EFFECT OF DIETARY INORGANIC AND ORGANIC ZINC SUPPLEMENTATION ON SEMEN QUALITY OF RABBIT BUCKS

Samia Z. Meshreky¹*, Sabbah M. Allam², El-Manilawy M², Amin H.F.¹

¹ Animal Production Research Institute, Dokki, ARC, Egypt.
² Faculty of Agriculture, Cairo University, Geza, Egypt.

* Corresponding author: Samia_Meshreky2010@hotmail.com

SUMMARY
Thirty New Zealand White rabbit bucks (at 5 month of age, 2.74±0.13 kg average body weight) were divided into three homogenous groups. The 1st group was fed basal diet as a control, whereas the 2nd and 3rd groups were fed the basal diet supplemented with 100 mg Zn from Zn sulfate (ZnSO₄, inorganic form) or Zn methionine (Zn-Met, organic form). Eighteen ejaculates from each buck (two ejaculates/day, three times in the 1st week of 7th, 8th and 9th months of age) were collected and evaluated for semen quality. At the end of the experimental period (2nd week of 9th months of age), bucks of each group were bred with 20 receptive nulliparous female rabbits. Mean values of semen characteristics were significantly different among the groups after 2 months of zinc supplementation. The results revealed that testosterone concentration in blood, sperm cell concentration, motility (%), live sperm (%) and intact acrosome (%) were significantly (P<0.05) higher in Zn-supplemented groups, especially with organic form (Zn-Met, 2.99±0.15 ng/mL, 348±12 x10⁶/ml, 87.9±1.39%, 88.2±1.35% and 88.6±1.43%, respectively) as compared to the control group (1.89±0.15, 237±12, 61.8±1.39, 63.4±1.35 and 72.8±1.43, respectively). Also, our data indicated that improvement (P<0.01) of semen characteristics with advancement of age. A positive correlation between testosterone concentration and sperm cell concentration, motility, live sperm and intact acrosome percentages (r= 0.344, 0.294, 0.360 and 0.406, respectively; P<0.01) was found. Fertility rate was increased from 65% (13/20) in control group to 85 (17/20) and 90% (18/20) in buck groups fed diet supplemented with ZnSO₄ and Zn-Met, respectively. Also, a positive correlation between semen quality and fertility was found. Only 58 kits were born alive in control group (5.8/ buck) compared to 101 and 118 bunny born alive in ZnSO₄ and Zn-Met groups (10.1 and 11.8/buck, respectively). We can concluded that the dietary Zn supplementation, especially organic form, improved semen characteristics and testosterone concentration in rabbit bucks, which could be related to better fertility and prolificacy.

Key words: rabbit, zinc organic, semen quality, testosterone, fertility.

INTRODUCTION
Zinc is an essential trace element required for the action of more than 200 metalloenzymes, supports the immune system (Shinde et al., 2006) and
plays an important role in polymeric organization of macromolecules like DNA and RNA (Garcia-Contreras et al., 2011), protein synthesis and cell division (Lukac and Massanyi, 2007). Zinc plays several roles in the male reproductive system, its participation in ribonuclease activity which is highly active during the mitosis of spermatogonia and meiosis of spermatocytes (Cheah and Yang, 2011), plays an important role in prostate, epididymal and testicular functions (Ebisch et al., 2003) and controls sperm motility (Wroblewski et al., 2003). Zinc levels in seminal plasma have been positively associated with sperm concentration and motility (Chia et al., 2000). Zinc supplementation enhances spermatogenesis, but the mechanism is not completely known (Oliveira et al., 2004). Lukac et al. (2009) reported that a concentration of zinc in rabbit semen of 81.2±59.4 mg/kg wet weight. Zinc requirement for rabbits, indicated in the literature, is 30-60 mg/kg dry matter, with suggestion of higher levels for breeders (Mateos and Blas, 1998). The commonly used grains in basal rabbit diets are rich in phytate content that may reduce availability and inhibit absorption of zinc (Baker and Halpin, 1988), since these levels was not adequate to compensate the insufficient zinc in the natural ingredients of the diet. Traditionally, Zn is supplemented in the animal diets as inorganic salt. However, recently the use of organic Zn for animals has gained popularity because of its reported higher bioavailability than inorganic sources (Droke et al., 1998). Very little information is available regarding the effect of organic zinc supplementation on semen quality and fertility of rabbit bucks. Therefore, the present experiment was conducted to study the effect of dietary Zn supplementation through inorganic (ZnSO₄) and organic (zinc methionine) sources on the semen characteristics, blood serum testosterone level and reproductive performance of rabbit bucks.

