Tj (4 ml / 100ml)	10.3 ± 0.8 [c]	16.3 ± 1.7 [c]	28.0 ± 1.1 [c]	41.1 ± 0.8 [c]
T3	AD2E + Tj (6 ml / 100ml)	9.1 ± 1.0 [c]	15.6 ± 1.2 [c]	27.5 ± 1.7 [c]	40.0 ± 1.7 [c]
T4	AD2E + Tj (8 ml / 100ml)	1.9 ± 0.8 [d]	6.1 ± 1.7 [d]	13.0 ± 0.8 [d]	26.0 ± 1.1 [d]

[a-d] Means in a column with no common superscript differ significantly ($P< 0.05$).
- AD2E , Al-Daraji 2 extender ; Tj , tomato juice.

In the present work, the addition of tomato juice to the semen extender led to increase quality of semen stored *in vitro* and this may be attributed to lycopene found in tomato juice. Lycopene possess a singlet-oxygen-quenching ability 10 times higher than that of α-tocopherol (14) and α-tocopherol has been demonstrated to reduce the susceptibility to lipid peroxidation and improved semen quality traits (10, 20, 23), therefore, the use of tomato juice as a lycopene-rich source in the semen extender in this study may be the probable reason for the amelioration occur in all semen characteristics studied.

In conclusion, enrichment of semen extender by adding tomato juice improves semen quality and can prolong the *in vitro* storage time of chickens' semen.

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