Effect of Supplementation of Garlic Extract in Diluent on Semen Quality of Roosters during Liquid Storage

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ABSTRACT

This study was designed to examine whether garlic extract (GE) supplementation could improve rooster sperm motility, viability, and morphology during in vitro storage for different periods (24, 48 or 72 h). A total of 42 White Leghorn roosters, 22 wk old, were randomly divided into 6 experimental pens (7 roosters each). The experimental groups were as follows: T1 = fresh, undiluted semen (control); T2 = semen diluted 1 : 1 with Lake diluent (LD) alone; T3 = semen diluted 1 : 1 with GE alone, while T4, T5 and T6 represented semen samples diluted 1 : 1 with LD and supplemented with 1, 2 and 4 ml GE/100 ml of diluent, respectively. Results denoted that semen incubation for 24, 48 or 72 h at the refrigerator temperature in the absence of GE (T1) was associated with a significant (P<0.05) decreased in the mass activity and individual motility, and significant (P<0.05) increased in the percentages of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities. The inclusion of GE into the LD (T4, T5 & T6) significantly (P<0.05) improved motility, viability and normality of acrosomes compared with control group (T1). Besides, T5 and T6 improved semen traits included in this study after in vitro storage for up to 72 h. In addition, T2 was superior to T3 in improving mass activity and individual motility, whereas there were no significant differences between these two treatments in percentages of live spermatozoa and normal spermatozoa and acrosomes for semen samples stored for 24, 48 or 72 h. In conclusion, supplementation of GE into avian semen diluents particularly at the doses of 2 and 4 ml GE/100 ml of diluent can be used as successful technique for suppressing the detrimental effects of lipid peroxidation which could lead to sperms deterioration during in vitro storage for up to 72 h.

Keywords: Garlic, diluents, liquid storage, semen quality, roosters

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INTRODUCTION

Spermatozoa are unique in structure and chemical composition and are characterized by high proportions of polyunsaturated fatty acids (PUFAs) in the phospholipid fraction of their membranes (Khan, 2011; Khan et al., 2012). This characteristic composition confers to sperm plasma membrane the fluidity they require to undergo the membrane fusion events that characterize fertilization (Zaniboni et al., 2004). However, high level of PUFAs increases the susceptibility of cells to free radical attack and lipid peroxidation. Therefore, antioxidant protection is a vital element in maintaining motility, viability, membrane integrity and fertilizing ability (Al-Daraji, 2004). The major fatty acyl components of avian spermatozoa are arachidonic (20: 4 n-6) and docosatetraenoic (22: 4 n-6) acids (Kelso et al., 1996). Thus avian spermatozoa are characterized by high amounts of C20-C22 polyunsaturates of the n-6 series, whereas long-chain fatty acids of the n-3 series predominate in mammalian spermatozoa. However, it appears that the 20: 4 n-6 and 22: 4 n-6 present in avian spermatozoa performs an essential function in promoting optimal spermatozoa motility, viability and fertilizing capacity, as marked reductions in the amounts of these fatty acids in spermatozoa as a result of lipid peroxidation are associated with impaired sperm number, motility, viability and fertilizing ability (Kelso et al., 1997).

Free radicals are formed when oxygen interacts with certain molecules during normal bodily processes or from exposure to several environments. Garlic is one antioxidant that defends against free radicals damage, thereby preserving the body’s healthy functioning (Munday et al., 1999). Garlic antioxidants help scavenges free radicals that can damage cell membranes, interact with genetic material and possibly contribute to the aging process as well as the development of a number of conditions including heart disease and cancer. Garlic antioxidants can neutralize free radicals and may reduce or even help prevent some of the damage they cause over time. In the study of Silagy and Haw (1994), a total of 261 patents from 30 general practices were given either garlic powder or placebo. After a 12 week treatment period mean serum cholesterol levels dropped by 12% in the garlic treated group and triglycerides and total lipid levels decreased by 17 and 19%, respectively compared to the placebo group.
Because garlic (*Allium sativum* L.) has antioxidant activity, this study examined the potential role of garlic extract (GE) as an antioxidant in preserving roosters’ spermatozoa during *in vitro* storage for certain storage periods (24, 48 or 72 h).

