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RESEARCH ARTICLE

EFFECT OF DIETARY SUPPLEMENTS OF ZINC-METHIONINE ON MILK COMPOSITION AND SUBCLINICAL MASTITIS IN EXTENSIVE DAIRY AWASSI SHEEP HERD OF BEKAA VALLEY OF LEBANON

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ABSTRACT

An experiment was conducted to investigate the effect of supplementation of organic chelated trace mineral Zinc-Methionine ZM complex (ZINPRO®, Zinpro Corporation, Eden Prairie, MN, USA) on extensive Awassi sheep herd submitted to subclinicalmastitis SCM in Bekaa valley of Lebanon. The effects of ZM complex on milk quality (fat, protein and somatic cell count SCC) were examined. 32 Awassi ewes were randomly assigned after separating them into two groups: ZM^- group of 16 ewes affected to a control group that were fed on basic ration with no mineral premix (barley, wheat bran, soybean meal, salt, vitamins and hay) and ZM^+ group of 16 ewes affected to an experimental group that received a supplement of ZM in their basic ration with an average of 600 mg/head/day for the first month then 1200 mg/head/day for the next month of the trial. Moreover, each group was separated into 2 sub-groups: CMT^+ group (n=8) positive to California mastitis test (CMT) and CMT^- group (n=8) negative to CMT. In total, 4 groups of 8 ewes each were assigned: ZM^+CMT^+, ZM^+CMT^-, ZM^-CMT^+ and ZM^-CMT^-. Treatment affected significantly milk protein percent (3.72 ± 0.03 vs 3.63 ± 0.03 % for ZM^+ vsZM^-,$p<0.05$), fat percent (5.60 ± 0.07 vs 5.35 ± 0.07 % for ZM^+ vs ZM^-, $p<0.05$), and SCC count (339.82 ± 4.48 vs 324.63 ± 4.48 $x10^3$ cells/ml for ZM^+ vs ZM^-, $p<0.05$). However, milk protein, fat percent and SCC count were significantly different in the infected halves (CMT^+) comparing to the non-infected halves (CMT^-) in both groups (milk protein and fat percent was lower in CMT^+ comparing to CMT^-; 3.55 ± 0.03 vs 3.80 ± 0.03, $p<0.001$ and 4.99 ± 0.07 vs 5.95 ± 0.07, $p<0.001$ respectively), while SCC count was higher in CMT^+ comparing to CMT^-; 377.70 ± 4.48 vs 286.76 ± 4.48 $x10^3$ cells/ml, $p<0.001$). Interestingly, the effect of ZM on the experimental affected group was significant ($p<0.05$) over time especially in the second month of the trial (increase of 1.83%, 4.55% and a decrease of 12.6% in milk protein, fat percent and SCC count in ZM^+CMT^+). Finally, supplementation of ZM improved dairy performance and udder health by reducing somatic cell count and increasing protein and fat contents in affected ewes.

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INTRODUCTION

The Awassi sheep is a breed of domestic sheep which was originated in the Syro-Arabian desert. It was actually developed as a nomadic sheep breed through centuries of natural and selective breeding for becoming the highest milk producing breed in the Middle East. This breed is of the Near Eastern Fat-tailed type and the only indigenous breed of sheep in Lebanon. Infectious mastitis has been described as one of the main diseases affecting animals during lactation. It costs the Dairy Industry enormous economic losses due to decrease in milk production and treatment of cases (Sordillo, 2011). The immune system is greatly affected, and losses of milk production and milk quality are clear during intramammary infections IMI. Nutritional strategies such as supplementation of different sources of micro minerals have been developed to strengthen the immune system, in order to diminish the negative effects of inflammatory stress and improve the resistance of the host against IMI. Ovine mastitis has been known and studied for many years in countries where the production of ewe's milk is economically important (Al Majali and Jawabri, 2003). Most of these studies have used either microbial culture as a determinant factor for subclinical cases or California Mastitis Test (CMT) and electronic cell counting estimates of SCC. The California Mastitis Test (CMT) is an indirect method for detecting IMI. This test measures the nuclear material of the cell present in milk (leucocytes and epithelial cells) that reacts to CMT reagent to form a gel, indicating an abnormal condition of the udder. Somatic cell count, also known as direct method, is one of the most

