Influence of Adding Licorice Extract to Diluents on Semen Quality and Storage Ability of Roosters’ Semen during Liquid Storage

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ABSTRACT

This study was undertaken as an attempt to enhance the resistance of roosters’ semen to peroxidative detriments by supplementing Beltsville Poultry Semen Extender (BPSE) diluent of roosters’ semen with liquorice extract (LE). Six treatment groups each of 7 White Leghorn cockerels, 22 weeks of age were used. Semen samples were collected from all roosters once a week throughout the experimental period (22 – 32 weeks of age). Treatment 1 was fresh semen and served as the control, T2 represented the semen diluted with BPSE diluent alone, while T3, T4, T5 and T6 were semen samples diluted with BPSE diluent and supplemented with 1, 3, 6 and 9 mg LE / 100 ml of diluent, respectively. Effects of diluent supplementation with LE on mass activity, individual motility and percentages of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities of roosters semen stored for different storage periods (0, 24, 48 and 72 hours) at refrigerator temperature (4-6 °C) were studied. Results revealed that inclusion of LE into BPSE diluent resulted in significant (p<0.05) improvement in spermatozoan motility, viability and morphology of spermatozoa and acrosomes of roosters semen stored for 24, 48 and 72 h at 4-6 °C compared with control group (T1). However, there were no significant differences between T2 and T3 in regard to traits mentioned hereinbefore. Furthermore, T5 and T6 surpass other treatments of LE (T3 and T4) with relation to these semen characteristics. In conclusion, involvement of LE in roosters semen diluent ameliorated semen quality of roosters' semen samples that stored at 4 – 6 °C unto 72 h. However, the levels of 6 and 9 mg LE / 100 ml of diluent recorded the best results regarding all semen characteristics included in this study in comparison with 1 and 3 mg LE / 100 ml of diluent.

Keywords: Licorice extract, diluents, liquid storage, semen quality, roosters

INTRODUCTION

The lipid composition of chicken semen is an important determinant of its quality and fertilizing capacity (Cerolini et al., 1997; Khan, 2011; Khan et al., 2012). Chicken spermatozoa are characterised by comparatively high levels of 20: n – 6 and 22: n – 6 fatty acids within their phospholipids (Blesbois et al., 1997). As a result of this high proportion of polyunsaturated fatty acids (PUFA) chicken semen is susceptible to lipid peroxidation (Surai et al., 1997), which could lead to sperm deterioration during storage (Surai et al., 1998). However, the high degree of PUFA typical of sperm lipids renders these gametes highly susceptible to lipid peroxidation, with the consequent risk of damage to cellular structures (Niki et al., 1993). Hammerstedt (1993) reported that lipid composition of the sperm membrane is a major determinant of motility, sperm membrane integrity, overall viability, cold sensitivity and fertilizing ability. The presence of such high concentrations of PUFA within the lipid fractions necessitates the presence of an efficient antioxidant system to protect against peroxidative damage and possible associated sperm dysfunction.

Suppression of lipid peroxidation through addition of antioxidants such as vitamins A, C or E to the sperm diluents, which block the production of reactive oxygen species or counteract oxygen toxicity, has been achieved with avian spermatozoa with good success (Al-Daraji, 2000; Al-Daraji, 2002; Al-Daraji, 2004).

Liquorice (Glycyrrhiza glabra) has been shown to reduce low density lipoprotein (LDL) cholesterol oxidation. The active components of liquorice inhibit the formation of lipid peroxides and protect LDL associated carotenoids (Belinky et al., 1998a). Belinky et al. (1998b) indicated that liquorice may complement other nutritional supplements in reducing LDL and PUFA oxidation. Murray (1995) concluded that glycyrrhizin, the chief substance in liquorice root, may protect vital organs from being damaged by oxidants. Bown (1995) reported that liquorice root is favourite of athletes, promotes endurance and vitality, oxygenates the genitalia and enhances the sexual potency. Al-Daraji et al. (2005) found that liquorice extract (LE) resulted in significant improvement in ejaculated volume, spermatozoa concentration, mass activity, individual motility and percentage of live and normal spermatozoa.
Our present objective was to determine the probable antioxidant role of LE in improving semen quality of roosters during in vitro storage for up to 72 h.