MATERIALS AND METHODS
This study was conducted on 30 New Zealand White rabbit bucks (at 5 month of age, 2.74±0.13 kg average body weight), divided into three equal groups. The 1st group was fed basal diet (Table 1) and served as a control, the 2nd and the 3rd groups were fed on the basal diet supplemented with 100 mg Zn/kg diet as zinc sulfate (0.441 g ZnSO₄.7H₂O, inorganic zinc, 287.54 AW/MW and the concentration 22.7%) and zinc methionine (1.0 g Zn-Met super, organic zinc, composition: 10% Zn; 20% methionine
and sepiolite to 100%, Spain), respectively. Clean and fresh drinking water was provided *ad lib*.

**Table 1**: Physical and chemical composition of the basal diet

| Ingredients: | clover hay (19%), barley (35.2%), wheat bran (26%), soybean meal 44% (14.9%), molasses (3%), common salt (0.3%), limestone (1%), premix (0.5%) and coccidiostat (0.1%); **Total** 100.0%. |
| Chemical composition: | DM, 89.2%; CP, 16.34%; EE, 2.41%; DE (kcal/kg) 2844; CF, 11.86%. Zinc content in the control diet was 58 mg from premix plus 49.34 mg from the other ingredients. |

*Supplied per kg diet to meet NRC’ (1984) vitamins & minerals recommended for male rabbits.

Chemical composition of the basal diet was determined according to AOAC (1995)

Eighteen ejaculates from each buck (two ejaculates/day, three times in the 1st week of the 7th, 8th and 9th months of age) from all the groups were collected using artificial vagina and evaluated for semen characteristics. The sperm cell concentration was measured in aliquots using fixed spermatozoa (2% glutaraldehyde) in a Thoma-Zeiss counting cell chamber (final dilution 1:50) and a light microscope (Olympus CH-2) at x400. Sperm motility was assessed at 37°C at x40 using a phase contrast microscope. For the morphological analyses, an aliquot from each ejaculate (20 μl) was fixed with 180 μl of a 2% solution of glutaraldehyde in DPBS. A minimum of 100 sperm cells was evaluated at a magnification of 500x with a differential interference contrast microscope. The status of the acrosome of the normal sperm (intact, AI, or damaged, AD), and the sperm abnormalities were evaluated (ANR). The percentage of sperm with intact acrosome was calculated as the ratio: [AI/(AI + AD)] x 100. Blood samples were collected from bucks of all groups at 7th, 8th and 9th months of age and serum was separated by centrifugation of samples at 3000 rpm for 20 min, stored at -20°C for analysis of testosterone. At the end of the experimental periods, bucks of each group were bred with 20 receptive nulliparous female rabbits from the same breed to evaluate of bucks fertility criteria (kindling rate) and prolificacy (number of kits born alive/ doe). The relative humidity and environmental temperature averaged 68±7% and 21±3°C, respectively during the experimental period. Data were statistically
analyzed using the GLM program of SAS (1998). Differences between means were analyzed by the Duncan's New Multiple Range test (Duncan, 1955). Chi-squared test was used to compare fertility results.