important factors to evaluate the quality and health of milk. Normal milk has less than 200,000 cells per ml. Increasing somatic cells causes significant reduction in producing amount and quality of milk. Also, it leads to reducing shelf life and flavor of milk and yogurt and reduces cheese yields.The severity of *subclinical* mastitis can be influenced by parity, stage of lactation, SCC, genetic resistance, nutritional status, pre-existing diseases, and others (Paape *et al*., 2002). Trace minerals are essential micro minerals needed for vital processes of the body, including functioning as antioxidants. According to (Wright *et al*., 2008), the ruminal absorption of trace minerals is minimal, whereas they are effectively absorbed by the intestine. Inorganic forms of minerals, such as sulfates, oxides, and carbonates, might have lower bioavailability compared to organic sources as they can dissociate in the upper gastrointestinal tract forming indigestible compounds that are unavailable for absorption by the intestine and thus excreted in the manure(Gressley, 2009). In contrast, organic forms of trace minerals have higher bioavailability compared to inorganic sources (Spears, 2003). Feeding organic trace minerals has been shown in several studies to improve immune function. and production variables in diverse species of animals(Nemec *et al*., 2012). Organic trace minerals OTM are typically bound to amino acids through complex, chelation process. Moreover, they become resilient enough to bypass the upper gastrointestinal tract, although dissociation still happens, thus remaining available for absorption by the gut tissues easier for organic than inorganic ions(Wright *et al*., 2008). Andrieu (2008) discussed many functions performed by one of these trace minerals which is Zn such as: involvement in keratin generation, appetite control, energy metabolism, and others. Supplementation of organic Zn was observed to reduce the incidence of IMI (Spain, 1993), but no difference in SCC or milk production was reported. While according to Kellogg (1990), the influence of Zn on SCC is contradictory. Feeding dietary organic trace minerals seems a reasonable technique to improve health. However, based on the information available to date, it is still unclear the effects of OTM on production. We hypothesized that animals supplemented with OTM would show an improved recovery against the IMI challenge due to a possible synergistic effect of the treatment.

MATERIALS AND METHODS

Animals and their management: The experiment was carried out at Nahle village in Bekaa valley- Lebanon at an altitude of 1360 meter between April and June 2017. The herd was composed of Awassi sheep specimens, local breed, for the production of milk and lambs. 32 Awassi dairy ewes in the early and mid-lactation period were included in the study. They were between 2 and 5 years old, with different number of lactations and number of born lambs, and none of them had been mechanically milked before. They were individually fed a basal diet (barley, wheat bran, soybean meal, salt, vitamins and hay) appropriate for physiological state formulated to meet or exceed NRC (2001) recommendations from day 1 to day 59 of the experiment. Ewes were randomly assigned to one of these 4 groups: as: 1) no supplemental control group ZM^- (CMT^+ and CMT^- positive and negative to california mastitis test CMT respectively; n=16), 2) trace minerals provided as 100% chelated Zn- methionine ZM^+ (CMT^+ and CMT^-**; n=16**). The dietary chelated Zn was supplied as Top Quality Organic Zinc Product Zinc-methionine ZM complex (ZINPRO®, Zinpro Corporation, Eden Prairie, MN, USA). Treatment was top-dressed and mixed into the top portion of the diet immediately prior to morning feeding to ewes of experimental group at a rate of 600 mg/head/day using a ground corn as carrier for the first month and 1200 mg/head/day for the second month of the trial. Ewes were fed twice daily (7:00 am and 2:00 pm) during experimental period. Milk samples for compositional analyses were collected every 4 days from the morning milking (6:00 am) during the period of the trial. Water was available for ad libitum consumption throughout the experimental period. A CMT was used to assign SCM during the experiment. Animals were independently assessed before each drawing sample. Animals were tested and CMT score was recorded immediately prior to the sample draw at the beginning of the trial.

