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 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 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 ^{0} 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 or 72 h at 4 – 6 ^{0} 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 semen quality of roosters semen samples that stored at 4 – 6 ^{0} 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.

تأثير إضافة مستخلص عرق السوس في مخففات المني في نوعية مني الديكة

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المستخلص

اجريت الدراسة الحالية كمحاولة لتعزيز مقاومة مني الديكة تجاه الاضرار الناجمة عن تكوين البيروكسيدات عن طريق اضافة مستخلص عرق السوس الى مخففات مني الديكة. واستخدم فيها 42 ديك لكهورن ابيض عمر 22 اسبوعاً ، وتم توزيعها على ست معاملات يتكون كل منها من ستة ديكة. وتم جمع المني من جميع الديكة لمرة واحدة اسبوعياً خلال مدة الدراسة الممتدة من عمر 22 – 32 اسبوعاً. وكانت المعاملة الاولى تمثل مجموعة المني الطازج والتي عدت كمجموعة سيطرة ، اما المعاملة الثانية فكانت تمثل مجموعة المني التي تم تخفيفها بمخفف BPSE من دون اية اضافة. في حين ان المعاملات 3 و 4 و 5 و 6 كانت تمثل مجاميع المني التي تم تخفيفها بمخفف BPSE مع اضافة مستخلص عرق السوس بتراكيز 1 و 3 و 6 و 9 ملغم / 100 مل من المخفف على التتابع. وتمت دراسة تأثير اضافة مستخلص عرق السوس في مخفف BPSE في الحركة الجماعية والفردية للنطف والنسبة المئوية للنطف الميتة والمشوهة وتشوهات الاكروسومات في مني الديكة الذي تم خزنه لعدد خزن مختلفة)0 و24 و48 و72 ساعة(بدرجة حرارة الثلاجة)4 – 6 ^{0} م.(

اظهرت النتائج ان ادخال مستخلص عرق السوس في مخفف BPSE ادى الى تحسن معنوي)أ < 0.05(في حركة وحيوية النطف ومظهر كل من النطف والاكروسومات للمني الذي تم خزنه بدرجة حرارة 4 – 6 ^{0} م ولمدة 24 أو 48 أو 72 ساعة مقارنة بمجموعة السيطرة. من ناحية ثانية ، لم تكن هناك فروق معنوية بين المعاملتين 2 و 3 فيما يتعلق بهذه الصفات. فضلاً على ذلك ، فأن المعاملتين T5 و T6 قد تفوقتا على باقي معاملات عرق السوس T3) و T4) فيما يخص الصفات المدروسة.

يستنتج من كل ذلك ، ان ادخال مستخلص عرق السوس في مخففات مني الديكة قد حسّن من نوعية المني الذي تم خزنه بدرجة حرارة 4 – 6 ^{0} م لغاية 72 ساعة. من ناحية اخرى ، فأن التراكيز 6 و 9 ملغم مستخلص عرق سوس / 100 مل من المخفف قد حققت افضل النتائج فيما يتعلق بجميع صفات المني التي شملتها الدراسة الحالية بالمقارنة مع التركيزين 1 و 3 ملغم مستخلص عرق سوس / 100 مل من المخفف.

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Introduction

The lipid composition of chicken semen is an important determinant of its quality and fertilizing capacity (16). Chicken spermatozoa are characterised 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 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 (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 (28) 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 ^{0} 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

