EFFECT OF DILUENT SUPPLEMENTATION WITH LIQUORICE EXTRACT ON SEMEN QUALITY OF ROOSTERS

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ABSTRACT

This study was undertaken as an attempt to enhance the resistance of roosters semen to peroxidative deterrents 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 or 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 or 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 spermatozoa motility, viability and morphology of spermatozoa and acrosomes of roosters semen stored for 24, 48 or 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 surpasses other treatments of LE (T3 or T4) with relation to these semen characteristics.

In conclusion, involvement of LE in roosters semen diluent ameliorated 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.

المستخلص

أجريت الدراسة الحالية كمحاولة لتحزيق مقاومة مني الديكة تواجه الإضرار الناجم عن تكوين البيروكسيديات عن طريق إضافة مستخلص عرق السوس في مخلطات مني الديكة. واستخدم فيها 36 ذكر لكونه أكثر عدد. فمثلاً على مستخلصة مستخلص عرق السوس إلى مخلطات مني الديكة. ونتيجة جمع المنية من مني الديكة لمحة واحدة يومياً خلال ما قد تكون منياً، وبيروكسيديات مني من 22 إلى 32 يوماً. وكانت المعاملات الأولى تمثل مجموعة مني الطازج والثاني عشرة مكتملة من بيروكسيديات مني من 100 مل من الصفن الذي تم تخفيفها باضافة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE مكتملة من BPSE من دون أي إضافة. ففي العينات من عرق السوس تم تخفيفها باضافة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE في مخلطات مني الديكة في منجات الغزالة والغذاء للغذاء المطبوخ للغذاء المطبوخ للمشروبات المكثوفة من مني الديكة BPSE في مخلطات مني الديكة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE من دون أي إضافة. ففي العينات من عرق السوس تم تخفيفها باضافة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE في منجات الغزالة والغذاء للغذاء المطبوخ للمشروبات المكثوفة من مني الديكة BPSE في مخلطات مني الديكة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE من دون أي إضافة. ففي العينات من عرق السوس تم تخفيفها باضافة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE في منجات الغزالة والغذاء للمشروبات المكثوفة من مني الديكة BPSE في مخلطات مني الديكة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE من دون أي إضافة. ففي العينات من عرق السوس تم تخفيفها باضافة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE في منجات الغزالة والغذاء للمشروبات المكثوفة من مني الديكة BPSE في مخلطات مني الديكة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE من دون أي إضافة. ففي العينات من عرق السوس تم تخفيفها باضافة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE في منجات الغزالة والغذاء للمشروبات المكثوفة من مني الديكة BPSE في مخلطات مني الديكة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE من دون أي إضافة. ففي العينات من عرق السوس تم تخفيفها باضافة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE في منجات الغزالة والغذاء للمشروبات المكثوفة من مني الديكة BPSE في مخلطات مني الديكة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE من دون أي إضافة. ففي العينات من عرق السوس تم تخفيفها باضافة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE في منجات الغزالة والغذاء للمشروبات المكثوفة من مني الديكة BPSE في مخلطات مني الديكة BPSE بنوع ديكة ضروري. وثبت أن المستخلصات من BPSE من دون أي إضافة. F
Introduction
The lipid composition of chicken semen is an important determinant of its quality and fertilizing capacity (16). Chicken spermatozoa are characterized by comparatively high levels of 20 : n - 6 and 22 : n - 6 fatty acids within their phospholipids (14). As a result of this high proportion of polyunsaturated fatty acids (PUFA), chicken semen is susceptible to lipid peroxidation (29), which could lead to sperm deterioration during storage (30). However, the high degree of PUFA typical of sperm lipids render these gametes highly susceptible to lipid peroxidation, with the consequent risk of damage to cellular structures (24). Hammerstedt (19) 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 fraction warrants 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 (3, 6, 7). However, liquorice 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 (11). Belinky et al. (12) indicated that liquorice may complement other nutritional supplements in reducing LDL and PUFA oxidation. Murray (23) concluded that glycyrrhizin, the chief substance in liquorice root may protect vital organs from being damaged by oxidants. Bown (15) reported that liquorice root (Glycyrrhiza glabra) is favored of athletes, promotes endurance and vitality so sex becomes that much better, oxygenates the genitalia and enhances the sexual potency. Al-Daraji et al. (9) found that liquorice extract (LE) drinking water supplementation resulted in significant improvement in ejaculate 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 72h.

Materials and Methods
Cockerels (White Leghorn, 22 weeks of age) were allocated to six treatment pens with 7 birds in each treatment pen. Birds fed a commercial layer ration ad libitum. Semen samples were collected on a weekly basis by abdominal massage (22) 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 (T2) 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 (27). Individual motility was also determined (4). The determination of number of dead spermatozoa was done by using a fast green stain – Eosin B stain – glutamate based extender (8). Percentage of abnormal spermatozoa was determined by using a Gentian violet – eosin stain (2). As an alternative to evaluation of avian spermatozoa for the acrosome reaction, staining procedure for fixed samples have been developed to distinguish which spermatozoa have retained or lost the acrosome (3, 5).