Milk Samples and laboratory analyses: For sampling, the udders were cleaned with water and dried with paper towels. Once dried, they were physically examined in order to detect clinical mastitis(Mota, 2008). Then the teats were disinfected using cotton wool soaked in alcohol 70%. The first jets of milk were stripped onto a paddle in order to perform the California Mastitis Test (CMT). Then, individual milk samples per animal were aseptically collected in sterilized tubes, kept in icebox in order to analyze fat and protein through Gerber (ISO 2446 /IDF 105)and Kjeldahl Methods(Lynch and Barbano, 1999)respectively, and the somatic cell count technique, which was carried out by an electronic counting device based on flow cytometry Ekomilk Scan (Thermo Fisher Scientific, Waltham, Massachusetts,US) at LARI (Lebanese agricultural research institute) milk analysis laboratory. Ewes with SCC > 350 000 cells/ml, were assumed to have affected halvespresenting a mastitis-positive counting (Forsback*et al.,* 2009)

Statistical Analysis: Data were analyzed as a completely randomized design using Statistica version10. Repeated measures ANOVA were used to analyze the effect of the treatment on the means of the measurements (milk fat, protein contentand SCC).The statistical model included the factorial effect of the experimental factors tested over time. Statistical significance was considered at $P<0.05$.

RESULTS

Effects of SCM, treatment ZM and time on milk fat, protein percent and SCC count variables have been presented in Figures 1, 2 and 3, respectively. Moreover, the *P values* for these variables have been shown in Table 1. Effects of treatment ZM, time, and SCM on milk protein variable are shown in Figure 1. Milk protein content significantly differed between ZM^+ group and ZM^- group (3.72 ± 0.03 vs 3.63 ± 0.03 % for ZM^+ vs ZM^-, $p<0.05$). Also, milk protein was affected by SCM with a significant difference ($P<0.001$) among CMT^+ compared to CMT^- ewes in the two groups from the beginning till the end of the trial. Protein percent in milk tended to be lower for CMT^+than CMT^- in both ZM^+ and ZM^- groups of ewes respectively (3.56 ± 0.03 vs 3.87 ± 0.03 for CMT^+ vs CMT^- in ZM^+, $P<0.001$ and 3.53 ± 0.04 vs 3.73 ± 0.04 for CMT^+ vs CMT^- in ZM^-, $P<0.001$). Quality of milk protein was not determined to check for casein or albumens. Treatment with ZM did not affect significantly milk protein in CMT^- ewes ($P>0.05$) over time. The difference in milk protein percent in the treated mastitic group was significant during the second month of the study ($P<0.05$). Moreover, in ZM^+CMT^+ group, milk protein was not differing significantly during the first five weeks of the trial (from day 1 until day 31).