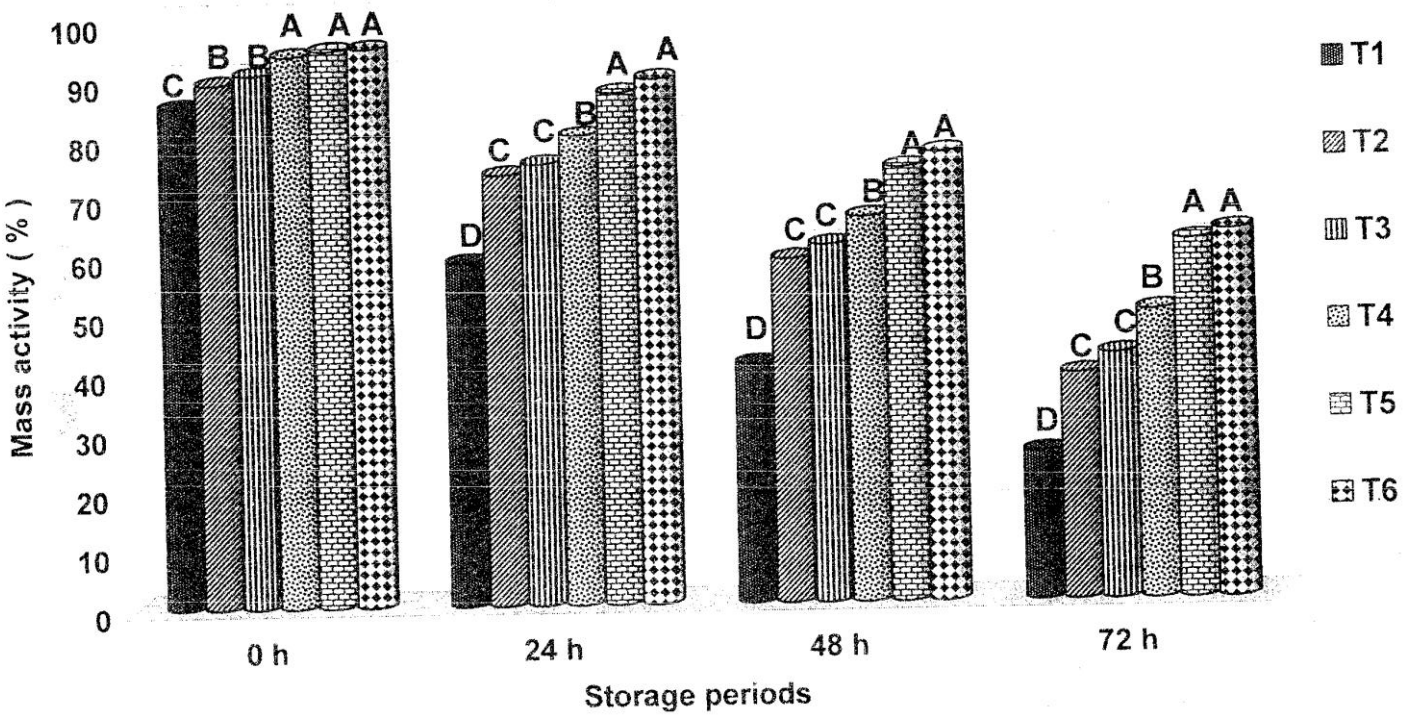
evaluated directly after collection were significantly ($p < 0.05$) higher in treatments T4, T5 and T6 in comparison with other treatments (T1, T2 and T3). However, T1 group recorded the poorest results as regards these two traits, while there were no significant differences between T2 and T3 groups. 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 other LE treatments (T3 and T4) in ameliorate 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 better than control group (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 spermatozoan viability, motility, morphology and fertilizing ability when semen stored at 4 – 6 ^{0} 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 liquorice 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 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 isoflavin glabridin, are powerful antioxidants against lipid peroxidation, therefore it protects certain vital organs from being harmed by oxidants (20, 25). Al – Haboby 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 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.

