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Effect of Zinc-Methionine Supplementation on Reproductive Performance, Kid's Performance, Minerals Profile and Milk Quality in Early Lactating Baladi Goats

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Abstract: Ten pregnant Baladi does were used in this experiment to evaluate the effects of organic zinc supplementation on reproductive performance, kid's performance, thyroid hormones levels, minerals profile, milk composition and somatic cell count (SCC) in early lactating Baladi goats. Does were stratified by the expected kidding date, age and body weight and assigned to the following dietary treatments: control group (control diet plus no supplement) and Zn-Met group (control diet plus 0.4 g/head/day zinc methionine). Dietary supplementation began approximately one month before the expected kidding date till two months post-kidding. Compared to the control group, diet supplementation with Zn-Met decreased ($P<0.05$) the number of days to conception (43.2 vs 54.4 days) and increased the conception rate (80 vs 60%).Weaning weight as well as average daily gain rate of the kids of the Zn-Met group was higher ($P<0.05$) than that of kids of the control one (11.39 vs 9.11 kg) and (158.89 vs 122.22 g/d), respectively. Zn-Met supplementation significantly ($P<0.05$ and $P<0.01$) increased serum T_3 level throughout the trial, meanwhile serum T_4 level remain unchanged. Serum calcium decreased ($P<0.05$) during the second week post-kidding in Zn-Met treated group. Serum Zn concentration was increased ($P<0.05$) throughout the trial in the supplemented group. Organic Zn had no effect on the levels of serum inorganic P, Cu and Fe. Also, serum nitric oxide level decreased ($P<0.05$) in Zn-Met treated group. The treatment with Zn- Mt had only effect on milk fat % which tends to be decreased. Meanwhile, milk SCC decreased (120×10^3 vs 155×10^3; $P<0.05$). It was concluded that supplementation of early lactating does with Zn-adequate ration resulted in early return to post-kidding estrus, improved the conception rate and enhance the growth rate as well as the weaning weight of kids, improved concentrations of serum zinc and T_3 levels and obviously decreased serum Ca two weeks post-kidding. Furthermore, Zn-Met supplementation improved milk quality by decreasing its SCC.

Key words: Goats · Zinc-Methionine · Reproduction · Milk

INTRODUCTION

Trace elements are needed for vitamin synthesis, hormone production, enzyme activity, collagen formation, tissue synthesis, oxygen transport, energy production and other physiological processes related to growth, reproduction and health. Using of organic trace minerals in the form of chelates for supplementation of ruminant rations has increased in recent years. Greater bioavailability and more positive effects on milk quality and udder health have been reported for organic forms of Zn when compared with inorganic forms [1]. Zinc (Zn) is known to affect growth, reproduction and immune system of the animals by influencing the enzyme activity and gene expression of proteins [2] or by its effect on mitogenic hormones, signal transduction, gene transcription and RNA synthesis [3]. However, recent studies showed that Zn supplemented through the organic sources had a higher retention [4] and tissue concentrations [5] relative to the inorganic sources ($ZnSO_4$ or ZnO). A greater bioavailability and more positive effects on milk quality and udder health have

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been reported for organic forms of Zn when compared to the inorganic forms [1, 6]. As Zn is needed to maintain the normal activity of lymphocytes [7], making it essential for integrity of immune system [8] and improves immunity in cattle [9]. Low zinc status leads to low quality milk with high somatic cell count (SCC) and increases incidence of mastitis [10, 11]. It was reported that supplementation with Zn-proteinates might enhance keratin synthesis in teat canal tissue and decrease the occurrence of new intramammary infection (IMI) [12]. In view of these facts, the present investigation was conducted to study the effect of zinc supplementation as Zn-methionine on reproductive performance, kid's performance, minerals profile, immune response, milk constituent, especially SCC in post-kidding Baladi goats.

