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Concentration of Zinc and Copper in Milk and Some Reproductive Aspects During Hot and Cold Seasons of Klebi Ewes Reared in South Egypt

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ABSTRACT

The present study was carried out to evaluate concentrations of zinc and copper in milk, some reproductive aspects of Klebi ewes during the hot and cold seasons in south Egypt. According to season of lambing, 48 ewes lambed in the cold months, while 47 ewes lambed during hot months were used in this study. Results showed that concentrations of zinc and copper in Klebi ewe milk were higher (2.23±0.3 and 0.48±0.05 mg/l) in cold than in hot months (1.33±0.2 and 0.2±0.03 mg/l, P<0.05), respectively. Lambing (P≥0.05) and twining (P<0.05) rates tended to be higher in cold than in hot months (122.9 and 106.4 and 37.3 and 12.0%, respectively). Stillbirth, abortion and retained placenta rates were more frequent in ewes lambed in hot than in cold months (8, 6, 4.3 vs. 5, 4, 2.1%, P≥0.05), respectively. Positive correlation coefficient (P<0.05) between each of zinc or copper concentration in Klebi ewe milk and both lambing and twining rates (r=0.615, 0.452, 0.631 and 0.684, respectively). Negative correlation coefficient (P<0.05) was recorded between each of zinc and copper concentration in Klebi ewe milk and stillbirth, abortion and retained placenta rates. The current study demonstrated seasonal changes in concentration of zinc and copper in Klebi ewe milk, which were associated with pronounced alteration in reproductive process of Klebi ewes. Reproductive performance of Klebi ewes in cold was better than in hot season. Therefore the present study may conclude that dietary supplementation of zinc and copper, particularly during the hot season is recommended for improving reproductive aspects of Klebi ewes under environmental conditions of south Egypt.

***Keywords*:** Klebi ewes, lambing season, milk, zinc, copper, reproduction.

INTRODUCTION

Sheep contribute to 1.3% from the total world milk production, according to FAO (2010). Majority of the sheep population in Egypt is in small holders (Ministry of agriculture, personal communication), most of the small holders depend on grazing for feeding their herds. Under extensive grazing system, the reproduction process of sheep was affected by nutrient availability, especially mineral contents of the forages. Machado *et al.* (2005) and Meeske *et al.* (2006) found fluctuation in total mineral content of ryegrass/clover pastures during different seasons. Michlova *et al.* (2016) reported that variability of the content of minerals in small ruminant milks related to the quality of feed and pasture, which is associated with fluctuations in vegetation and climate conditions.

The environmental conditions of south Egypt are characterized by widely seasonal variation, especially the ambient temperature (AT). The ambient temperature reached to 44.3 ^{o}C in hot and dropped sharply to 8.8 ^{o}C) in cold months, particularly at night. This fluctuation in seasonal conditions reflected seriously on the quality and quantity of the pasture and the green fodders which consequently greatly reflected on the animal performance. The Klebi sheep is one of the local breeds that reared in south Egypt for meat production. Many investigations interested with the role of zinc and copper on the reproductive performance of sheep (Hemingway *et al.*, 2001; Vázquez-Armijo *et al*., 2011; Abd-El-Monem *et al*., 2015). The physiological functions of zinc is component of numerous metallo enzymes, influences transcription and cell replication and the deficiency lead to impaired spermatogenesis and development of secondary sex organs in males, reduced fertility in sheep and goats (Vázquez-Armijo *et al*., 2011). While, copper is enzyme component and catalyst involved in steroidogenesis and prostaglandin synthesis and its deficiency lead to delayed and depressed estrus, abortion, death fetuses, infertility, and congenital ataxia (Vázquez-Armijo *et al*., 2011). Sales *et al.* (2011) reported that zinc and copper play main role in regulating progesterone production from corpus luteum via enzyme superoxide dismutase of crossbred heifers. Ceylan *et al*. (2008) reported that zinc effect on the secretion and function of testosterone by the enzymes that control the arachidonic acid. Zinc plays major role in the repair and maintenance of the uterine layer after parturition, which is necessary for embryo implantation (Hostetler *et al*., 2003; Robinson *et al*., 2006). Deficiency of copper in sheep diets lead to embryo loss, failures embryo implantation and fetal death (Hidiroglou, 1979).