RESULT AND DISCUSSION
Dietary Zn supplementation in the diet of rabbit bucks revealed a significant (P<0.01) improvement in sperm cell concentration, especially Zn-Met group (organic form, 348±12), compared to the control one (237±12, Table 2). These results are in agreement with the findings of Mocz et al. (2000) who observed an increase of spermatozoa concentration in the ejaculates of rabbit bucks fed the supplemental zinc (levels from 35 to 100 ppm) as compared to non-supplemented ones. Oliveira et al. (2004) also observed increase of spermatozoa concentration in rabbits supplemented with 50, 100 and 150 ppm zinc. The production of sperm necessitates extensive cell division and Zn plays a significant role in this process by influencing mitotic and meiotic cell divisions, along with synthesis of DNA and RNA by enhancing the activity of DNA polymerase and RNA polymerase (Cheah and Yang, 2011). In the present study, it was observed that supplementation of Zn above the recommended level in the diet of rabbit bucks resulted in a highly significant (P<0.01) increase in motility and live sperm percentage (Table 2). These results were in harmony with Alavi-Shoushtari et al. (2009) who found that seminal plasma zinc content is correlated with semen characteristics and synergistically act to preserve motility and viability of the spermatozoa after ejaculation in bulls. Our results revealed significantly lower abnormal sperm percentage in Zn supplemented groups (11.2 and 10.2±0.32 for ZnSO4 and Zn-Met, respectively) compared to the control group (16.6±0.32, Table 2). Also, results demonstrated increased (P< 0.05) intact acrosome percentage in Zn supplemented groups as compared to the control group, could be this related to the anti-oxidant properties and membrane stabilizing action of Zn that modulates the stability of the acrosomal membrane by inhibiting lipid peroxidation through influencing phospholipase activity, resulting in a membrane fluidity change. Zn has been found to stabilize various acrosomal enzymes like acrosin, acid phosphatase and phospholipase, which may account for improved intact acrosome percentage (Eggert et al., 2002). As shown in Table 2, semen characteristics have been improved with advancement of age (time of semen collection). The mean blood serum

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testosterone concentration (ng/ml) in Zn-supplemented groups, especially with organic form was significantly (P<0.01) higher than the control group (2.99, 2.46 and 1.89±0.15 ng/ml in Zn-Met, ZnSO₄ and control groups, respectively). These results are in close agreement with El-Masry et al. (1994) who recorded increased serum testosterone levels with Zn supplementation in diets of rabbits. Imam et al. (2009) reported that supplementation of zinc in the diet improved blood testosterone level and in this regard zinc propionate (organic form) at half the dose of zinc sulphate (inorganic) supplementation. The improved values of testosterone in Zn-supplemented groups might be due to stimulation Leydig cells of the testis and enhancing the production of testosterone (Fang and Furushasi, 1978).

Data (Table 3) revealed positive correlation between testosterone concentration and sperm cell concentration, motility, live sperm and intact acrosome percentages (r= 0.344, 0.294, 0.360 and 0.406, respectively; P<0.01). Wong et al. (2002) observed that Zn is involved in the activation and maintenance of the germinal epithelium of semineferous tubules and also stimulates production and secretion of testosterone, which influences spermatogenesis. The interaction between Zn supplemental source and the time of semen collection (age) was significant (P<0.01) only with abnormal sperm percentage, intact acrosome percentage and sperm cell concentrations.

Table 2: Effect of dietary Zn supplementation on semen characteristics, blood serum testosterone level and reproductive performance of New Zealand White rabbit bucks.

