Effect of Copper Sulphate Supplementation on Semen Quality, Ovarian Activities and Reproductive Performance of Egyptian Baladi Sheep

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Abstract: The present work was performed to study the effect of dietary copper sulphate ($CuSO_4$) supplementation on the performance of ewes and rams during the period from March to December. A total number of forty Baladi (25 ewes and 15 rams) were randomly distributed into five treatment groups (5 ewes and 3 rams each). The first group was kept as a control and was fed the basal diet hay (64.2%) and barley grain (35.0%) plus minerals and vitamins (0.8%). Groups 2, 3, 4 and 5 were fed the basal diet supplemented with 5, 10, 15 and 20 mg of copper sulphate ($CuSO_4$) per kg of diet /ewe/day, respectively. Reproductive performance, some physiological and blood parameters and semen traits were studied. The results showed that increasing the levels of $CuSO_4$ in the diet had the tendency to improve oestrus response, pregnancy and lambing rates, however, the differences between the control and the supplemented groups were not significant. The litter size at lambing as well as at weaning tended to be higher in supplemented copper groups than control one, however the differences failed to reach significant. At birth, lambs born from ewes fed the highest level of Cu SO_4 (20 mg/kg) had higher average body weight than those born from ewes fed 0 (control), 5 or 10 mg/kg of Cu SO_4. At weaning, average body weight of lambs nursing ewes supplemented with 5, 10, 15, and 20 mg/kg of Cu SO_4 was significantly (P<0.05) higher than lambs of control ewes. Pre weaning gains of lambs were significantly (P<0.05) improved in the groups supplemented with 5, 10, 15, and 20 mg/kg of Cu SO_4 as compared to control group.Supplementation of copper to the dietary increased ($P < 0.05$) ejaculate volume, sperm concentration, sperm mass motility and individual sperm motility with all treated groups (5, 10, 15, and 20 mg/kg of Cu SO_4) as compared to the control group. The results showed that increasing the levels of $CuSO_4$ in the dietary had the tendency to effect packed cell volume (PCV), red blood cell count (RBC), white blood cell counts (WBC) and hemoglobin (Hb) content as compared to the control group. No significant differences were observed in mean PCV among supplemented and non supplemented CuSO4 groups. RBC and Plasma Hb values increased significantly ($P < 0.05$) with increasing Cu SO_4 levels in the dietary. In contrast, WBC values decreased ($P < 0.05$) gradually with increasing Cu SO_4 contents in the diets.

[Abd El-Monem, U.M, Peris, S.A. and Amal I.A. El-Shorbagy. **Effect of Copper Sulphate Supplementation on Semen Quality, Ovarian Activities and Reproductive Performance of Egyptian Baladi Sheep.** *J Am Sci* 2015;11(10):42-50]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 6

Keywords: Copper Sulphate; Supplementation; Semen; Ovarian; Reproductive; Egyptian Baladi Sheep

1. Introduction

Trace minerals are required in small amounts to maintain productivity, health and reproductive efficiency in farm animals. Trace minerals including copper, manganese, zinc, iodine, cobalt and selenium have been found to be very importance for normal livestock growth (Akhtar *et al.,* 2009). It has been stated that, copper plays an essential role as a micronutrient and the deficiency of this element (hypocuprosis) has a severe impact on growth as well as on reproduction in domestic animals (Abba ***et al.,*** 2000). Copper deficiency may be primary due to a lack of copper in the ration or a secondary deficiency due to reduce Cu absorption and utilization by the animals. Lower utilization of Cu has been blamed to the antagonistic minerals such as molybdenum, sulphur and iron (Pavlata *et al.,* 2005).

Growth retardation in calves from Cu deficient cows has already been observed, but in most studies (Wittenberg and Devlin, 1987 and Underwood and Suttle, 1999), Cu deficiency was secondary due to dietary molybdenum excess. Furthermore, Keles *et al.* (2006) stated that, inadequate and unbalanced feeding, specifically in Cu and Zn content, may lead to absorption disorders in the digestive system, reduction in feed efficiency and, retardation of growth in the growing heifers. On the other hand, Sprinkle *et al.* (2006) found that weaning and postweaning weights did not differ between calves nursing Cu-supplemented and control cows.

It has been found that lower copper dietary intake either prevent implantation or induce embryonic loss and fetal death in ewes (Mcchowell, *1968*).

Unanian and Feliciano-Silva (*1984*) found that copper status was lower in aborted goats and the high incidence of early abortion could be associated to copper deficiency. Moreover, Anke *et al. (*1977) identified copper deficiency in ruminants as cause of abortion. Also, based on information from 148 aborting ewes, Naziroğlu *et al.* (1998), reported that the most common cause of abortion, was the low levels of micronutrients, and among them, was low Cu concentration. However, the serum level of copper was not associated with the abortion in sheep (Aytekin and Aypak, 2011). Additionally, it was reported that Cu deficiency in cattle has been associated with delayed or suppressed estrus, reduced conception rates, infertility, and embryo death. Furthermore, the reproductive efficiency of cattle and buffaloes was decreased due to copper and phosphorus deficiencies (Pederson, 1989, Ahmed *et al.,* 2009).

Diet and feedstuffs deficient in trace minerals requirements can have deleterious effects not only on female reproduction functions, but also on male fertility. In this regard, copper deficiency can result in decreased libido, lower semen quality, and severe damage of testicular tissue may render the bull sterile. (Kreplin,1992; Nix, 2002). Additionally, the fertility of rams suffering from copper deficiency was also severely affected, as the ejaculate volume, sperm concentration, and sperm motility have been reduced. However, after the copper deficiency was reversed, the above parameters reverted to normal (Van Niekierk and Van Niekerk, 1989). In light of the relationship between trace minerals supplementation and the productive and reproductive performance of sheep, we have investigated the effect of copper supplementation as copper sulfate on 1) reproductive performance of Egyptian Baladi ram and ewe, 2) blood picture and plasma cu concentration of Egyptian Baladi ewe and 3) growth performance of their lambs.