Recently many investigation determined concentrations of minerals in milk of experimental animals as mirror of blood (Ranjith and Pandey, 2015; Farzad *et al.*, 2016; Michlova *et al.*, 2016). The present study aimed to spot light on concentration of zinc and copper in milk of Klebi sheep reared under traditional conditions (small holders and grazing system) and their relationships with some reproductive aspects under conditions of south Egypt.

MATERIALS AND METHODS

Location and climatic conditions:

This study was carried out in Aswan governorate; it is far from Cairo city by about 890 km. Sheep farm located in Kom Ombou city. The climatic conditions of Aswan governorate are famous for dry weather. The months of the year were divided into two seasons cold (November to April) and hot (May to October). Throughout the experimental period, ambient temperature, relative humidity and rainfall of cold and hot seasons were collected from Meteorological Authority and presented in Table (1).

Table 1. Ambient temperature (^{o} C), relative humidity (%) and rainfall of cold and hot seasons throughout the experimental period.

Season	Ambient Temperature (^{o} C) Max.	Min.	Relative Humidity (%)	Rainfall mm./month
Hot	35.9 – 44.3	20.8 – 26.2	16 – 26	0.0 – 1.0
Cold	22.9 - 35.0	8.8 - 19.1	18 – 40	0.1 – 1

Animals and management:

A total number of 50 Klebi ewes were used in the present study. The age of animals ranged between 2 to 4 years and the parity ranged between 3rd and 5th. Live body weight of ewes ranged between 30 and 40 kg. Total of 48 ewes lambed in the cold months and 47 ewes lambed during the hot months. Animals were reared in traditional farm as semi-shaded yards. Ewes were fed on concentrate feed mixture (corn and wheat bran) at night and in the morning on residues of crops and plants for each season.

Reproductive aspects:

Reproductive parameters included: Rate of lambing, stillbirth, abortion, retained placenta and twining) were recorded according to Tadeg *et al*. (2015).

Lambing rate (%) = Number of lambs born/total number of lambed ewes. Stillbirth rate (%) = Number of lambs stillborn/total number of lambs born.

Abortion rate (%) = Number of ewes aborted/total number of pregnant ewes.

Retained placenta rate (%) = Number of ewes with retained placenta (≥12 hr)/number of lambed ewes.

Twining rate (%) = Number of twins/total number of lambs born

Determination of zinc and copper:

Milk samples were collected from 48 ewes lambed in the cold months and 47 ewes lambed in hot months. Milk samples were taken at 6a.m. before drinking, after two weeks of post-partum period. The samples were analyzed quantitatively for zinc (Zn) and copper (Cu) based on the procedure outlined by the manufacture using atomic absorption (AA) flame (ICE 3000C113500040 v1.30, England) according to AOAC (2000).

Statistical analysis:

Statistical model included the effect of season of the year on different parameters by T. test using SAS (2002). Chi Squire was used to compare between the percentage values, while person's correlation coefficients were calculated. The used model was:

$$\mathbf{Y_{ij} = \mu + B_i + e_{ij}}$$

Where**:** Y_{ij} = = the observations, μ = overall mean, B_i =effect of lambing season (cold =1, hot = 2) and e_{ij} = experimental error assumed to be randomly distributed $(0, \partial^2)$.

RESULTS AND DISCUSSION

Concentration of Zn and Cu in Klebi ewe milk:

Overall mean of zinc and copper concentrations in Klebi sheep milk was 1.78±0.3 and 0.35±0.04 mg/l, respectively (Table, 2). The obtained value of zinc is within a range of 1.29 and 3.09 mg/l as reported by Khan *et al*. (2007) and Al-Wabel (2008), respectively. Meanwhile, the concentration of copper is within a range of 0.20 and 0.40 mg/l as reported by Barłowska *et al.* (2013) and El-Bagermi *et al*. (2014), respectively. The concentration of zinc and copper was lower than 24.14 and 0.63 mg/l (Michlova *et al*., 2016), while was higher than 0.79 and 0.212 mg/l (Abed-Al-Helaly *et al*., 2013), respectively.