MATERIALS AND METHODS

This study was conducted during the period from April to August, 2008 at the experimental goat farm of the Animal Reproduction Research Institute (ARRI), El-Haram, Giza, Egypt.

Animals and Experimental Design: In the present study, a total of ten pregnant Baladi does (2 -3 years old) during the 4th month of pregnancy were used. Animals were assigned into two groups (each of five animals), each group was kept in a separate, well-ventilated animal shed, under conditions of natural day light and temperature. All does were proved to be clinically healthy and free from external or internal parasites. The control group fed a standard total mixed ration according to the managemental system of ARRI, consisted of: ground yellow corn 31%, soybean meal 16%, sunflower meal 23%, wheat bran 20.7 %, molasses 5%, lime stone 2%, common salt 2% and mineral mixture 0.3%, in addition to green corn (darawa). The basal diet contain 24 mg/kg Zn. Additionally, does in the treated group were orally supplemented with 0.4 g/head/day of zinc-methionine from the last month of pregnancy up to day 60 post-kidding. Water was offered *ad libitum*. Zinc-Methionine was available from Biomet-zinc 10% (Norel and Nature, Norel–Miser Suez Gulf P.O.B.157 Suez).

Reproductive Performance: Two fertile bucks were used for estrous detection and natural mating for each group beginning from day 15 post-kidding. Once the doe came in estrus (first detected estrus) it was bred naturally, ultrasonography was performed for pregnancy diagnosis using a real time B-mode (Vetson, Kontron France) equipped with 6.5 MHz MC probe about one month post-mating. Day of conception as well as number of days to conception was recorded for each doe separately. Also, conception rate was calculated for the two groups.

Lamb Performance: Born kids were numbered, sexed, weighed at day of birth (Birth Weight, BW) and then at day 60 of age (Weaning Weight, WW).

Blood Sampling and Measurements: Blood samples were collected from all does at day of kidding and then each two weeks until two months post-kidding from jugular vein in the morning (fasting samples). Serum samples were separated and stored at -20°C for hormonal assay (T_3 and T_4) by ELISA technique (DRG Instruments GnbH, Germany. Division of DKG–international Inc. Fravenberg Str.18, D. 325039 Marburg), according to Walker, [13] for T_3 and Katt *et al.* [14] for T_4. The minimum detectable concentrations were 0.2 ng/ml for T_3, 0.4 ug/dl for T_4. Blood serum samples were analyzed, for estimation of calcium and phosphorus using diagnostic reagent kits according to Young [15] using spectrophotometer (Dr. Lang 400 Spain) and zinc; copper and iron using atomic absorption (Model X3300 Perkin Elmer U.S.A) and for Nitric oxide (NO) using ELISA reader [16].

Milk Sampling: Milk samples were taken every two weeks from day 15 till day 60 post-kidding and analyzed automatically using Bentley-150 milk scan which consists of Bentley soma count and Bentley infrared milk analyzer (Bentley France) to estimate somatic cell count (SCC) and milk parameter (fat, protein, lactose, urea (mg), total solid (TS), solid not fat (SNF) percentage).

Statistical Analysis: All data were subjected to statistical analysis according to Snedecor and Cochran [17]. Data were analyzed by one way ANOVA implying a completely randomized design using Costat version 3.03.

RESULTS

Results in the present study revealed that Zn-Met treated does had fewer ($P<0.05$) days to conception as well as a higher conception rate as compared to the control group (Table 1). As shown in Table 2 kids borne from Zn-Met treated dams had a higher weaning weight ($P<0.05$) and faster daily gain rate ($P<0.05$) than those from the control group. Meanwhile, it was found that kids BW

Table 1: The effect of zinc–methionine treatment on conception rate and number of days to conception in does

		Conception rate	Days to conception
•	Control	3/5 (60%)	54.4 ± 3.41^{a}
•	Zn-Met	4/5 (80%)	43.2 ± 3.07^{b}

Means with different superscripts letters in the same column are significantly different at $P<0.05$