2. Materials and Methods

2.1 Experimental location

This study was conducted at special farm in Sharkia province, Egypt located in the north part of Nile Delta (latitude 30' 01" N; longitude 31'21" E). The experiment started in March and lasted till December.

2.2. Experimental animals, Feeding and Design

2.2.1. First experiment:

Twenty five Baladi ewes aged between 1 to 2 years old weighting 36 kg average body weight. The animals were randomly allotted into 5 equal groups of 5 ewes each. Group 1 was kept as a control and was fed the basal diet hay (64.2%) and barley grain (35.0%) plus minerals and vitamins (0.8%). Groups 2, 3, 4 and 5 were fed the basal diet supplemented with 5, 10, 15 and 20 mg of copper sulphate ($CuSO_4$) per kg of diet /ewe/day, respectively.

2.2.2. Second experiment:

Fifteen Baladi rams aged between 1 to 2 years old weighting 42 kg average body weight. The animals were randomly allotted into 5 equal groups of 3 rams each. Group 1 was kept as a control and was fed the basal diet hay (64.2%) and barley grain (35.0%) plus minerals and vitamins (0.8%). Groups 2, 3, 4 and 5 were fed the basal diet supplemented with 5, 10, 15 and 20 mg of copper sulphate ($CuSO_4$) per kg of diet /ram/day, respectively.

2.2.3. Experimental Feeding and Design:

Experimental diet was formulated to meet the nutrient requirements of NRC (1985) for sheep. All ewes were healthy and clinically free of external and internal parasites. Animals were housed in semi open yards. The supplementation period was start two weeks before mating and extended thought pregnancy till occurrence of lambing. All the experimental ewes were detected for the onset of estrus two times daily 30 minute for each using intact ram. Ewes came in estrus were naturally bred by proven fertile ram. Fertility measures including estrus response, time interval between the treatment to onset of estrus, pregnancy and lambing rates were recorded. Ewes lambed indoors and lamb weights were calculated at birth and monthly till weaning (Four months). The number of lambs, sex ratio (male/female) and frequency of single, twin and mortality rate were recorded among different groups.

2.3 Serum collection and analysis

Blood samples were collected via jugular vein puncture at 14-day intervals from the beginning of the study, (pre-supplementation) till the end of the experimental period. Blood samples were allowed to clot and sera were separated by centrifugation at 3000 rpm for 15 minutes. Sera were divided into aliquots and frozen at –20oC until analysis.

2.4 Statistical analysis

Data were expressed as mean ± SEM.The data were analyzed statistically by ANOVA method and Duncan's test was used to detect differences among means using SPSS® Statistical Software (SPSS ®11.01 for Windows, 2003).

3. Results

Reproductive performance of ewe including, oestrus response, onset of oestrus, number of service per conception, pregnancy and lambing rates for control and supplemented copper groups are presented in Table 1. As shown in this table, increasing the levels of $CuSO_4$ in the dietary had the tendency to improve oestrus response, pregnancy and lambing rates, however, the differences between the control and the supplemented groups were not significant. The highest oestrus response was observed in the group supplemented with 15 mg $CuSO_4$ (100.0 %) and the lowest in control (80.0 %). Inclusion of 10, 15, and 20 mg $CuSO_4$ in the dietary improved both of pregnancy and lambing rates (100.0 %**)** compared to control

(87.5%), however, this improvement was not significant.

Results presented in Table 1 revealed that, ewes supplemented with 10, 15, and 20 mg $CuSO_4$ showed oestrus symptoms earlier (20.5 ± 0.50, 19.9 ± 0.57, 18.0 ± 0.67 days, respectively; $P<0.05$) than the control (22.6 ± 0.81 days). However, no significant difference was observed between group supplemented with 5 mg $CuSO_4$ (22.4 ± 0.54 days) and control (22.6 ± 0.81 days) with respect to onset of oestrus.

In this study, litter size at lambing as well as at weaning tended to be higher in supplemented copper groups than control one (Table 2), however, the differences failed to reach significant. At birth, lambs born from ewes fed the highest level of Cu SO_4 (20 mg/kg) had higher average body weight (4.3 ± 0.14; $P < 0.05$; Table 2) than those born from ewes fed 0 (control), 5 or 10 mg/kg of Cu SO_4 (2.6 ± 0.02, 3.1 ± 0.08, 3.4 ± 0.08 kg, respectively). Despite, there was no difference ($P > 0.05$) in average body weight of lambs of ewes fed either 15 or 20 mg/kg of Cu SO_4. At weaning, average body weight of lambs nursing ewes supplemented with 5, 10, 15, and 20 mg/kg of Cu SO_4 was significantly ($P<0.05$; Table 3) higher (13.1 ± 0.27, 13.8 ± 0.31, 15.7 ± 0.20, 17.1 ± 0.34 kg, respectively) than lambs of control ewes (10.7 ± 0.40 kg). Pre weaning gains of lambs were significantly ($P<0.05$) improved in the groups supplemented with 5, 10, 15, and 20 mg/kg of Cu SO_4 (10.0 ± 0.24, 10.4 ± 0.28, 11.5 ± 0.19, 12.8 ± 0.37 kg, respectively) as compared to control group (8.1 ± 0.38 kg).