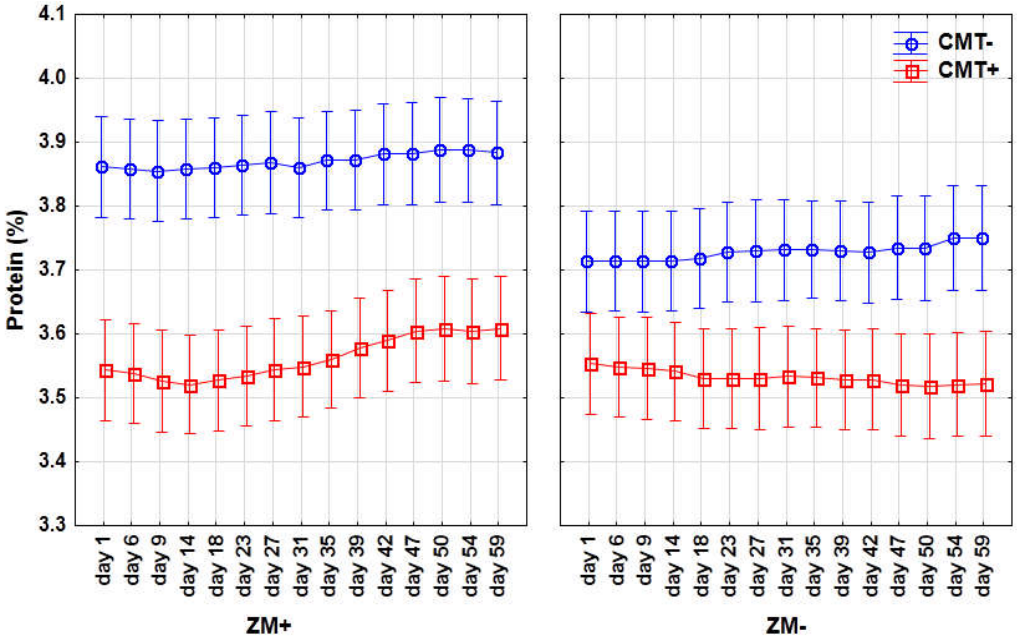

Figure 1. Means and their 95% confidence intervals (middle markers and vertical bars respectively) of the protein content (%) as a function of treatment (in ZM^+ and ZM^- groups; ○ for CMT^- and □ for CMT^+) and time

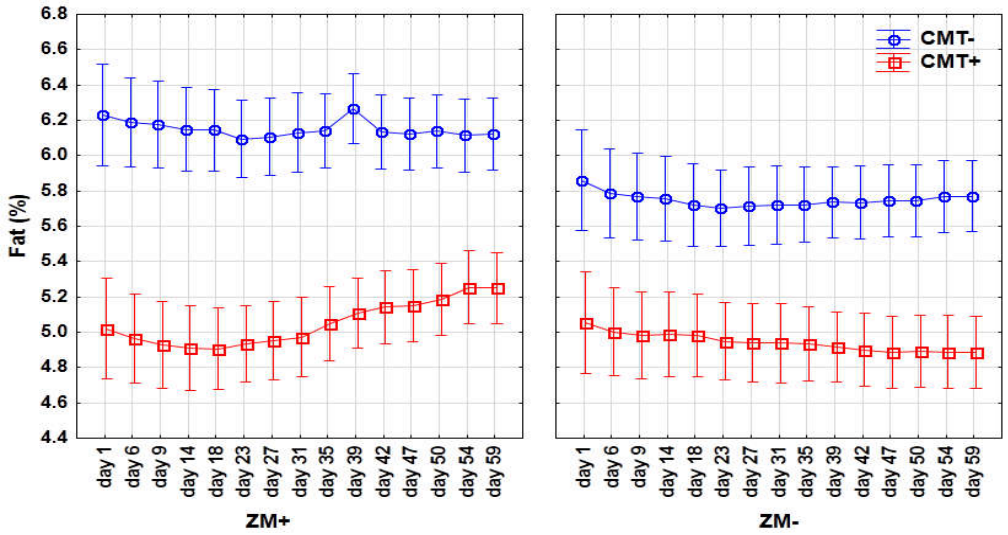

Figure 2. Means and their 95% confidence intervals (middle markers and vertical bars respectively) of the fat content (%) as a function of treatment (in ZM^+ and ZM^- groups; ○ for CMT^- and □ for CMT^+) and time.

Table 1. Milk variable among different treatment groups ZM^+ and ZM^-, with p values

	Treatment			P value						
Variable	**ZM^+**	**ZM^-**	**SEM**	**Treatment**	**SCM***	**Time**	**Treatment x SCM**	**Treatment x Time**	**SCM x Time**	**Treatment x Time x SCM**
Protein, (%)	3.72	3.63	0.03	<0.05	<0.001	<0.001	0.14	<0.001	0.97	<0.001
Fat, (%)	5.60	5.35	0.07	<0.05	<0.001	<0.001	0.17	<0.001	<0.05	<0.001
SCC, ($x10^3$ cells/ml)	339.82	324.63	4.48	<0.05	<0.001	0.25	0.67	0.06	<0.001	<0.001