<table>
<thead>
<tr>
<th>Items</th>
<th>Testosterone concentration (ng/ml)</th>
<th>Sperm cell concentration (x10⁶/ml)</th>
<th>Motility (%)</th>
<th>Live sperm (%)</th>
<th>Abnormal sperm (%)</th>
<th>Intact acrosome (%)</th>
<th>Fertility No. (%)</th>
<th>Prolificacy (kits born alive/do)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zinc supplemental source</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.89⁵</td>
<td>237⁵</td>
<td>61.8⁵</td>
<td>63.4⁵</td>
<td>16.6⁶</td>
<td>72.8⁵</td>
<td>13/20 (65)</td>
<td>4.46⁵±0.46</td>
</tr>
<tr>
<td>Zn-SO₄</td>
<td>2.46⁶</td>
<td>312²</td>
<td>83.4⁶</td>
<td>86.8⁶</td>
<td>11.2²</td>
<td>83.2⁶</td>
<td>17/20 (85)</td>
<td>5.94⁶±0.40</td>
</tr>
<tr>
<td>Zn-Met</td>
<td>2.99⁷</td>
<td>348¹</td>
<td>87.9⁷</td>
<td>88.2⁷</td>
<td>10.2²</td>
<td>88.6⁷</td>
<td>18/20 (90)</td>
<td>6.56⁷±0.39</td>
</tr>
<tr>
<td>Standerr error</td>
<td>±0.15</td>
<td>±12</td>
<td>±1.39</td>
<td>±1.35</td>
<td>±0.32</td>
<td>±1.43</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| **Time of semen collection at (1st week)** | | | | | | | | |
| 7th months of age | 2.33³ | 272³ | 73.5³ | 75.7³ | 13.9³ | 77.2³ | - | - |
| 8th months of age | 2.43³ | 295³ | 78.0³ | 80.9³ | 12.7³ | 81.4³ | - | - |
| 9th months of age | 2.58³ | 330³ | 81.6³ | 81.8³ | 11.4³ | 86.1³ | - | - |
| Standerr error | ±0.15 | ±12 | ±1.39 | ±1.35 | ±0.32 | ±1.43 | - | - |
Table 3: Pearson's correlation coefficients (r) between testosterone level and semen characteristics and reproductive performance.

<table>
<thead>
<tr>
<th></th>
<th>Testosterone concentration (ng/ml)</th>
<th>Sperm cell concentration (x10^6/ml)</th>
<th>Motility (%)</th>
<th>Live sperm (%)</th>
<th>Abnormal sperm (%)</th>
<th>Intact acrosome (%)</th>
<th>Fertility No. (%)</th>
<th>Prolificacy (kits born alive/doe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/ml)</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm cell (x10^6/ml)</td>
<td>0.344**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Motility (%)</td>
<td>0.294**</td>
<td>0.804**</td>
<td>1.000</td>
<td></td>
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<tr>
<td>Live sperm (%)</td>
<td>0.360**</td>
<td>0.783**</td>
<td>0.872**</td>
<td>1.000</td>
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<tr>
<td>Abnormal sperm (%)</td>
<td>-0.367**</td>
<td>-0.696**</td>
<td>-0.686**</td>
<td>-0.705**</td>
<td>1.000</td>
<td></td>
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</tr>
<tr>
<td>Intact acrosome (%)</td>
<td>0.406**</td>
<td>0.744**</td>
<td>0.759**</td>
<td>0.726**</td>
<td>-0.697**</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertility (%)</td>
<td>0.722</td>
<td>0.429</td>
<td>0.675</td>
<td>0.327</td>
<td>-0.976</td>
<td>0.888</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Prolificacy</td>
<td>0.223</td>
<td>0.296*</td>
<td>-0.068</td>
<td>0.142</td>
<td>0.059</td>
<td>-0.034</td>
<td>-0.189</td>
<td>1.000</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level. and ** at 0.01 level.

Our results showed that fertility rate was increased from 65% (13/20) in control group to 85 (17/20) and 90% (18/20) in bucks groups fed diet supplemented with ZnSO4 and Zn-Met, respectively. Supplementation of Zn-Met in the diet of rabbit bucks improved significantly (P<0.01) prolificacy (kits born alive/doe) (6.56±0.39), compared to the control group (4.46±0.46, Table 2). Only 58 kits born alive in control group (5.8/ buck) compared to 101 and 118 kits born alive in ZnSO4 and Zn-Met groups (10.1 and 11.8/buck, respectively). A reduced rate of fertility in same species has been attributed to a reduction of total RNA and protein content in spermatozoa which further emphasizes the vital function of zinc in polymerase activities (Hidiroglou and Knipfel, 1984). Result in Table (3) revealed positive correlation between sperm cell concentration and live sperm with fertility rate (r= 0.429 and 0.327, respectively). Generally, our results indicated a better effect of organic Zn (Zn-Met) as compared to inorganic Zn (ZnSO4) on most of the semen characteristics studied, testosterone concentration and reproductive performance. This might be due to the fact that Zn methionine has got more bioavailability than Zn sulfate. As a result, there might have been more absorption, distribution and uptake of Zn in the Zn methionine supplemented group, which accounted for its better effect over Zn sulfate. Wedekind et al. (1992) observed that the bioavailability of Zn from Zn-Met was 228% relative to ZnSO4 (100%). Also, Kumar et al. (2006) observed that supplementation of Zn in the organic form improved the semen quality of cattle bulls compared to inorganic form. Horky et al. (2011) found that feeding the organic zinc
form has a beneficial influence on the volume of ejaculate in breeding boars and hence on the production of insemination doses.