Semen characteristics including ejaculate volume, sperm concentration, sperm mass motility, individual sperm motility and percentage of viable sperm of ram fed copper supplemented diets are shown in Table 3. As shown in this Table, all semen characteristics improved with the increase in dietary supplemented copper levels until 20 mg/kg of $CuSO_4$. For example, ejaculate volume increased ($p<0.05$) from 0.78± 0.61 ml for control group to 1.8 ± 0.03 ml for the group supplemented with 20 mg $CuSO_4$. Similarly, sperm concentration increased ($p<0.05$; Table 3) from 293.7 ± 5.8 x (10^7 x ml) for control group to 337.7± 2.3 x (10^7 x ml) for the group supplemented with 20 mg $CuSO_4$ (Table 3). Supplementation of copper to the dietary increased ($P < 0.05$; Table 3) both kind of sperm motility (sperm mass motility and individual sperm motility) with all treated groups (5, 10, 15, and 20 mg/kg of Cu SO_4) as compared to the control group. Comparing the increase in sperm motility among the four levels of supplemented copper, motility was different ($P < 0.05$; Table 3) only between the groups supplemented with 5 mg/kg and 20 mg/kg of Cu SO_4, but not different among the other groups (10, 15, 20 mg/kg of Cu SO_4) or (5, 10, 15 mg/kg of Cu SO_4).

With respect to percentage of viable sperm, results presented in Table 3 showed that percentage of viable sperm was significantly ($P<0.05$) improved in the group supplemented with 10, 15, and 20 mg $CuSO_4$ (81.0 ±1.2, 87.3 ±1.2, 92.3 ± 1.2, respectively) as compared to control group and group that received 5 mg $CuSO_4$ (72.3 ± 2.96, 77.33 ±1.5, respectively).

Haematological parameters such as packed cell volume (PCV), red blood cell count (RBC), white blood cell counts (WBC), and hemoglobin (Hb) content of ewes fed supplemented (diets 2, 3, 4 and 5) and non supplemented (diet 1) Cu SO_4 diets are shown in Table 4. No significant differences were observed in mean PCV among supplemented and non supplemented $CuSO_4$ groups. As shown in Table 4, both RBC and Plasma Hb values increased significantly ($P < 0.05$) with increasing Cu SO_4 levels in the dietary. For example, RBC values increased from 8.3 ± 0.31 to 11.3 ± 0.35 and Hb values increased from 13.0 ± 1.15 to 22.9 ± 0.70 for the control and ewes supplemented with 20 mg $CuSO_4$ groups, respectively. In contrast, WBC values decreased ($P < 0.05$; Table 4) gradually with increasing Cu SO_4 contents in the diets. These values decreased from 6.5 ± 0.26 for control to 4.97 ± 0.15 for the group supplemented with 20 mg $CuSO_4$. Plasma Cu levels increased significantly ($P < 0.05$) as dietary Cu SO_4 levels increased (Table 4).

Table 1. Effect of copper sulfate supplementation on the reproductive performance of ewe

Reproductive performance	Control	Cu 5 mg	Cu 10 mg	Cu 15 mg	Cu 20 mg
Ewe weight	27.4 ± 0.94 ^{a}	29.5 ± 0.89 ^{a}	31.1±0.64 ^{ab}	31.9±0.90 ^{b}	32.9±1.3 ^{b}
Oestrus response (%)	8 (80.0) ^{a}	9 (90.0) ^{a}	9 (90.0) ^{a}	10 (100.0) ^{a}	9 (90.0) ^{a}
Onset of estrus (days)	22.6 ± 0.81^{a}	22.4 ± 0.54^{a}	20.5 ± 0.50^{b}	19.9 ± 0.57^{b}	18.0 ± 0.67^{c}
Number of service per conception	2.4 ± 0.37^{a}	2.0 ± 0.26^{ab}	1.8 ± 0.25^{ab}	1.6 ± 0.22^{ab}	1.3 ± 0.15^{ab}
Pregnancy rate (%)	7 (87.50)^{a}	8 (88.9)^{a}	9 (100.0)^{a}	10 (100.0)^{a}	10.9 (100.0)^{a}
Lambing rate (%)	7 (87.50)^{a}	8 (88.9)^{a}	9 (100.0)^{a}	10 (100.0)^{a}	10?9 (100.0) ^{a}

Within the same row with different superscripts (a, b and c) are different (p<0.05).

Table 2. Effect of copper sulfate supplementation on the production performance of lambs up to weaning age (mean±SEM)

Traits	Control	Cu 5 mg	Cu 10 mg	Cu 15 mg	Cu 20 mg
No. of lambs born	8	11	13	15	17
No. of lambs / ewe at:					
Birth	1.14	1.38	1.44	1.50	1.70
Weaning	1.00	1.25	1.44	1.50	1.60
Sex of lambs:					
Males	4	6	7	9	9
Female	4	5	6	6	8
Weight of lambs (kg) at					
Birth*	2.6 ± 0.0^{a}	3.1 ± 0.08^{b}	3.4 ± 0.08^{b}	4.2 ± 0.09^{c}	4.3 ± 0.14^{c}
Weaning*	10.7 ± 0.4^{a}	13.1 ± 0.27^{b}	13.8 ± 0.31^{b}	15.7 ± 0.20^{c}	17.1 ± 0.34^{d}
Pre weaning gain (kg) *	8.1 ± 0.38^{a}	10.0 ± 0.24^{b}	10.4 ± 0.28^{b}	11.5 ± 0.19^{c}	12.8 ± 0.37^{d}

Within the same row with different superscripts (a, b, c, d) are different ($p<0.05$).
*BWG (body weight gain)

Table 3. Effect of copper sulfate supplementation on the semen characteristics of ram.