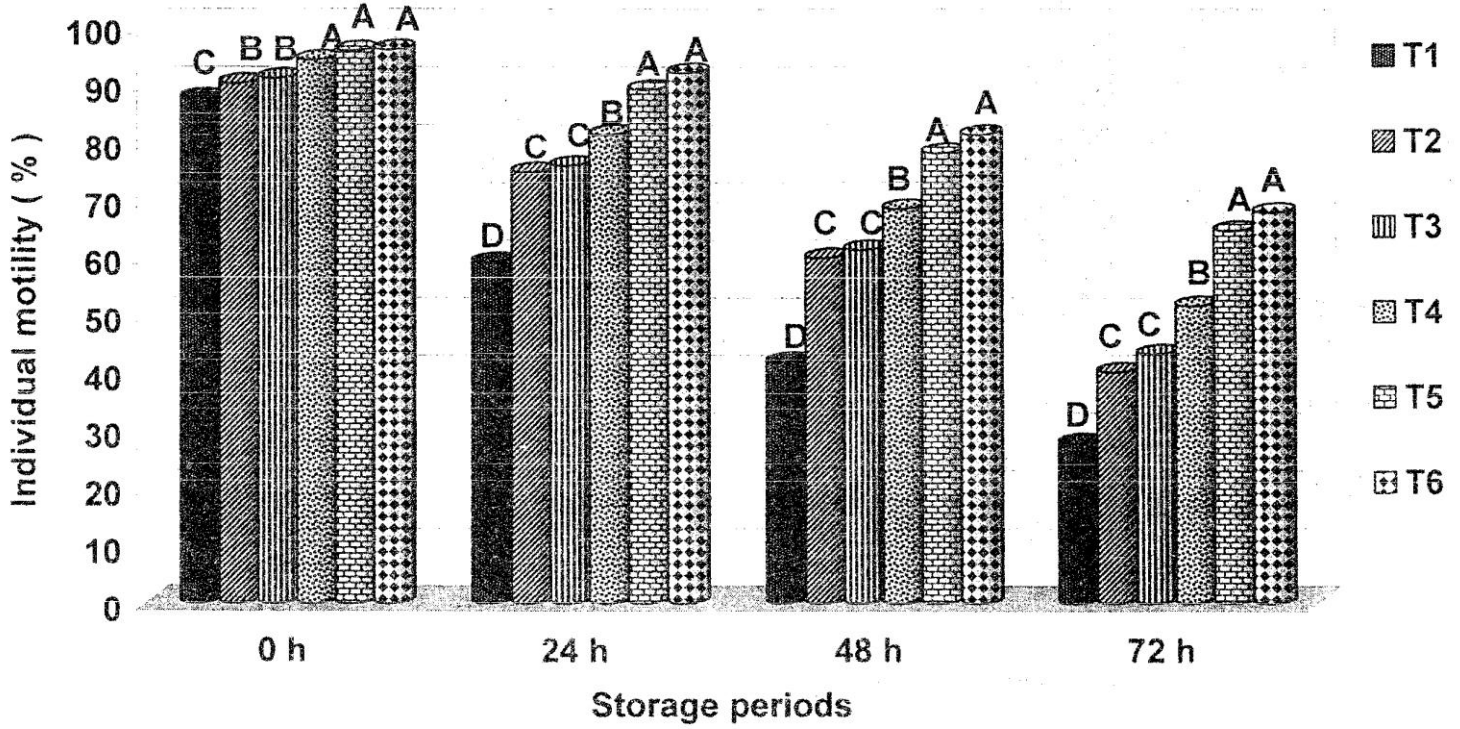
It was concluded from this study that the antioxidant / proxidant balance in roosters semen is an important element in maintaining spermatozoa viability, motility and morphology. The antioxidant system can be suggested to be a crucial element of such a regulation.However, inclusion of liquorice root 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.