MATERIAL AND METHODS

Forty two White Leghorn Cockerels (22 weeks of age) were allocated to six treatments with 7 birds in each treatment. Birds were fed a commercial layer ration ad libitum. Semen samples were collected on a weekly basis by abdominal massage (Lake and Stewart, 1978) during the first part of the reproductive period (22–32 weeks of age). 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. However, semen samples were collected for 10 times during the experimental period (22-32 weeks of age); therefore, there were 30 replicates for each treatment group. Fresh semen served as a control (T1), treatments were semen diluted 1:1 in BPSE diluent (Sexton, 1977) alone (T2), semen diluted with BPSE and supplemented with LE (1 mg/100 ml of diluent; T3). The other semen treatments were diluted with BPSE and supplemented with 3, 6 and 9 mg LE/100 ml of diluent for T4, T5 and T6, respectively. Treatments were individually stored at the refrigerator temperature (4–6°C) for different storage periods (0, 24, 48 and 72 h). An aliquot of semen from each treatment group was evaluated at 0, 24, 48 and 72 h of in vitro storage for mass activity, individual motility and percentages of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities.

Mass activity of spermatozoa cells (movement in a forward motion) was estimated on a percentage basis (Sexton, 1976). Individual motility was also determined (Al-Daraji, 2000). The determination of number of dead spermatozoa was done by using eosin B stain – glutamate based extender (Al-Daraji et al., 2002). Percentage of abnormal spermatozoa was determined by using a Gentian violet – eosin stain (Al-Daraji, 1998). As an alternative to evaluation of avian spermatozoa for the acrosome reaction, staining procedure was used (Al-Daraji, 2000, 2001).

Results were evaluated by analysis of variance. Differences between treatments means were analyzed by using the ANOVA procedure in Statistical Analysis System (SAS, 1989). Duncan’s multiple range test was used to know the statistical difference between treatments.

RESULTS

The traits of the samples from treated groups, in terms of mass activity and individual motility of spermatozoa are shown in Figures 1 and 2. The mass activity and individual motility of spermatozoa evaluated directly after collection were significantly (P<0.05) higher in treatments T4, T5 and T6 in comparison with other treatments (T1, T2 and T3). However, T1 group recorded the poorest, while there were no significant differences between T2 and T3 groups. When evaluated at 24, 48 and 72 h after initiation of in vitro storage, treatments 4, 5 and 6 surpassed other treatments in mass activity and individual motility (Fig. 1 & 2). However, T5 and T6 showed the best results (P<0.05) for these two characteristics compared with other LE treatments (T3 and T4), whereas, there were no significant differences between T2 and T3 groups.

Spermatozoa incubated for 24, 48 and 72 h at the refrigerator temperature in the absence of added LE was associated with a significant (P<0.05) increase in the percentages of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities (Fig. 4, 5 & 6). The inclusion of LE in the BPSE diluent significantly (P<0.05) decreased the percentages of these three characters in comparison with control group (T1). However, T5 and

Figure 1: Effect of diluent supplementation with licorice extract on mass activity of roosters semen
T1=fresh semen; T2 = semen diluted with BPSE diluent alone; while T3, T4,T5 and T6 = semen diluted with BPSE diluent and supplemented with 1, 3, 6 and 9 mg LE / 100 ml of diluent, respectively; Bars with different superscripts differ significantly (P<0.05)
Figure 2: Effect of diluent supplementation with licorice extract on individual motility of roosters semen
T1 = fresh semen; T2 = semen diluted with BPSE diluent alone; while T3, T4, T5 and T6 = semen diluted with BPSE diluent and supplemented with 1, 3, 6 and 9 mg LE / 100 ml of diluent, respectively; Bars with different superscripts differ significantly (P<0.05).