Semen characteristics	Control	Cu 5 mg	Cu 10 mg	Cu 15 mg	Cu 20 mg
Ejaculate volume	0.78 ± 0.61^{a}	0.98 ± 0.63^{b}	1.2 ± 0.06^{c}	1.5 ± 0.03^{d}	1.8 ± 0.03^{e}
Sperm concentration (10^7 x ml)	293.7 ± 5.8^{a}	313.3 ± 2.9^{b}	326.7 ± 4.8^{c}	332.7 ± 3.7^{c}	337.7 ± 2.3^{c}
Sperm mass motility (score 0-5)	3.13 ± 0.19^{a}	3.8 ± 0.17^{b}	4.1 ± 0.13^{bc}	4.3 ± 0.20^{bc}	4.6 ± 0.08^{c}
Sperm individual motility (%)	56.7 ± 5.0^{a}	70.0 ± 1.2^{b}	76.3 ± 1.8^{bc}	74.7 ± 2.7^{bc}	82.0 ± 4.9^{c}
Sperm live (%)	72.3 ± 2.96^{a}	77.33 ± 1.5^{ab}	81.0 ± 1.2^{b}	87.3 ± 1.2^{c}	92.3 ± 1.2^{c}

Within the same row with different superscripts (a, b) are different ($p<0.05$).

Table 4. Haematological and plasma Cu values of ewes fed varied levels of dietary copper.

Traits	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	Control	Cu 5 mg	Cu 10 mg	Cu 15 mg	Cu 20 mg
PCV %^{NS}	33.3 ± 2.96	37.3 ± 1.1	39.6 ± 0.84	39.4 ± 0.87	40.1 ± 0.41
WBC's*	6.5 ± 0.26^{a}	5.8 ± 0.14^{b}	5.7 ± 0.21^{bc}	5.3 ± 0.33^{bc}	4.97 ± 0.15^{c}
RBC's*	8.3 ± 0.31^{a}	8.8 ± 0.05^{a}	10.0 ± 0.19^{b}	11.1 ± 0.35^{bc}	11.3 ± 0.35^{c}
Plasma haemoglobin*	13.0 ± 1.15^{a}	17.0 ± 0.58^{b}	18.4 ± 0.58^{bc}	20.5 ± 0.79^{cd}	22.9 ± 0.70^{d}
Plasma CU*	0.90 ± 0.06^{a}	1.07 ± 0.02^{b}	1.13 ± 0.03^{bc}	1.14 ± 0.02^{bc}	1.22 ± 0.03^{c}

abc: Means on same row with different superscripts differ significantly ($P<0.05$).

4. Discussion

The role of copper in male reproductive performance seems to be unknown, however copper appears to be involved in sperm motility and it may act at the pituitary receptors which control the release of LH (Roychoudhury *et al.,* 2008; Eidi *et al.,* 2010). Study of Van Niekerk and Van Niekerk (1989b) showed that the testes development of rams suffering from Cu deficiency was slower than those of the control group. Also, histological examinations of the testes of the rams suffering from a severe Cu deficiency showed that the seminiferous tubules were less developed and less active than that in the control group. This was mainly due to the inactivity of the Sertoli cells which contained only a small volume of cytoplasm in Cu deficient rams.

Many studies in animals and humans found a positive correlation between Cu concentrations in blood or semen and sperm quality (Jockenhovel *et al.,* 1990; Wong *et al.,* 2001; Tvrdá *et al.,* 2012). Results of the present study showed that all semen characteristics studies including ejaculate volume, sperm concentration, sperm motility and percentage of viable sperm of ram improved significantly ($p<0.05$) with increasing copper contents in the diets. In this respect, Jockenhövel *et al.* (1990) showed significant positive correlations between human seminal plasma Cu concentrations and sperm count, motility and normal morphology. Also, Wong *et al.* (2001) showed a significant positive correlation between blood Cu concentrations and sperm motility. Additionally, Machal *et al.* (2002) observed positive association between Cu concentration in seminal plasma and ejaculate volume, sperm motility and number of sperm with progressive motility and between Cu concentration in blood, sperm count in the ejaculate, and number of sperm with progressive motility. Moreover, positive influence of Cu content in bovine seminal plasma on total and progressive sperm motility was noticed (Tvrdá *et al.,* 2012). *In vitro* study with water buffaloes concluded that addition of $CuSO_4$ (0.032 mg/l) to semen extenders led to a significant increase in sperm progressive motility, viability, membrane integrity and total antioxidant capacity during freezing processes and reduce the percentage of sperm with damaged DNA after semen freeze-thawing, which in turn, led to improve semen fertility. However, addition of higher Cu concentrations (0.064 mg/l) was detrimental to spermatozoa (Tabassomi and Alavi-Shoushtari, 2013)

The findings of other studies are however controversial in the comparison with our results. For example, Eidi *et al.* (2010) demonstrated significant negative correlations between seminal plasma Cu concentration and sperm concentration, motility, vitality and normal morphology in human semen. Also, Knazicka *et al.* (2013) found a strong negative correlation between bovine seminal plasma Cu concentration and both of percentage of motile sperm and progressive motile sperm.

Copper is an essential trace element that plays a major role as a cofactor of some enzymes such as superoxide dismutase (Cu/Zn SOD). Moreover, it is known that mammalian spermatozoa are highly susceptible to oxidative damage because they contain high quantities of polyunsaturated fatty acids and also have the ability to produce reactive oxygen species (ROS), mainly superoxide anion and hydrogen peroxide (Tvrdá *et al.,* 2012). Spermatozoa are vulnerable to the potential detrimental effects of ROS and may thus require antioxidant protection at sites of gamete production, maturation and storage and embryo implantation (Taylor, 2001; Eidi *et al.,* 2010). Superoxide dismutase plays a major role in the protection of spermatozoa from this oxidative damage.