Figure 1.Effect of diluent supplementation with licorice extrct on mass activity of roosters semen



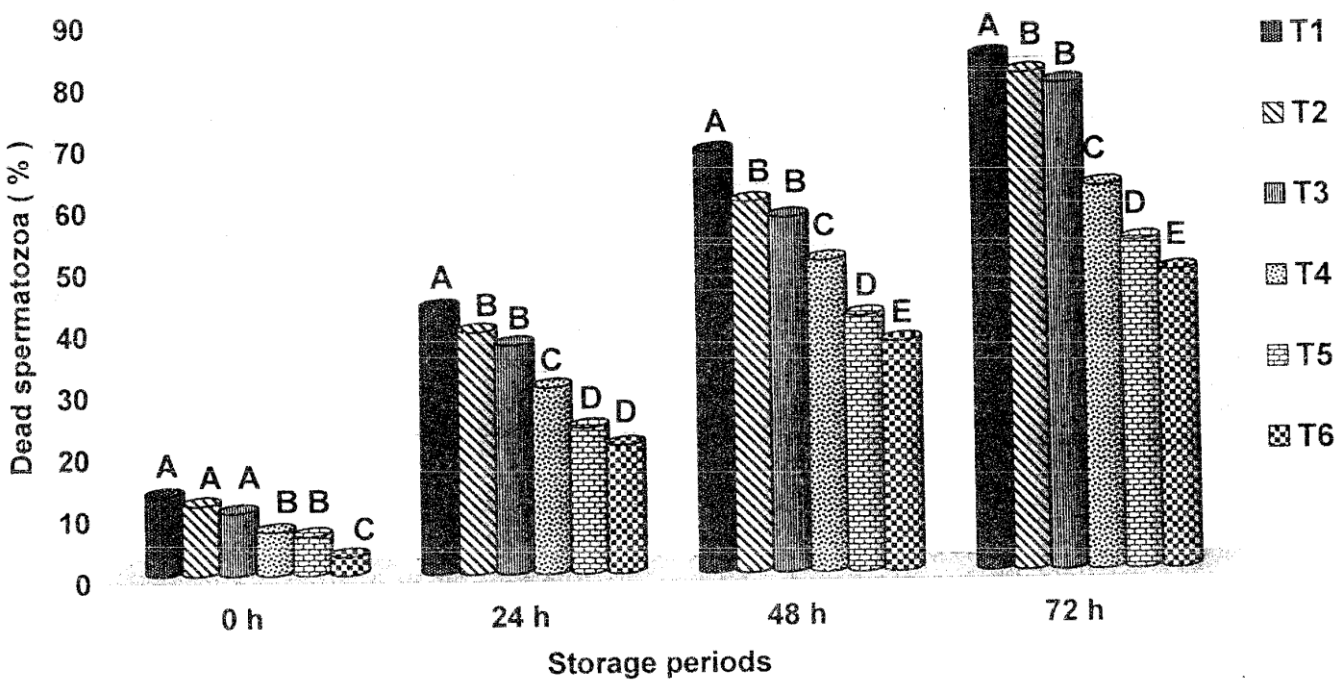
T1=fresh semen , T2 = semen diluted with BPSE diluent alone , while T3 , T4 ,T5 and T6 = semen diluted with BPSE diluent and supplemented with 1 , 3 , 6 and 9 mg LE / 100 ml of diluent , respectively .
Bars with different superscripts differ significantly ($p < 0.05$) .

Figure 2.Effect of diluent supplementation with licorice extract on individual motility of roosters semen