Concentrations of zinc and copper in Klebi sheep milk are significantly (*P <0.05*) higher in cold (2.23±0.3 and 0.48± 0.05 mg/l) than in hot months (1.33±0.2 and 0.21±0.03 mg/l), respectively (Table 2). This result agreed with that reported by Khan *et al*. (2003), who found that concentration of zinc in sheep milk during the cold season was higher (1.29 mg/l) than in hot season (0.56 mg/l). In similar trend, Dar *et al*. (2014) found that concentrations of zinc and copper in ewe blood plasma in cold season was higher (1.17 and 1.38 mg/l) than in hot season (0.69 and 0.68 mg/l), respectively.

The present result is in agreement with that reported by Farzad *et al*. (2016), who found the grazing season was effective on variations of milk composition of mineral contents in Moghani sheep. They attributed the differences in milk mineral content to the soil and grazing plant. In addition, Haenlein (1996) reported that lactation, season, feeding and health of the animal cause difference in the composition of milk macro- or micro-elements. Suttle (2010) reported that some variables affect plant mineral composition such as plant genotype, soil environment and climate**.**

Table 2. Concentration (mg/l) of Zn and Cu in Klebi ewe milk during hot and cold months (mean ± SE).

Element	Hot months	Cold months	Overall mean
Zn (mg/l)	1.33 ± 0.212^{a}	2.23 ± 0.343^{b}	1.78 ± 0.323
Cu (mg/l)	0.21 ± 0.032^{a}	0.48 ± 0.052^{b}	0.35 ± 0.041

$^{a, b}$: values within the same row having different superscripts are significantly different at (*P <0.05*).

Effect of season of lambing on some reproductive aspects of Klebi ewes:

Lambing rate of Klebi ewes was 114.7% (Table 3). The present rate, was higher than that reported by Koyuncu and Yerlikaya (2007) and Aldomy *et al*. (2009), they found that the lambing rate ranged between 96.7 and 100%. Meanwhile the present values are lower than that reported by Teleb *et al.* (2009), being 150% in Saidi ewes. Lambing rate of Klebi ewes was higher in cold (122.9%) than in hot months (106.4%), but the differences were not significant (Table 3). The obtained result is in agreement with that reported by Teleb *et al.* (2009), who found that lambing rate was higher (160%) in winter than in summer season (140%) in Saidi ewes. Similar trend was reported by Koycegiz *et al. (*2009), who found that lambing rate during cold months was (127%) and in hot one (126%).

Percentage of stillbirth in Klebi ewes was 6.5% (Table, 3). These results are in agreement with the findings of Teleb *et al.* (2009), who reported that 6.1% of lambs were stillbirth in Saidi ewes. The obtained result is lower than that reported by Casellas *et al*. (2014), who found that stillbirth percentage was (9.6%) in lambs under semi-intensive system. Percentage of stillbirth of Klebi ewes was lower in cold (5%) than in hot months (8%), but the difference was not significant (Table 3). This result agreed with that reported by Teleb *et al.* (2009), who found that stillbirth was higher in summer (12.2) than in winter (1.5%) in Saidi ewes. Abortion rate of Klebi ewes was 5% (Table 3). The present result was lower than 7.5% as reported by Aldomy *et al*. (2009) and 7.6% (Zahraddeen *et al*., 2010). Abortion rate was lower (4%) in cold than in hot months (6%), but the differences were not significant. In this respect, Miller *et al*. (1988) reported that deficiency of copper is one of the important factors responsible for early embryonic death. O'Donoghue and Boland (2002) found that lower zinc levels in the diet was associated with decreased in fertility and increase abortions in cattle. Percentage of retained placenta of Klebi ewes was 3.2% (Table 3). This result is lower than that reported by Hussain *et al*. (2013) and Alenyorege and Mensah (2015), who found the percentage of retained placenta in ewes ranged between 10.5 and 30.4%. Retained placenta percentage of Klebi ewes is higher (4.3%) in hot than in cold months (2.1%), while the differences was insignificant. The present result agreed with that reported by Alenyorege and Mensah (2015), who found that incidence of retained placenta was higher (36.3%) in hot than in cold months (34.5%). Campbell *et al*. (1999) reported that decrease in retained placentas in dairy cows fed diet supplemented with zinc and copper.