Table 2: Birth weight (BW), Weaning Weight (WW) and Daily Gains Rate (DGR) of kids as affected by treatment of dams with zinc–methionine during late pregnancy and suckling periods (Means ± SE)

	Weight		
Main Effect	BW (kg)	WW (kg)	D G R (g/d)
Treatment			
Control	1.78 ± 0.04	9.11 ± 0.51^{b}	122.22 ±7.99^{b}
Zn-Met	1.86 ± 0.09	11.39 ± 0.64^{a}	158.89 ± 9.75^{a}

▪ Weaning weight adjusted at 60 days after birth

▪ Means with different superscripts letters in the same column are significantly different at $P<0.05$

was not affected by Zn-Met maternal supplementation. With regard to thyroid hormones, serum T3 level was elevated ($P<0.05$) throughout the trial in Zn-Met treated group compared to the control one, meanwhile T_4 level remain unchanged (Table 3). In respect to serum minerals profile, serum calcium (Ca) level was decreased ($P<0.05$) during the first two weeks post-kidding in the treated group (Table 4), serum Zn level was increased ($P<0.05$) through out the experiment and there were no effect on serum Cu, Fe and inorganic P values as compared to the control group. The effect of Zn-Met supplementation on nitric oxide is shown in Table 4. It was noticed that Zn-Met supplementation decreased ($P<0.05$) the nitric oxide level as compared to the control value.

With regard to milk composition, Table 5 reveals that Zn-Met supplementation resulted in a slight, non-significant decrease in milk fat percentage than that of the control group, but had no effect on milk protein, lactose and urea, total solid and solid not fat percentage. Meanwhile, milk SCC decreased ($P<0.01$) in Zn-Met supplemented group as compared to the control value.

Table 3: Serum Triiodothironin (T_3) and Thyroxin (T_4) concentrations of control and zinc–methionine treated does (Means ± SE)

	Day 15	Day 30	Day 45	Day 60
	T3 (pg/ml)			
Control	1.41 ± 0.07^{b}	1.42 ± 0.07^{b}	1.37 ± 0.09^{b}	1.32 ± 0.09^{b}
Zn-Met	1.74 ± 0.08^{a}	1.71 ± 0.08^{a}	1.82 ± 0.08^{a}	1.81 ± 0.07^{a}
	T4 (ug/dl)			
Control	10.57 ± 0.62	10.86 ± 0.42	11.19 ± 0.42	12.99 ± 0.79
Zn-Met	11.43 ± 0.77	12.56 ± 0.66	12.59 ± 0.74	13.12 ± 0.58

Means with different alphabetical letters in the same column are significantly different at $P<0.05$ and $P< 0.01$

Table 4: Serum Ca, inorganic P, Zn, Cu, Fe and NO concentrations of control and zinc –methionine treated does(Means ± SE)