**MATERIALS AND METHODS**

Forty two White Leghorn roosters (22 wk old) were randomly divided into 6 experimental pens (7 each). Cocks were fed a commercial layer ration *ad libitum*. Semen samples were collected from all roosters once a week for 10 consecutive weeks (22–32 weeks of age) by using the method of Lake and Stewart (1978). Semen samples in each treatment pen were divided into 3 test tubes of 1 ml each to provide 3 replicates pooled samples per each treatment group. Therefore, there were 30 replicates for each treatment. The experimental groups were as follows: T1 = fresh, undiluted semen (control group); T2 = semen diluted 1:1 with LD alone; T3 = semen diluted 1:1 with GE alone; T4 = semen diluted with LD and supplemented with GE (1 ml/100 ml of diluent); T5 = semen diluted with LD and supplemented with GE (2 ml/100 ml of diluent) and T6 = semen diluted with LD and supplemented with GE (4 ml/100 ml of diluent). Experimental samples were stored at the refrigerator temperature (4–6°C) for different storage times (24, 48 or 72 h). An aliquot of semen from each group was evaluated at 24, 48 and 72 h of *in vitro* storage for mass activity, individual motility and percentages of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities.

Spermatozoa motility (movement in a forward) was estimated on a percentage basis by using the microscopic method of Sexton (1976). The determination of the percentage of dead spermatozoa was done by using a Fast green, Eosin B stain – glutamate extender (Al-Daraji et al., 2002). Percentage of abnormal spermatozoa was evaluated by using a Gentian violet–eosin stain (Al-Daraji, 1998). As an alternative to evaluate the percentage of acrosomal abnormalities, staining procedure for fixed samples have been developed to distinguish which spermatozoa have retained or lost the acrosome (Al-Daraji, 2001). However, the extraction of garlic components was achieved according to the procedure mentioned by Ohnishi and Ohnishi (2001). Results were evaluated by analysis of variance. Differences between experimental group were analyzed by Duncan’s multiple range test, using the ANOVA procedure in Statistical Analysis System (SAS, 1996).

**RESULTS**

The overall means of the treated groups regarding mass activity and individual motility at 0 h showed that the differences between treated groups and the control group were not statistically significant (Fig. 1 & 2). However, in semen samples evaluated after 24, 48 or 72 h *in vitro* storage at the refrigerator temperature, diluent supplemented with GE (T4, T5 and T6) surpassed mass activity and individual motility in T1 and T3 groups. However, there were no significant differences between T2 and T4 groups regarding these two traits when semen samples were stored for 24 or 48 h. Besides, T5 and T6 surpassed all other treatments in mass activity and individual motility during all storage periods (Fig. 1 & 2).

As shown in Figures 3, 4 and 5, there were no significant differences between T1, T2, T3 and T4 groups, and between T5 and T6 groups as regards percentages of dead, abnormal spermatozoa and acrosomal abnormalities.

**Fig. 1:** Effect of supplementation of different levels of garlic extract into LD on mass activity of roosters semen stored for different periods.

T1 = Fresh semen, T2 = LD, T3 = G, T4 = LD + G (1 ml / 100 ml), T5 = LD + G (2 ml / 100 ml) and T6 = LD + G (4 ml / 100 ml); Bars with different superscripts differ significantly (P<0.05).
Fig. 2: Effect of supplementation of different levels of garlic extract into LD on individual motility of roosters semen stored for different periods. 

T1 = Fresh semen, T2 = LD, T3 = G, T4 = LD + G (1 ml / 100 ml), T5 = LD + G (2 ml / 100 ml) and T6 = LD + G (4 ml / 100 ml); Bars with different superscript differ significantly (P<0.05).

Fig. 3: Effect of supplementation of different levels of garlic extract into LD on dead spermatozoa of roosters semen stored for different periods.

Fresh semen, T2 = LD, T3 = G, T4 = LD + G (1 ml / 100 ml), T5 = LD + G (2 ml / 100 ml) and T6 = LD + G (4 ml / 100 ml); Bars with different superscripts differ significantly (P<0.05).

Fig. 4: Effect of supplementation of different levels of garlic extract into LD on abnormal spermatozoa of roosters semen stored for different periods.