Reproductive performance of ewe including, oestrus response, onset of oestrus, number of service per conception, pregnancy and lambing rates for control and supplemented copper groups are presented in Table 1.

As shown in this table, increasing the levels of $CuSO_4$ in the dietary had the tendency to improve oestrus response, pregnancy and lambing rates, however, the differences between the control and the supplemented groups were not significant. The highest oestrus response was observed in the group supplemented with 15 mg $CuSO_4$ (100.0 %) and the lowest in control (80.0 %). Inclusion of 10, 15, and 20 mg $CuSO_4$ in the dietary improved both of pregnancy and lambing rates (100.0 %**)** compared to control (87.5%), however, this improvement was not significant.

Results presented in Table 1 revealed that, ewes supplemented with 10, 15, and 20 mg $CuSO_4$ showed oestrus symptoms earlier (20.5 ± 0.50, 19.9 ± 0.57, 18.0 ± 0.67 days, respectively; $P<0.05$) than the control (22.6 ± 0.81 days). However, no significant difference was observed between group supplemented with 5 mg $CuSO_4$ (22.4 ± 0.54 days) and control (22.6 ± 0.81 days) with respect to onset of oestrus.

In this study, litter size at lambing as well as at weaning tended to be higher in supplemented copper groups than control one (Table 2), however, the differences failed to reach significant. At birth, lambs born from ewes fed the highest level of Cu SO_4 (20 mg/kg) had higher average body weight (4.3 ± 0.14; $P < 0.05$; Table 3) than those born from ewes fed 0 (control), 5 or 10 mg/kg of Cu SO_4 (2.6 ± 0.02, 3.1 ± 0.08, 3.4 ± 0.08 kg, respectively). Despite, there was no difference ($P > 0.05$) in average body weight of lambs of ewes fed either 15 or 20 mg/kg of Cu SO_4. At weaning, average body weight of lambs nursing ewes supplemented with 5, 10, 15, and 20 mg/kg of Cu SO_4 was significantly ($P<0.05$; Table 3) higher (13.1 ± 0.27, 13.8 ± 0.31, 15.7 ± 0.20, 17.1 ± 0.34 kg, respectively) than lambs of control ewes (10.7 ± 0.40 kg). Pre weaning gains of lambs were significantly ($P<0.05$) improved in the groups supplemented with 5, 10, 15, and 20 mg/kg of Cu SO_4 (10.0 ± 0.24, 10.4 ± 0.28, 11.5 ± 0.19, 12.8 ± 0.37 kg, respectively) as compared to control group (8.1 ± 0.38 kg).

The results of the present study showed that $CuSO_4$ supplementation had a beneficial effect on the reproductive performance of Baladi ewes as indicated by decreasing ($P < 0.05$; Table 1) number of service per conception, time interval to onset of estrus ($P < 0.05$; Table 1), and the tendency to improve all of oestrus response, pregnancy and lambing rates, however, the differences between the supplemented and the control groups were not significant for the last three parameters.

In this regard, Cu deficiency has been associated with delayed or suppressed estrus, reduced conception rates, infertility, and embryo death in cattle and buffalo (Ahmed *et al.,* 2009). Moreover, Akhtar *et al.* (2009) concluded that the deficiencies of trace minerals (copper, iron, zinc and selenium) either singly or in combination could be responsible for anoestrus condition in Nili- Ravi buffaloes and by improving the nutritional status the fertility can be improved in females of this specie. Our results showed that pregnancy and lambing rates were 100% in ewes supplemented with 10, 15, and 20 gm $CuSO_4$ compared to 87.5% in controls, however, this increasing was not significant. Ahola *et al.* (2004*) found that cows receiving trace minerals (Cu, Zn and Mn) had higher pregnancy rates than non-supplemented. In contrast, supplementation of Cu in the organic or inorganic form did not improve pregnancy rates of cows (Muehlenbein *et al.,* 2001). To our knowledge, few studies were conducted to investigate the effect of copper supplementation on reproductive performance of ewe. Therefore, we could not have a chance to compare the results obtained in our study with those in other similar studies. Van Niekerak and Van Niekerk, (1990) found that supplementation of copper had no effect on conception or fecundity of the ewes. Hemingway *et al.* (2001) concluded that the multi-trace element/vitamin ruminal bolus given to ewes before mating significantly increased the lambing percentage. The ruminal bolus was composed of copper, zinc, manganese, selenium, cobalt, iodine, vitamin A, D3 and E. Controversy, Van Niekerak and Van Niekerk, (1990) found that supplementation of copper had no effect on conception or fecundity of the ewes. The mechanism by which Cu affects female reproduction is not well established. However, studies showed that ovaries of ewes were reduced in size and have a decreased response to FSH-induced superovulation regime in the molybdenum and sulphur supplemented ewes. Whilst molybdenum-induced Cu deficiency may also have central effects via the hypothalamus-pituitary axis on LH secretion leading to reduced ovarian oestradiol secretion and absence of estrus in animals (Kendall *et al.,* 2006). Copper acts at the level of hypothalamus through the modulation of neural activity, modification of GnRH granules stability and modulation of neurohormone release (Michaluk and Kochman, 2007), these authors revealed that copper complexes with GnRH are more effective than native GnRH in the release of LH and FSH from the anterior pituitary *in vivo*. In addition, Cu plays also a significant role in maintaining normal fetus development in mammals.