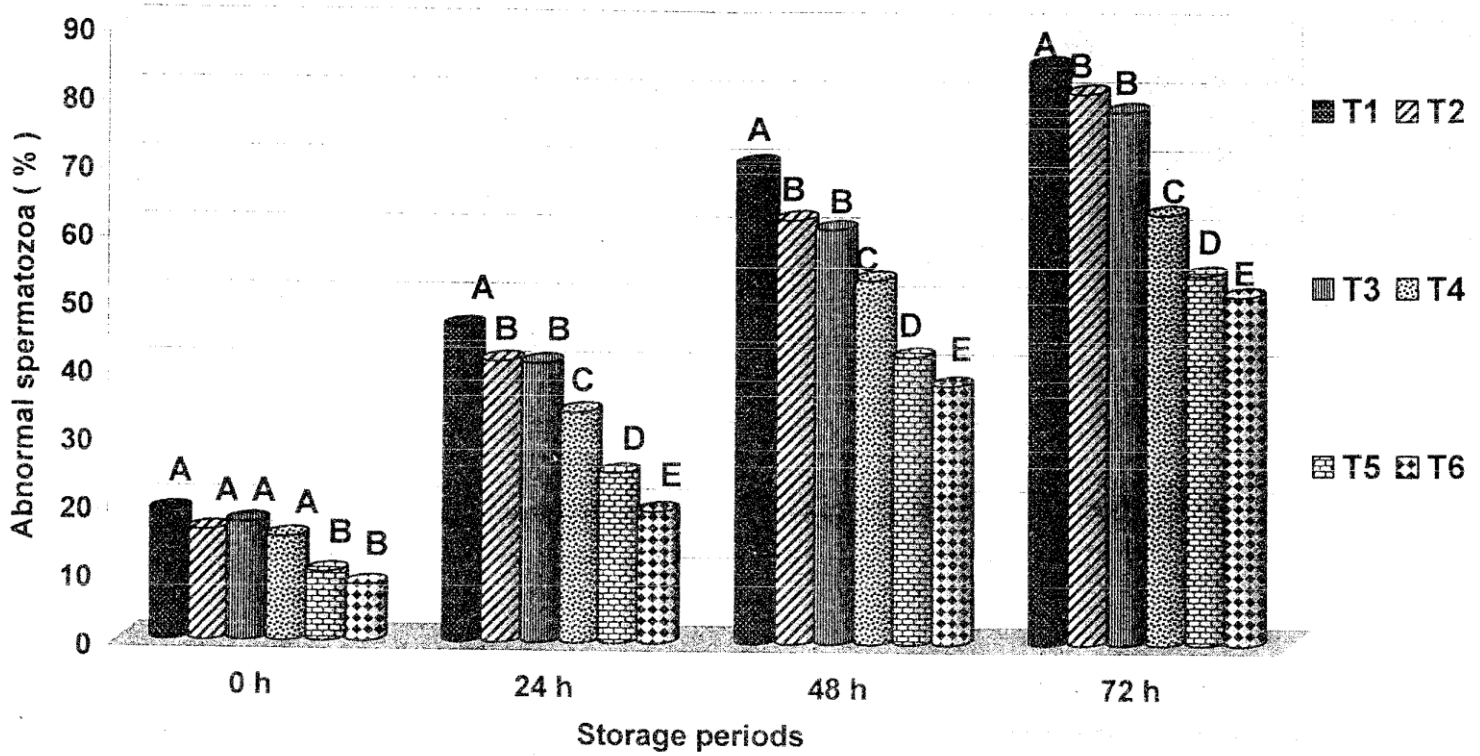
T1 = fresh semen , T2 = semen diluted wit BPSE diluent alone , while T3 , T4 , T5 and T6 = semen diluted with BPSE diluent and supplemented with 1 , 3 , 6 and 9 mg LE / 100 ml of diluent .
Bars with different superscripts differ significantly ($p < 0.05$) .

Figure 3.Effect of diluent supplementation with licorice extract on percentage of dead spermatozoa of roosters semen