T1 = Fresh semen, T2 = LD, T3 = G, T4 = LD + G (1 ml / 100 ml), T5 = LD + G (2 ml / 100 ml) and T6 = LD + G (4 ml / 100 ml); Bars with different superscripts differ significantly (P<0.05).
abnormalities for semen samples evaluated directly after collection (0 h). However, during this time of storage (0 h) T5 and T6 surpassed other treatments in relation to these three characteristics. In addition, supplementation of the diluent with GE (T4, T5 & T6) significantly improved the percentages of live spermatozoa and normal spermatozoa and acrosomes when semen samples were stored for 24, 48 or 72 h. The most efficient levels of extract were 2 ml/100 ml of diluent (T5) and 4 ml/100 ml of diluent (T6). On the other hand, there were no significant differences between T2 and T3 groups regarding these 3 characters (Figures 3, 4 & 5).

**DISCUSSION**

It is interestingly obvious from the results of this study that inclusion of GE into LD maintained activity, viability and normality of sperms and acrosomes. Our results are in accordance with the results of previous authors (Al-Daraji, 2000; Al-Daraji, 2002; AlDaraji, 2004 and El-Nasry et al., 2004) who found that the addition of certain antioxidants (vitamins A, C or E) to the avian semen diluents preserved motility, viability, morphology and fertilizing capacity of semen stored for different storage periods at 4-6°C. Furthermore, the amelioration in semen characteristics noticed in the present experiment may be due to the antioxidant effect of GE that limit the detrimental effects of lipid peroxidation during in vitro storage. Wishart (1989) reported that lipid peroxidation is already initiated in fresh ejaculates and is able to develop during incubation even at low temperatures. Donoghue and Donoghue (1997) pointed out that antioxidant activity in seminal plasma and sperm is not high enough to prevent lipid peroxide damage after extension and in vitro storage, and that supplemental antioxidants could improve semen shelf life.

Extracts of fresh garlic contain antioxidant phytochemicals that prevent oxidant damage. These include unique water-soluble organosulfur compounds, lipid-soluble organosulfur components and flavonoids, notably allixin and selenium (Ohnishi and Ohnishi, 2001). A mounting body of research indicates that garlic act as a potent antioxidant, decreasing lipid peroxidation, increasing free radical scavenging and glutathione, lowering high cholesterol by interfering with its metabolism in the liver and lowering LDL cholesterol and triglyceride levels while raising the level of HDL cholesterol (Geng and Lau, 1997; Reeve et al., 1993; Wei and Lau, 1998). Ohnishi and Kojima (1997) concluded that aged garlic extract has a strong antioxidant effect. However, GE exerts antioxidant action by scavenging reactive oxygen species (ROS), enhancing the cellular antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase, and increasing glutathione in the cells (Ryu et al., 2001). GE inhibits lipid peroxidation, reducing ischemic/reperfusion damage and inhibiting oxidative modification of LDL, thus protecting endothelial cells from the injury by the oxidized molecules (Borek, 2001). However, phytochemicals from plant-rich diets, including garlic, provide important additional protection against oxidant damage (Borek, 1997). The variety of antioxidant phytochemicals in GE, which protect against detriments-causing oxidative damage (Ide and Lau, 1997), may act in single and combined fashion (Amagase et al., 1996). The antioxidative actions of GE and its components are determined by their ability to scavenge ROS and inhibit the formation of lipid peroxides. These effects are determined by measuring decrease in ROS-induced chemiluminescence, inhibition of thiobarbituric acid reactive substances (lipid peroxides), and in vitro inhibition of the release of pentane, a product of oxidized lipids, in the breath of an animal exposed to oxidative
stress (Awazu and Horie, 1997; Imai et al., 1994). On the other hand, Pizzorno et al. (1999) reported that garlic has always been known as an aphrodisiac and from a medical point of view it can improve blood circulation significantly. Now appear that an enzyme called nitric oxide synthase is primarily responsible for the mechanism of erection. Studies have recently shown that garlic in certain forms can stimulate the production of nitric oxide synthase particularly in individuals who have low levels of this enzyme. Clearly folklore is now being proven correct.

Conclusions
In the light of the results of the present study, it can be concluded that developing a defence system against lipid peroxide damages of practical importance to improve the extended liquid storage of rooster semen. The present experiment demonstrated improved motility, membrane integrity, survival, and normality of spermatozoa and their acrosomes after cold storage for up to 72 h of roosters’ sperms with garlic.

REFERENCES