Litter size and birth weight are among the most important economic traits in several species of multiparous mammals. The three major factors that contribute to litter size are ovulation rate, embryonic survival, and fetal survival (Martin *et al.,* 2004). Moreover, it was observed that low birth weight negatively related to lambs' survival (Fogarty *et al.,* 2000). The present study showed that, litter size at lambing as well as at weaning tended to be higher in supplemented copper groups than control. At birth, lambs born from ewes fed the highest level of Cu SO_4 (20 mg/kg) had higher average body weight than those born from ewes fed 0 (control), 5 or 10 mg/kg of Cu SO_4. At weaning, average body weight of lambs nursing ewes supplemented with 5, 10, 15, and 20 mg/kg of Cu SO_4 was significantly higher than lambs of control ewes. Pre weaning gains of lambs were significantly ($P<0.05$) improved in all supplemented copper groups as compared to control group. In this regard, Hill *et al.* (1969) found significant improvement in twinning rate for ewes treated with selenium or selenium plus copper, however, copper alone did not induce any improvement. These authors assumed that, such improvement in twinning rate was due to the improved live weight and hence ovulation rate of treated ewes, rather than being a direct effect on reproductive physiology. Additionally, sows fed high Cu diets farrowed larger litters of pigs and the pigs were heavier at birth (Cromwell *et al.,* 1993; Adu *et al.,* 2011). Hemingway *et al.* (2001) reported that, the multi-trace element/vitamin ruminal bolus administered to ewes before mating period improved lambing percentage. The ruminal bolus was composed of copper, selenium, cobalt, iodine, manganese, zinc, vitamin A, D3 and E. For birth weight, Berger (2004) found that birth weight was increased with increasing copper concentration in pig. On the other hand, nutritional supplement containing vitamins, amino acids and minerals (including copper) did not improve litter size or birth weights of lambs (Kara *et al.,* 2010).

Keles *et al.* (2006*) stated that, inadequate and unbalanced feeding, specifically in Cu and Zn content, may lead to absorption disorders in the digestive system, reduction in feed efficiency and in the thyroid hormones, which are the stimulator of growth hormone, retardation of growth in the growing heifers. Cromwell *et al,* (1993) found that, feeding high dietary Cu (250 ppm) to sows increased birth and weaning weights of their pigs.

The increase in pig weaning weight in the high Cu treatment may be due to the pharmacological effects of Cu and its potential effect on milk production in sows. Moreover, Arthington et al. (1995) evaluated the effect of Cu bolus administration before weaning. The authors found that, weaning weights were heavier in calves that received supplemental Cu compared with unsupplemented controls. On the other hand, Sprinkle *et al.* (2006) found that weaning and postweaning weights did not differ between calves nursing Cu-supplemented and control cows.

It has been reported that copper produces a growth promoting effects through the microbial gut flora (Shurson *et al.,* 1990). The mechanisms involved remain not well understood. Shurson *et al.* (1990) found a positive effect of high concentration of copper (283ppm) in the diet on the daily growth rate and feed conversion rate in pigs. Furthermore, the addition of 200 ppm copper to weanling pig diets improved growth rate and feed intake, however, 400 ppm copper depressed growth and feed intake (Kornegay *et al.,* 1989). In rabbit, (Adu and Egbunike, 2010; Adu *et al.,* 2010) showed that daily weight gain, final weight and feed to gain ratio increased significantly with the increase in dietary supplemented copper levels until 200 ppm. Copper is believed to stimulate growth due to its bacteriostatic properties, which are similar to those of antibiotics (Kornegay *et al.,* 1989).

Evaluation of hematological parameters showed that, plasma Cu, RBC and hemoglobin concentration increased significantly ($P < 0.05$) with increasing $CuSO_4$ levels in the dietary. These findings are in agreement with those recorded in sheep and goats (Islam *et al.,* 2005), cattle (Kegley and Spears, 1994), rabbits (Bassuny, 1991) and broiler chicken (Samanta *et al.,* 2011). Increasing RBC and Hb values could be as a result of the subsequent production of more copper transporting protein ceruloplasmin, which is required for normal RBC formation by allowing more iron absorption from the small intestine and release of iron in the tissue into the blood plasma as observed by Cromwell *et al.* (1989).

Kegley and Spears (1994), found an increase in plasma Cu concentrations of growing cattle when the level of Cu supplementation in calves was increased to 30 mg of daily Cu intake from both $CuSO_4$ and Cu lysine.

Eckert *et al*. (1999) found that ewes fed 20 mg/kg Cu had lower PCV, RBC, and Hb than those fed 10 or 30 mg/kg Cu diets. On contrast, Adu and Egbunike (2010) and Adu *et al.* (2010) reported that, the supplementation of feeds with Cu for rabbits had no significant effect on haematological parameters i.e. Hb, PCV, RBC. Furthermore, hemoglobin concentration was depressed for pigs fed diets supplemented with 400 ppm Cu compared with those fed 200 ppm or the control (Kornegay *et al.,* 1989). The reduction in Hb concentration at high Cu level could be due to reduced absorption of iron from the gut.

White blood cells decreased ($P < 0.05$) gradually with increasing $CuSO_4$ contents in the diets. This result agreed with the findings of Murawski *et al.* (2006) who found a reduction in WBC in ewes treated with 50 mg of copper for 30 days. Similar results with significant reduction of WBC in rabbits exposed to different levels of copper have been reported previously by Adu and Egbunike, (2010) and Ojiezeh *et al.* (2011). However, Adu *et al.* (2010) reported that diets supplemented with copper had no effects on WBC values when fed to rabbits. The reduction in WBC observed in our study may be due to its defense nature in fighting the inclusion of copper and /or probably due to the low or the slow rate of production in the bone marrow (Adu and Egbunike, 2010).