CONCLUSION
Dietary Zn supplementation in the diet of rabbit bucks improved testosterone hormone concentration and semen quality, which reflected on reproductive efficiency as compared to the control one. The organic form of Zn (Zn methionine) presented a better response in improving all traits studied as compared to the inorganic form (Zn sulfate).

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التفاعل بين أضافة فيتامين ه وانخفاضات العضوية لأكسدة ذاتية الأرانب

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١ معهد بحوث الإنتاج الحيواني، مركز البحوث الزراعية، الجيزة، مصر
٢ كلية الزراعة، جامعة القاهرة، الجيزة، مصر

تم تقسيم ثلاثين أرنب ذكر نويزيلاند أبيض (عمر 5 أشهر، المتوسط وزن الجسم ± 2،74 جرام) إلى ثلاث مجموعات تجانية. تم تغذية المجموعة الأولى على الزيت الأساسية كمجموعة ضابطة، في حين غذت المجموعات 2 و3 على الزيت نفسه ضاضفاً اليميا 100 ملجرام زنك من كبريتات الزنك (شكل غير عضوي) أو زنك ميثيلين (شكل عضوي). تم جمع ثمانية عشر قذفة من كل (ذئبة منوية / يوم، ثلاث مرات في الأسبوع الأول عند عمر 7 و 9 أشهر) وتقييمها لجودة السائل المنوي. في نهاية الفترة التجريبي، تم تزويج كل مجموعة بعشرون أنثى أرنب. اختلاف معنى في متوسط قيم خصائص السائل المنوي بين ذكور المجموعات المختلفة بعد 2 شهور من أضافة الزنك. أظهرت النتائج أن تركيز هرمون التنستريون في الدم، تركيز الزيوت وانتاج السائل المنوي الحية (%) وجسم طريقي سليمة (μm). كان أعلى معنويو (P<0.05) في مجموعات المضاعف اليميا للكبريتات في الزنك ميثيلين، 2،99 ± 0.99 نانوجرام/مل (P<0.05) و1،43 ± 0.99 يع/مل (P<0.05). نواتج السائل المنوي إيجابي، معنويو (P<0.05) في مجموعات السائل المنوي، ومعدل نجاح التبويض في العائلة المعروفة (P<0.05) في مجموعات السائل المنوي، ذو اهتمام إيجابي. تركيز هرمون التستستيرون وتركيز الزيوت وانتاج السائل المنوي الحية (%) يع/مل (P<0.05) في مجموعات السائل المنوي، ذو اهتمام إيجابي. ظل معدل الخصوبة في المجموعة الضابطة (25%) في المجموعة الضابطة إلى (20% / 17) في ذكور الجامع الذي تم تغذيةها مع أضافة كبريتات الزنك وزيوت الميثيلين، على التوالي. وتشمل النتائج ارتباط إيجابي بين زكريا مشرقي، صباح محمود علام، محمد أحمد المنيلاوي، حمدى فاروق أمين، ومحمد أحمد المنيلاوى (2003). الأثر الإيجابي على ارتباط إيجابي بين جودة السائل المنوي والخصوبة. الذي قد تكون له صلة مع أفضل الخصوبة والخليات.

Effect of dietary inorganic..