Twining rate of Klebi ewes was 25.7% (Table 3). The obtained result is higher than that reported by Aldomy *et al*. (2009) and Hussain *et al*. (2013), being 5 and 15%). Twining rate of Klebi ewes was significantly (P<0.05) higher in cold (37.3%) than in hot months (12%). This result agreed with that reported by Teleb *et al.* (2009), who found twining rate in Saidi ewes was 63.6 and 40.8 in cold and hot season, respectively.

Table 3. Percentage of some reproductive aspects of Klebi ewes during hot and cold months.

Rate (%)	Hot months	Cold months	Overall mean
Lambing	106.4 (50/47)	122.9 (59/48)	114.7 (109/ 95)
Stillbirth	8 (4/50)	5 (3/59)	6.5 (7/109)
Abortion	6 (3/50)	4 (2/50)	5 (5/100)
Retained placenta	4.3 (2/47)	2.1 (1/48)	3.2 (3/95)
Twining	12[a] (6/50)	37.3[b] (22/59)	25.7 (28/109)

[a,b]:values within the same row having different superscripts are significantly different at (*P <0.05*).

The correlation between concentration of zinc in Klebi ewe milk and reproductive aspects:

The physiological functions of zinc is component of numerous metalloenzymes, influences transcription and cell replication and the deficiency lead to impaired spermatogenesis and development of secondary sex organs in males, reduced fertility in sheep and goats (Vázquez-Armijo *et al*., 2011). Positive correlation coefficient (P<0.05) was recorded between each of lambing and twinning rates and concentration of zinc in Klebi ewe milk. While negative correlation was observed between each of stillbirth, abortion and retained placenta rates and concentration of zinc in Klebi ewe milk (Table, 4).

The present results are in agreement with that reported by Vázquez-Armijo *et al.* (2011), who found that dietary Zn supplementation improved reproductive performance of ewes. Also, El-Nour *et al.* (2010) reported that **c**onception rate of Baladi goats was increased from 60 to 80% after Zn treatment. In addition zinc plays major role in maintenance the epithelia layer of the reproductive organs, which is necessary for embryo implantation (Hostetler *et al*., 2003; Robinson *et al*., 2006). Zn deficiency affect the reproductive process in males and females like spermatogenesis, secondary sex organs development, estrus and gestation (Smith and Akinbamijo, 2000).

Table 4. Correlation coefficients between concentration of zinc in Klebi ewe milk and reproductive aspects.

Element	Reproductive aspect rates (%)				
	Lambing	Stillbirth	Abortion	Retained placenta	Twining
Zn	0.615*	-0.739*	-0.327	-0.497	0.452

*** values with having superscripts are significantly different at (P<0.05).**

The correlation between concentration of copper in Klebi ewe milk and reproductive aspects:

The physiological functions of copper is enzyme component and catalyst involved in steroidogenesis and prostaglandin synthesis and the deficiency lead to delayed and depressed estrus, abortion, death fetuses, infertility, congenital ataxia (Vázquez-Armijo *et al*., 2011). Positive correlation coefficient (P <0.05) was observed between each of lambing and twinning rates and concentration of copper in Klebi ewe milk. While negative correlation was observed between each of stillbirth, abortion and retained placenta and concentration of copper in Klebi ewe milk (Table, 5). The obtained results are in agreement with that reported by Abd El-Monem *et al*. (2015), who found that supplementation of copper to the diet of Baladi ewes improved estrus response, pregnancy, lambing and twinning rates. Vázquez-Armijo *et al.* (2011) found that supplementation of copper to diet of sheep affected reproductive process, like expression of estrus, embryo implantation and reduction in spermatogenesis. Ahmed *et al.* (2009) found that deficiency of copper has been associated with delayed estrus, low conception rates, infertility, and embryonic mortality in cattle and buffalo. Supplementation of copper and zinc to diets during spring and summer seasons improved the physiological status of sheep (Dar *et al*. 2014). Sales *et al*. (2011) reported that zinc and copper play main role in regulating progesterone production from corpus luteum via enzyme superoxide dismutase of crossbred heifers. Deficiency of copper in sheep diets lead to embryo loss, failures embryo implantation and fetal death (Hidiroglou, 1979).