References

1. Abba M, De Luca JC, Mattioli G, Zaccardi E, Dulout FN. Clastogenic effect of copper deficiency in cattle. Mutat. Res. 2000; 466: 51-55.
2. Adu OA, Akinfemi A, Adebiyi OA. Effect of varied levels of dietary copper on performance and blood chemistry of growing female rabbits. Tropical and Subtropical Agroecosystems. 2010;12:313-319.
3. Adu OA, Egbunike GN. Enhancing growing rabbits performance with diets supplemented with copper. Advances in Biological Research 2010; 4: 18-22.
4. Adu OA, Omoleye OS, Adebiyi OA, Asolo OH. Influence of dietary copper supplementation of puberty boars on the fertility of sows. Elixir Agriculture. 2011; 36: 3168-3170.
5. Ahmed WM, El Khadrawy HH, Hanafi EM, Abd El Hameed AR, Sabra HA. Effect of copper deficiency on ovarian activity in Egyptian buffalo-cows. World J. Zool. 2009; 4:1-8.
6. Ahola JK, Baker DS, Burns PD, Mortimer RG, Enns RM, Whittier JC, Geary TW, Engle TE. Effect of copper, zinc, and manganese supplementation and source on reproduction, mineral status, and performance in grazing beef cattle over a two-year period. J Anim. Sci. 2004; 82: 2375-2383.
7. Akhtar MS, Farooq AA, Mushtaq M. Serum concentrations of copper, iron, zinc and selenium in cyclic and anoestrus Nili–Ravi buffalos kept under farm conditions. Pakistan Vet. J. 2009; 29: 47-48.
8. Anke M, Henning A, Grun M, Partschefeld M, Groppel B. Influence of Mn, Zn, Cu, I, Se, Mo and Ni deficiencies on the fertility of ruminants. Mathematics- Naturwissensch of liche-Reihe. 1977; 26:283–292.
9. Arthington JD, Larson RL, Corah LR. The effects of slow-release copper boluses on cow reproductive performance and calf growth. Prof. Anim. Sci. 1995; 11:219–222.
10. Aytekin I, Aypak SU. Levels of selected minerals, nitric oxide, and vitamins in aborted Sakis sheep raised under semitropical conditions. Trop. Anim. Health Prod. 2011; 43:511-514.
11. Bassuny SM. The effect of copper supplementation on rabbit performance under Egyptian conditions. J. of Appl. Rabbit Res. 1991; 14: 93-97.
12. Berger LL. Trace minerals and reproduction in livestock. Salt and Trace Minerals. 2004, 36: 1-4.

13. Cromwell GL, Monegue HJ, Stahly TS. Long-term effects of feeding a high copper diet to sows during gestation and lactation. J. Anim. Sci. 1993; 71:2996-3002.
14. Cromwell GL, Stahly TS, Monegue HJ. Effects of source and level of copper on performance and liver copper stores in weanling pigs. J Anim Sci. 1989; 67: 2996-3002.
15. Eckert GE, Greene LW, Carstens GE, Ramsey WS. Copper status of ewes fed increasing amounts of copper from copper sulfate or copper proteinate. J Anim Sci. 1999; 77:244-249.
16. Eidi M, Eidi A, Pouyan O, Shahmohammadi, Fazaeli R, Bahar M. Seminal plasma levels of copper and its relationship with seminal parameters. Iranian Journal of Reproductive Medicine. 2010; 8: 60-65.
17. Fogarty NM, Hopkins DL, van der Van R. Lamb production from diverse genotypes, 1. Lamb growth and survival and ewe performance. Anim Sci. 2000; 70:135-145.
18. Hemingway RG, Parkins JJ, Ritchie NS. Enhanced reproductive performance of ewes given a sustained-release multi-trace element/vitamin ruminal bolus. Small Ruminant Research. 2001; 39:25-30.
19. Hill MK, Walker SD, Taylor AG. Effects of "marginal" deficiences of copper and selenium on growth and productivity of sheep. New Zealand Journal of Agricultural Research. 1969; 12: 261-270.
20. Islam R, Rashid SMH, Hossain MK, M. Rahman M. Effects of Hematinics on body weight and some hematological values in sheep and goats. Int. J. Agri. Biol. 2005; 7:582-584.
21. Jockenhovel F, Bals-Pratsch M, Bertram HP, Nieschlag E. Seminal lead and copper in fertile and infertile men. Andrologia. 1990; 22:503-511.
22. Kara C, Orman A, Topal E, Carkungoz E. Effects of supplementary nutrition in Awassi ewes on sexual behaviors and reproductive traits. J. Biol. Environ. Sci. 2010; 10:15-21.
23. Kegley EB; Spears JW. Bioavailability of feed-grade copper sources (oxide, sulfate, and lysine) in growing cattle. J. Anim. Sci. 1994; 72:2728-2734.
24. Keles I, Donmez N, Altug N, Ceylan E. Serum zinc, copper and thyroid hormone concentrations in heifers with retarded growth. YYU. Vet. Fak. Derg. 2006; 17: 103-105.
25. Kendall NR, Marsters P, Guo L, Scaramuzzi RJ, Campbell BK. Effect of copper and thiomolybdates on bovine theca cell differentiation *in vitro.* J Endocrinol. 2006;189:455-463.
26. Kendall NR, Marsters P, Scaramuzzi, Campbell BK. Expression of lysyl oxidase and effect of copper chloride and ammonium tetrathiomolybdate on bovine ovarian follicle granulosa cells cultured in serum-free media. Reproduction 2003; 125:657-665.
27. Knazicka Z, Lukacova J, Gren A, Formicki G, Massanyi P, Lukac N. Relationship between level of copper in bovine seminal plasma and spermatozoa motility. Journal of Microbiology, Biotechnology and Food Sciences. 2013; 2: 1351-1362.
28. Kornegay ET, van Heugten PHG, Lindemann MD, Blodgett DJ. Effects of biotin and high copper levels on performance and immune response of weanling pigs. J Anim. Sci. 1989; 67:1471-1477.
29. Kreplin C. (1992). Breeding soundness of bulls. Practical information for Alberta's agriculture industry, Agri facts. http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex3545.
30. Machal L, Chladek G, Strakova E. 2002. Copper, phosphorus and calcium in bovine blood and seminal plasma in relation to semen quality. Journal of Animal and Feed Sciences. 2002; 11:425-435.
31. Martin GB, Milton JTB, Davidson RH, Banchero Hunzicker GE, Lindsay DR, Blache D. Natural methods for increasing reproductive efficiency in small ruminants. Anim Rep Sci. 2004; 82–83: 231-246.
32. McChowell, JA, 1968. The effect of experimental copper deficiency on growth, reproduction and haemopoieses in the sheep. Vet. Rec. 1968; 83:226-227.
33. Michaluk A, Kochman K. Involvement of copper in female reproduction. Reprod. Biol. 2007;7:193-205.
34. Muehlenbein EL, Brink DR, Deutscher GH, Carlson MP, Johnson AB. Effects of inorganic and organic copper supplemented to first-calf cows on cow reproduction and calf health and performance. J. Anim. Sci. 2001; 79:1650-1659.
35. Murawski M, Bydłoń G, Sawicka-Kapusta K,Wierzchoś E, Zakrzewska M, Włodarczyk S, Molik E, Zięba D. The effect of long term exposure to copper on physiological condition and reproduction of sheep. Reprod Biol. 2006; 6:201-206.
36. Naziroglu M, Cay M, Karataş F, Cimtay I, Aksakal M. Plasma levels of some vitamins and elements in aborted ewes in Elazig region. Turkish Journal of Veterinary and Animal Sciences. 1998; 22:171-174.