Results were evaluated by analysis of variance. Differences between treatments means were analyzed by Duncan’s Multiple Range Test, using the ANOVA procedure in Statistical Analysis System (26).

Results and Discussion
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 sperms
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 results as regards these two traits, while there were no significant differences between T2 and T6 groups. When evaluated 24, 48 and 72 h after initiation of in vitro storage, treatments 4, 5 and 6 surpasses other treatments with relation to mass activity and individual motility (Figures 1 and 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 incubation 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 (Figures 4, 5 and 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 T6 were superior to LE treatments (T3 and T4) inameliorate the deterioration that occurred in the percentages of live spermatozoa and normal spermatozoa and acrosomes. Besides, there were no significant differences between T2 and T3 groups regarding these three traits (Figures 3, 4 and 5).

Results of the present study clearly denoted that addition of appropriate concentration of LE into BPSE diluent maintained motility, viability and morphology of roosters spermatozoa till 72 h in vitro storage. Sexual performance (T1) or semen diluted with BPSE alone (T2). It is speculated that endogenous antioxidants activity in roosters seminal plasma may be not 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 a result of LE antioxidants suppressing 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 (17) and Al-Daraji (3, 6, 7) who demonstrated that the supplementation of antioxidants had maintained spermatozoa viability, motility, morphology and fertilizing ability when semen stored at 4 – 6 °C for different storage periods. Etches (18) reported that when maintained in vitro at room temperature, however, the fertilizing capacity and motility of avian spermatozoa begins to decline within 15 min. following ejaculation. Furthermore, the mechanisms responsible for liquefactive protection of LDL and PUFA against oxidation are its ability to bind LDL, scavenge free radicals, and protect other oxidants associated with LDL, the carotinoids, from oxidation (13). Vaya et al. (32) reported that some dietary nutrients such as liquorice isoflavones are potent antioxidants against LDL and PUFA oxidation. Flavonoids of liquorice root extract (glabridin, glabrene) were shown to have antimicrobial, anti-inflammatory, and antioxidant activity. Liquorice root extract, as well as its major flavonoid, the isoflavonoid glabridin, are powerful antioxidants against lipid peroxidation, therefore it protects certain vital organs from being harmed by oxidants (20, 25). Al-Hobey et al. (10) found that orally treatment of Awassi rams with LE resulted in significant improvement in semen quality and libido. However, these authors concluded that these amelioration in semen quality and sexual activity may be due to the role of liquorice as antioxidant agent, which might improve the stages of spermatogenesis, maintained LH receptors and increase FSH and testosterone concentrations (1, 21). On the other hand, Tamir (31) indicated that using the men erection capsule (power of love) which contain 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 control and sexual performance and increase libido. Individuals who are presently using this formula of liquorice root extract and some natural herbs reported that they satisfy their partner more often, enjoy better orgasms, and have stiffer erections and feel sexier.

It was concluded from this study that the antioxidant / prooxidant balance in roosters semen is an important element in maintaining spermatozoa viability, motility and morphology. The antioxidant system can be suggested to be a crucial element of such a regulation. However, 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 successful tool for repress the nocuous effects of lipid peroxidation which could lead to spermatozoa deterioration during in vitro storage.
Bars with different superscripts differ significantly (p < 0.05).

Supplemented with I, 3, 6, and 9 μg of different, respectively. 1T = fresh semen, 1T = semen diluted with HPSFE diluted and 1T = semen diluted with HPSFE diluted and

Storage periods

0 h 24 h 48 h 72 h

Mass activity (%) 0 10 20 30 40 50 60 70 80 90 100

Activity of roosters semen

Figure 1. Effect of different supplementation with HPSFE extract on mass activity.
Bars with different superscripts differ significantly ($p > 0.05$).

Supplemented with 1, 3, 6, and 9 mL L$^{-1}$ of chitin.

TI = fresh semen, T2 = semen diluted with BPE before diluent and
T3, T4, T5, T6 = semen diluted with BPE after diluent and

Storage periods:
- 72 h
- 48 h
- 24 h
- 0 h

Individual motility of roosters' semen

Figure 2: Effect of different supplementation with licorice extract on
Percentage of dead spermatozoa of roosters semen 

Figure 3: Effect of different supplementations with licorice extract on 

Bars with different superscripts differ significantly (p < 0.05) 

Supplemented with 1, 3, 6 and 9 mg of licorice, respectively. 

T1 = Fresh semen; T2 = semen diluted with BPSE different alone; while T3, T4, T5 and T6 = semen diluted with BPSE different and
Bars with different superscripts differ significantly (p < 0.05).

I = control, 12 = semen diluted with BPF in enteric-coated gel, 24 h = semen stored for 24 h, 48 h = semen stored for 48 h.

Figure 4. Effect of diuretic supplementation with bacopa extract on
Percentage of abnormal spermatozoa of roosters semen.
Bars with different superscripts differ significantly (p < 0.05).

Storage periods:
- 72 h
- 48 h
- 24 h
- 0 h

Percentage of acrosomal abnormalities of roosters semen.

Figure 5: Effect of diluent supplementation with licorice extract on acrosomal abnormalities (%).
References