Table 5. Correlation coefficient between concentration of copper in Klebi ewe milk and reproductive aspects.

	Reproductive aspects rates %				
Element	**Lambing**	**Stillbirth**	**Abortion**	**Retained placenta**	**Twining**
Cu	0.631*	-0.837*	-0.709*	-0.815*	0.684*

*values with having superscripts are significantly different at (P <0.05).

CONCLUSION

The current study demonstrated seasonal changes in concentration of zinc and copper in Klebi ewe milk, which were associated with pronounced alteration in reproductive process of Klebi ewes. Reproductive performance of Klebi ewes in cold was better than in hot season. Therefore the present study may conclude that dietary supplementation of zinc and copper, particularly during the hot season is recommended for improving reproductive aspects of Klebi ewes under environmental conditions of south Egypt.

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تركيزات الزنك والنحاس في اللبن وبعض المظاهر التناسلية خلال المواسم الحاره والبارده لنعاج الكليبى و المرباه فى جنوب مصر

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أجرى هذا البحث لدراسة تركيزات الزنك و النحاس فى لبن نعاج الكليبى و بعض المظاهر التناسلية للنعاج تحت الظروف البيئية فى جنوب مصر خلال مواسم السنة. تم تقسيم السنة إلى موسمين بارد وحار. بناء على موسم الولادة تم تقسيم النعاج الى مجموعتين الأولى هى مجموعة النعاج التى ولدت خلال الموسم البارد , والثانية النعاج التي ولدت خلال الموسم الحار. أظهرت النتائج ان تركيزات الزنك والنحاس كان أعلى معنويا فى عينات اللبن المأخوذة من نعاج الكليبى خلال الموسم البارد ٢.٢٣ ± ٠.٣ و ٠.٤٨ ± ٠.٠٥ مليجرام /لتر عن الموسم الحار ١.٣٣ ± ٠.٢ و ٠.٢ ± ٠.٠٣ مليجرام /لتر على التوالي. كان معدل الولادات والتوائم أعلى فى النعاج التي ولدت خلال الموسم البارد عنه فى النعاج التي ولدت خلال الموسم الحار (١٢٢.٩, ١٠٦.٤ , ١٨.٦ و ٦% على التوالي). سجل كلا من معدل نفوق المواليد بعد الولادة, والإجهاض و احتباس المشيمة اتجاها معاكس حيث كانت هذه المقاييس أكثر تكرارا خلال الموسم الحار عنه فى الموسم البارد (٨, ٦, ٤.٣ و ٥, ٤, ٢.١ % على التوالي). اتضح ان هناك معامل ارتباط معنوي موجب بين تركيز كل من الزنك والنحاس و معدل الولادات وتركيز النحاس و معدل التوائم (٠.٦٨٤ , ٠.٦٣١ , r = ٠.٦١٦). ايضا لوحظ أن هناك معامل ارتباط معنوي سالب بين تركيزات النحاس فى عينات لبن النعاج وكل من معدل نفوق المواليد بعد الولادة, الإجهاض و احتباس المشيمة و كذلك بين تركيز الزنك و معدل نفوق المواليد. أظهرت الدراسة ان هناك تغيرات موسمية فى تركيزات الزنك والنحاس فى لبن نعاج الكليبى مرتبطه بتغيرات فى بعض المظاهر التناسلية. كان الأداء التناسلي لنعاج الكليبى التي ولدت خلال الموسم البارد أفضل من التى ولدت فى

الموسم الحار. وتوصى الدراسة بأهمية إضافة الزنك والنحاس إلى العلائق خلال الموسم الحارحيث انها قد تؤدى إلى تحسين بعض المظاهر التناسلية لنعاج الكليبى تحت الظروف البيئية لجنوب مصر .