37. Nix J, 2002. Trace minerals important for goat reproduction. Sweetlix Livestock Supplement System.www.sweetlix.com/media/ documents/articles/Goat/013.pdf.
38. Ojiezeh TI, Ibeh NI, Okoko FJ. Effects of cations on the haematological indices of *salmonella enterica* challenged rabbits. Tanzania Journal of Natural and Applied Sciences. 2011; 2: 304 -309.
39. Pavlata L, Podhorsky A, Pechova A,Chomat P. Differences in the occurrence of selenium, copper and zinc deficiencies in dairy cows, calves, heifers and bulls. Vet. Med. -Czech. 2005; 50:390-400.
40. Pederson CH. Infertility and disease surveillance using a milk recording scheme in the Sahiwal district of Pakistan. Trop. Anim. Health Prod. 1989; 22:263-272.
41. Roychoudhury S, Slivkovf J, Bulla, J, Massinyi P. Copper administration alters fine parameters of spermatozoa motility *in vitro*. Folia Veterinaria. 2008; 52:64-68.
42. Samanta B; Ghosh PR; Biswas A; Das SK. The effects of copper supplementation on the performance and hematological parameters of broiler chickens. Asian Aust. J. Anim. Sci. 2011; 24: 1001-1006.
43. Shurson GC; Ku PK, Warler GL, Yokoyama MT, Miller ER. Physiological relationships between microbiological status and dietary copper in the pig. J. Anim. Sci. 1990; 68: 1061-1071.
44. Sprinkle JE, Cuneo SP, Frederick HM, Enns RM, Schafer DW, Carstens GE, Daugherty SB, Noon TH, Rickert BM, Reggiardo C. Effects of a long-acting, trace mineral, reticulorumen bolus on range cow productivity and trace mineral profiles. J Anim Sci. 2006; 84:1439-1453.
45. Tabassomi M, Alavi-Shoushtari SM. Effects of in vitro copper sulphate supplementation on the ejaculated sperm characteristics in water buffaloes (Bubalus bubalis). Veterinary Research Forum. 2013; 4: 31-36.
46. Taylor CT. Antioxidants and reactive oxygen species in human fertility. Environmental Toxicology and Pharmacology. 2001; 10: 189-198.
47. Tvrda E, Knazicka Z, Lukacova J, Schneidgenova M, Massanyi P, Goc Z, Stawarz R, Lukac N. Relationships between iron and copper content, motility characteristics and antioxidant status in bovine seminal plasma. Journal of Microbiology, Biotechnology and Food Sciences. 2012; 2: 536-547.
48. Unanian MD, Feliciano-Silva AE. Trace elements deficiency: association with early abortion in goats. International Goat and Sheep Research. 1984; 2: 129-134.
49. Underwood EJ, Suttle NF, 1999: The Mineral Nutrition of Livestock. 3rd edn, CABI Publishing, Wallingford.
50. Van Niekerak FE, van Niekerk CH. Copper and selenium supplementation of ewes grazing on pastures low in copper and selenium: Effect on reproduction and concentration of plasma copper and blood selenium during pregnancy. S.-Afr Tydskr.Veek. 1990; 20: 246-249.
51. Van Niekerk FE, Van Niekerk CH. The influence of experimentally induced copper deficiency on the fertility of rams. I. Semen parameters and peripheral plasma androgen concentration. J. South Afr. Vet. Assoc.1989a; 60: 28-31.
52. Van Niekerk FE, Van Niekerk CH. The influence of experimentally induced copper deficiency on the fertility of rams. II. Macro- and Microscopic changes in the testes J. South Afr. Vet. Assoc. 1989b; 60: 32-35.
53. Wittenberg KM, Devlin TJ. Effects of dietary molybdenum on productivity and metabolic parameters of lactating beef cows and their offspring. Canadian Journal of Animal Science 1987; 67:1055-1066.
54. Wong WY, Flik G, Groenen PM, Swinkels DW, Thomas CM. The impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on semen parameters in men. Reprod Toxicol. 2001; 15:131-136.

9/8/2015