	Day Birth	Day 15	Day 30	Day 45	Day 60
	Ca(mg/dl)				
Control	10.07 ± 0.54	8.21 ± 0.45^{b}	9.02 ± 0.47	9.73 ± 0.50	10.01 ± 0.59
Zn-Met	10.50 ± 0.54	6.76 ± 0.43^{a}	8.28 ± 0.55	8.96 ± 0.49	9.06 ± 0.67
	Inorganic P(mg/dl)				
Control	6.32 ± 0.52	6.00 ± 0.47	7.34 ± 0.55	5.90 ± 0.56	6.16± 0.50
Zn-Met	6.26 ± 0.49	6.75 ± 0.52	6.12 ± 0.54	6.00 ± 0.44	6.14 ± 0.54
	Zn (mg/l) $P<0.05$				
Control	0.67 ± 0.07^{b}	0.80 ± 0.04^{b}	0.73 ± 0.04^{b}	0.74 ± 0.04^{b}	0.71 ± 0.04^{b}
Zn-Met	0.89 ± 0.03^{a}	1.01 ± 0.08^{a}	0.91 ± 0.05^{a}	0.94 ± 0.06^{a}	0.89 ± 0.05^{a}
	Cu (mg/l)				
Control	1.59 ± 0.10	1.18 ± 0.06	1.63 ± 0.13	1.60 ± 0.08	1.24 ± 0.03
Zn-Met	1.35 ± 0.08	1.27 ± 0.05	1.31 ± 0.06	1.40 ± 0.08	1.30 ± 0.09
	Fe(mg/l)				
Control	0.11 ± 0.01	0.10 ± 0.009	0.12 ± 0.009	0.12 ± 0.009	0.12 ± 0.01
Zn-Met	0.10 ± 0.01	0.12 ± 0.01^{a}	0.15 ± 0.01	0.11 ± 0.01	0.13 ± 0.01
	NO (μn/ml)				
Control	26.88 ± 1.00^{b}	30.17 ± 1.08^{b}	30.22 ± 1.92^{b}	32.80 ± 1.09^{b}	28.24 ± 1.30^{b}
Zn-Met	23.31 ± 1.10^{a}	24.90 ± 1.32^{a}	23.03 ± 1.13^{a}	26.11 ± 1.82^{a}	22.92 ± 0.93^{a}

Means with different alphabetical letters in the same column are significantly different at $P<0.05$

Table 5: Means ± SE of milk fat, protein, lactose, urea, total solids and SNF of control and zinc –methionine treated does (Means ± SE)

	Control	Zn-Met
Fat (%)	3.28 ± 0.20	2.83 ± 0.14
Protein (%)	2.75 ± 0.17	2.86 ± 0.17
Lactose (%)	3.92 ± 0.13	3.40 ± 0.20
Urea (mg %)	24.71 ± 0.98	23.26 ± 0.99
Total Solid (%)	11.31 ± 0.57	10.26 ± 0.46
Solid Not Fat (%)	7.78 ± 0.50	8.2 ± 0.57
SCC x10^3	120 ± 5.69^b	155 ± 6.11^a

Means with different alphabetical letters in the same raw are significantly different at $P< 0.01$

DISCUSSION

This work focuses on the effect of supplementation with organic zinc on post-kidding reproductive performance, offspring growth, thyroid gland functions, minerals profile and milk quality in native breed goat. Results in the present study revealed that there is a benefit effect for Zn-Met supplementation on post-kidding reproductive performance indicated by a fewer days to conception and an increased conception rate in the treated does as compared to the control group. These results agreed with Abou-Zeina *et al.* [18] who reported that treatment with Zn-Met alone for a short period improved the fertility of anestrous buffalo-cows and enhance the ovarian activity for about 20% (1/5) of the treated cows, Campbell and Miller [19] stated that cows and heifers fed supplemental Zn had fewer days to first estrus than the control group. As well as Socha and Johnson [20] found that dairy cows fed organic trace minerals had a fewer days to the first service and a fewer days open than cows fed inorganic trace minerals. This condition was attributed to the improvement and early repair of damaged uterine tissue following parturition as Zn had a critical role in the repair and maintenance of the uterine lining following parturition, speeding return to the normal reproductive function and estrus [21]. Also, Zn-Met was shown to improve pregnancy rate within the first 21 days of breeding season in beef cows [22], on the same hand, Ahola *et al.* [23] found that trace minerals (Zn, Cu and Mn) supplemented cows had a higher pregnancy rate than the control group. Improved reproductive performance was reported in dairy cows receiving organic mineral supplements [24]; this may be due to increase level of plasma beta carotene by Zn supplementation [25], as increased plasma beta carotene is directly correlated to the improved conception rates and embryonic development.