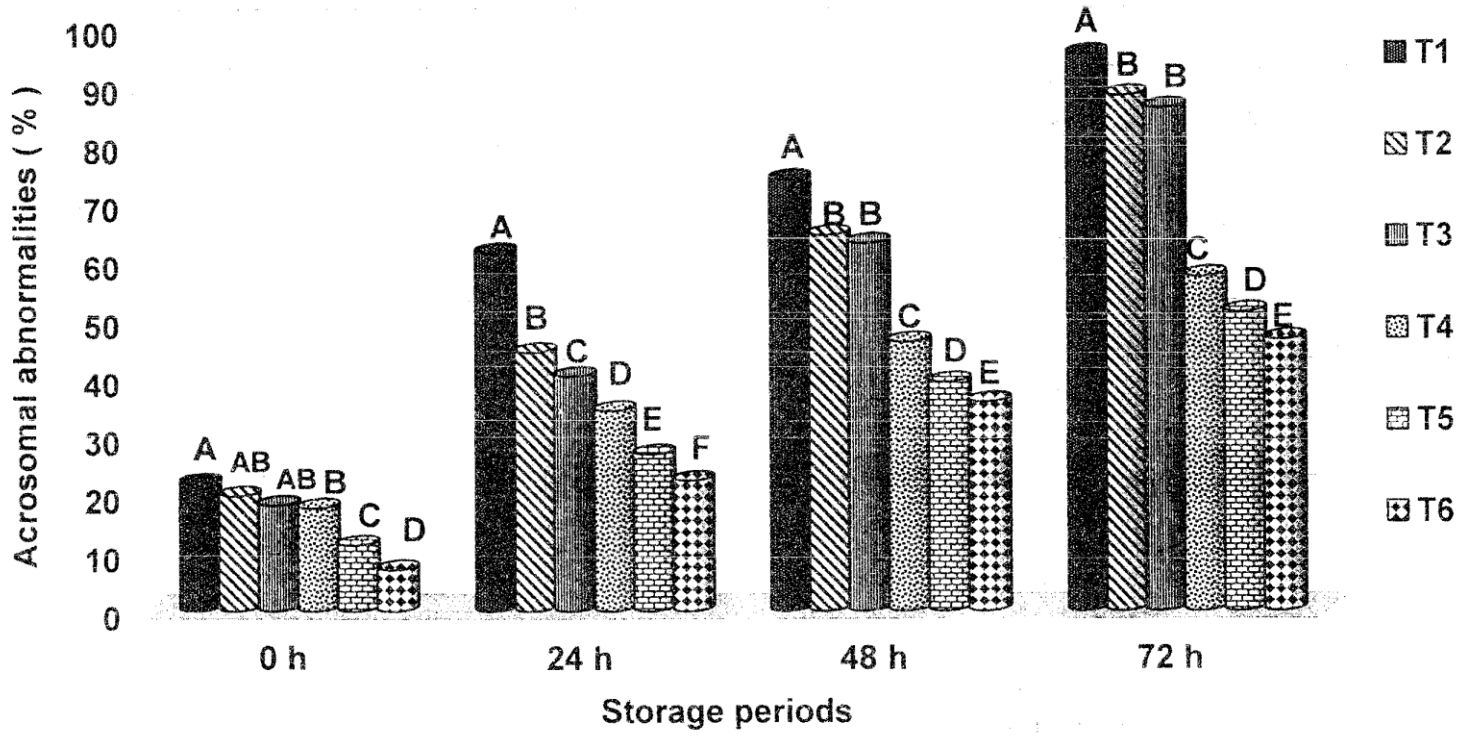
T1=fresh semen , T2 = semen diluted with BPSE diluent alone , while T3 , T4 , T5 and T6 = semen diluted with BPSE diluent and supplemented with 1 , 3 , 6 and 9 mg LE / 100 ml of diluent , respectively .
Bars with different superscripts differ significantly ($p < 0.05$) .

Figure 4.Effect of diluent supplementation with licorice extract on percentage of abnormal spermatozoa of roosters semen



T1=freshsemen , T2 = semen diluted with BPSE diluent alone , while T3 , T4 , T5 and T6 = semen diluted with BPSE diluent and diluent with 1 , 3 , 6 and 9 mg LE / 100 ml of diluent , respectively .
Bars with different superscripts differ significantly ($p < 0.05$) .

Figure 5.Effect of diluent supplementation with licorice extract on percentage of acrosomal abnormalties of roosters semen



T1=fresh semen , T2 = semen diluted with BPSE 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 ($p < 0.05$) .

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