Figure 3: Effect of diluent supplementation with licorice extract on percentage of dead spermatozoa of roosters semen
T1 = fresh semen; T2 = semen diluted with BPSE diluent alone; while T3, T4, T5 and T6 = semen diluted with BPSE diluent and supplemented with 1, 3, 6 and 9 mg LE / 100 ml of diluent, respectively; Bars with different superscripts differ significantly (P<0.05).

Figure 4: Effect of diluent supplementation with licorice extract on percentage of abnormal spermatozoa of roosters semen
T1 = fresh semen; T2 = semen diluted with BPSE diluent alone; while T3, T4, T5 and T6 = semen diluted with BPSE diluent and supplemented with 1, 3, 6 and 9 mg LE / 100 ml of diluent, respectively; Bars with different superscripts differ significantly (P<0.05).
T6 were superior to other LE treatments (T3 & T4) to ameliorate the deterioration that occurred in the percentages of live spermatozoa, normal spermatozoa and acrosomes. Besides, there were no significant differences between T2 and T3 groups regarding these three traits (Fig. 3, 4 & 5).

**DISCUSSION**

Results of the present study clearly indicated that addition of appropriate concentration of LE into BPSE diluent maintained motility, viability and morphology of roosters spermatozoa till 72 h in vitro storage better than control group (T1) or semen diluted with BPSE alone (T2). It is speculated that endogenous antioxidants activity in roosters' seminal plasma may not be enough to prevent the lipid peroxide damage after dilution and in vitro storage. However, the improvement in sperm characteristics noticed in our study could be the result of LE antioxidants enhancing or limiting the damaging effects of lipid peroxidation in vitro. The improvements in spermatozoa motility, liveability and morphology with LE treatments during in vitro storage were in a good agreement with the results of Donoghue and Donoghue (1997) and Al-Daraji (2000, 2002, 2004) who demonstrated that the supplementation of antioxidants maintained spermatozoan viability, motility, morphology and fertilizing ability when semen was stored at 4-6°C for different storage periods. Etches (2000) reported that the fertilizing capacity and motility of avian spermatozoa begins to decline within 15 min after storing them in vitro. Furthermore, the mechanisms responsible for liquorice protection of LDL and PUFA against oxidation are its ability to bind LDL, scavenge free radicals, and protect other oxidants associated with LDL from oxidation (Bianca et al., 2002). Vaya et al. (1997) reported that some dietary nutrients such as liquorice isoflavanes are potent antioxidants against LDL and PUFA oxidation. Flavonoid components of liquorice root extract (glabridin, glabrene) were shown to have antimicrobial, anti-inflammatory and antioxidative activity. Liquorice root extract, as well as its major flavonoid, the isoflavain glabridin, are powerful antioxidants against lipid peroxidation, therefore it protects certain vital organs from being harmed by oxidants (Kent et al., 2002; Rosenblat et al., 1999). Al-Haboby et al. (2003) found that oral treatment of Awassi rams with LE resulted in significant improvement in semen quality and *libido*. It is speculated that amelioration in semen quality and sexual activity may be due to the role of liquorice as antioxidant agent. Some authors have also suggested that it improves the stages of spermatogenesis, maintains LH receptors and increase FSH and testosterone concentrations (Aitken et al., 1989, Kilgour et al., 1993). On the other hand, Tamir (2000) indicated that using the men erection capsule (power of love) which contains liquorice root extract in its formula enhances short-term activity while providing support to the kidney/adrenal system for long-term sexual health. Formulated with certain natural, traditionally used herbs, they work together for increased sexual health, enhancing stamina and sexual performance and increase *libido*.

**Conclusion**

It was concluded from this study that inclusion of liquorice root extract into semen diluents especially at the levels of 6 and 9 mg/100 ml of diluent can be used as an efficient tool to prevent spermatozoa deterioration during in vitro storage.

**REFERENCES**

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