With regard to kids BW, WW and DGR, the results revealed a significantly higher WW and faster DGR of kids borne from treated dams as compared to those from the control one. Meanwhile, kids BW was not affected by treatment of their dams. This increase in WW and DGR of kids from Zn-Met treated dams may be attributed to the increase in milk yield in the treated dams which previously reported by Salama *et al.* [26] in goat and Aguilar and Jordan [27] in dairy cows, as a result of dietary Zn-Met supplementation. This result agreed with Mayland *et al.* [28] who reported that preweaning ADG improved when Zn was supplemented to the dams from the time of bull removal until weaning. Treacher [29] stated that milk is essential in the first 3 to 4 wk of the lamb's life and during this period there was a highly positive correlation between milk intake and body weight gaining. Another explanation of higher WW and faster DGR of kids from treated dams, may be due to increased goat milk Zn concentration due to Zn-Met supplementation which was previously stated by Strusinska *et al.* [30] who found an increase in colostrum Zn concentration by 3-10% when supplementing organic microelements and Benuska *et al.* [31] observed an increase in Zn concentration of cows milk after intramuscular administration of Zn as well as Hermansen *et al.* [32] who found that feeding a high concentration of Zn only tend to increase the concentration of Zn in the whole milk but significantly increased its concentration in the cream fraction. This high Zn level in milk consumed by kids enhance their DGA and resulted in increased their WW as Hatfield *et al.* [33, 34] suggested that Zn supplement increased feedlot lamb performance and had a positive influence on lamb weaning weight [35], on the same hand, Abdelrahman *et al.* [36] and Puchala *et al.* [37] reported a higher average daily gain and feed conversion efficiency of Zn-Met treated lambs as compared to the control group.

Concerning thyroid hormones analysis, results of the present study revealed that Zn-Met had a positive effect on total T_3 which was significantly elevated due to treatment, meanwhile, the mean concentration of total T_4 kept unchanged throughout the trial (Table 3). This result was in agreement with other studies denoting that Zn supplementation increased total T_3 [18]. In addition to its participation in protein synthesis, Zn is essential for proper thyroid function. It is involved in T_3 binding to its nuclear receptor [38]. Also, Zn participates in synthesis and action of thyrotropin-releasing hormone (TRH). Pekary *et al.* [39] reported that the processing of prepro-TRH to form TRH is Zn dependant via post-translational processing enzymes such as carboxypeptidase. Also, El-Tohamy [40] indicated that Zn alone or combined with Se deficiencies resulted in a decrease in thyroid status in rams. In addition to its direct effect on thyroid function, Zn deficiency can indirectly affect thyroid hormone status by decreasing energy intake [41]. There was a significant decrease in serum calcium level after two weeks post-kidding. This result agreed with that obtained by Daghash and Mousa [42] in buffaloes supplemented with Zn. This decrease might be due to the increase of milk yield due to Zn-Met supplementation which led to increase calcium let down in milk. Contrary to this observation, Bedi [43] and Khan [44] reported an increase in serum Ca level upon supplementation of Zn in growing calves. This disagreement might be due to different dose and sex. However, the obtained results revealed that there was no effect on serum inorganic P level due to Zn-Met supplementation, which was previously stated by Garg *et al.* [36] in lambs, Mandal *et al.* [45] in calves and Chirase *et al.* [46] who did not find any significant difference in serum inorganic P level in steers. Results in the present work showed that level of serum Zn was significantly increased in Zn-Met supplemented group as compared to the control one, this result was in agreement with Abou-Zeina *et al.* [18] in buffalos, Garg *et al.* [35] in lambs and Huerta *et al.* [47] in beef steers. This finding indicating a higher bioavailability of Zn from Zn-Met supplementation and effective absorption via intestinal transport mechanisms. Also, Hempe and Cousins [48] reported that Zn-Met complex is transported intact from the intestinal lumen into mucosal cells, which increasing tissue supply of Zn and thereby improving animal productivity. In respect to serum Cu and Fe concentrations, the results revealed that Zn-Met supplementation had no effect on serum Cu concentration throughout the trial, which were supported by Garg *et al.* [35] and Abdelrahman *et al.* [36] in lambs, Chirase *et al.* [46] in beef steers and Puchala *et al.*[37] in Angora goats. On the same hand, there was no adverse effect of Zn-Met supplementation on serum Fe contents. Similar to our results, Mandal *et al.* [45] did not find any effect on serum Fe content in relation to dietary Zn supplementation in calves. The non-significant effect of Zn-Met supplementation on serum inorganic P, Cu and Fe concentrations indicated that Zn-Met had no antagonistic effect on absorption and metabolism of these minerals.

Nitric oxide (NO) is one of the most widespread signaling molecules and participates in virtually every cellular and organ function in the body [49]. Physiological levels of NO produced by endothelial cells are essential for vasodilatation, regulation of angiogenesis and blood flow in many tissues, including the ovary [49-51]. However, as oxidant and inhibitor of enzymes containing an iron-sulfur center, free radicals and other reactive species cause the oxidation of bio-molecules (e.g., protein, amino acids, lipids and DNA), which leads to cell injury and death [49,52]. Comparing to the control group, there is marked decrease in the NO activity in the treated group throughout the trial which was previously stated by Abou-Zeina *et al.* [18] who detected a decrease in serum NO level due to Zn-Met treatment, which was attributed to the anti oxidative effects of the metaloenzymes; superoxide dismutase (Zn) which had an important role in scavenging harmful free radicals [53].

In the present work, there was a slight and non-significant decrease in milk fat percentage for the supplemented group. This finding agreed with the results of Salama *et al.* [26] who reported a reduced milk fat by 8.8% in Zn-Met supplemented goats. Also supplementation with Zn-Met reduced milk fat by 9.6 % in dairy ewes [32] and by 2.3% in dairy cows [54]. This decrease is probably due to the dilution effect, as milk yield increased non-significantly for diets supplemented with Zn-Met [26] in goats and [33] in ewe. Also this condition may be attributed to the increased molar proportion of ruminal propionate and the decreased butyrogenic ratio after Zn supplementation to the diets for steers [55]. The percentage of protein, lactose, total solids, solid not fat and urea of milk were not significantly affected by Zn-Met treatment. Similarly, Salama *et al.* [26] did not observe any difference in goat's milk compositions due to Zn-Met treatment. The amount of methionine in the Zn-Met supplement used in this study was probably too low to affect the percentage of milk proteins and also Zn-Met supplementation had no effect on plasma glucose and urea concentration [37], which explains the none obvious effect of Zn-Met supplementation on milk lactose and urea percentage of the treated goats. On the other hand, there was a significant decrease in SCC in the milk of the treated group when compared to the control one, which was similar to the results of other studies, as SCC decreased by (32 vs 45%) in the milk of cows supplemented with Zn-Met as compared to the control group in the experiments of Kellog [6] and O'Donoghue *et al.* [54]. There were several plausible means by which increasing Zn status reduces SCC. Zinc is important in maintenance of health and integrity of epithelial tissues, such as skin (teat) and mammary tissues, because of its role in cell division and protein synthesis [56]. Jones [57] stated that reduced SCC was due to increased keratin synthesis and improved immune function. On the same hand, Zn is required for the incorporation of cystine into keratin, the fibrous protein that lines the teat canal [58]. The keratin lining the teat canal entraps bacteria and prevents their upward movement into the mammary gland [59-61].

In conclusion, on the basis of the present experiment, it may be concluded that supplementation of 0.4g/head/day Zn-Met to Zn adequate basal diet of 4 months pregnant does till 2 months post-kidding significantly improved the reproductive performance by decreasing the number of days to conception, increased the conception rate and kids weaning weight, also increased serum T_3 and serum zinc levels throughout the trials. Also, it decreased both serum nitric oxide level and milk SCC. So using zinc-methionine as a feed additive improved the animal productivity.

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