*SCM: according to California mastitis test CMT, separating into 2 subgroups: CMT^+ positive and CMT^- negative.
ZM^+: experimental group; ZM^-: control group
SEM: standard error of the mean

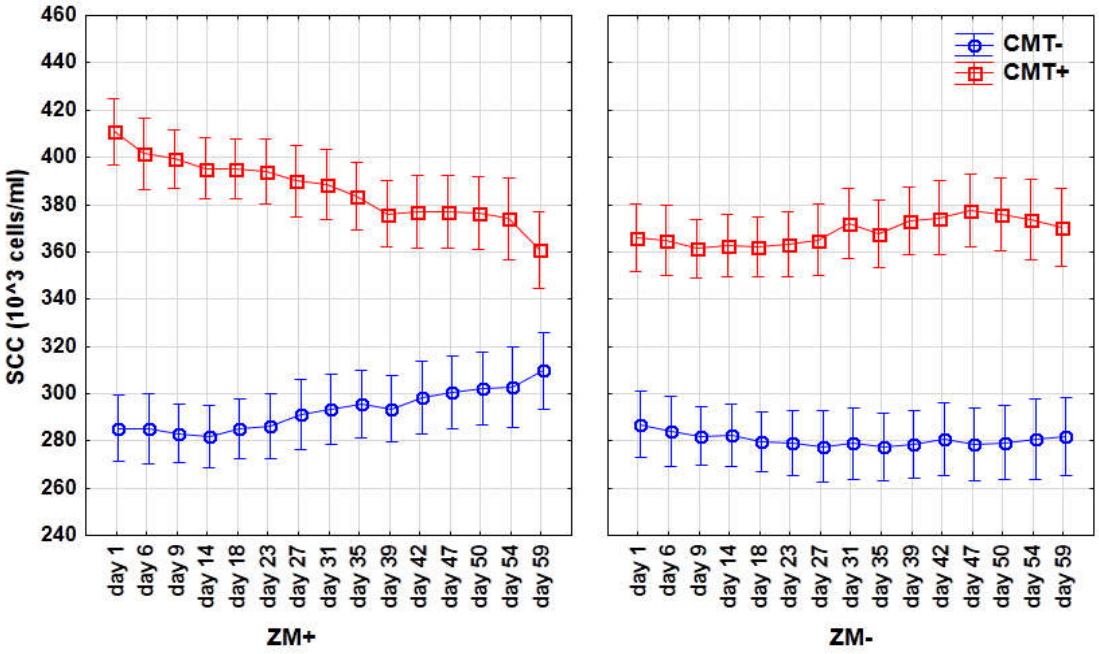

Figure 3. Means and their 95% confidence intervals (middle markers and vertical bars respectively) of the SCC (x10^3 cells/ml) as a function of treatment (in ZM$^+$ and ZM$^-$ groups; ○ for CMT$^-$ and □ for CMT$^+$) and time

However, it had significantly increase of 1.83% (*P<0.05)* during the last three weeks of the study (from day 35 till day 59).Moreover, significant results with time effect, treatment × time effect and treatment × SCM x time interactions were observed for protein percent (*P*<0.001; table 1). Effects of ZM treatment, time, and SCM (CMT$^{\pm}$) on milk fat variable are shown in Figure 2. Milk fat significantly differed between ZM$^+$ group and ZM$^-$ group (5.60 ± 0.07 vs 5.35 ± 0.07 for ZM$^+$ vs ZM$^-$, *p<0.05*). Also, milk fat was affected by SCM with a significant difference (*P<0.001)* among CMT$^+$ compared to CMT$^-$ ewes in the two groups from the beginning till the end of the trial (5.05 ± 0.10 vs 6.15 ± 0.10 for CMT$^+$ vs CMT$^-$ in ZM$^+$, *P<0.001* and 4.24 ± 0.10 vs 5.75 ± 0.10 for CMT$^+$ vs CMT$^-$ in ZM$^-$, *P<0.001*). An effect of time was also observed for this variable during the study only for ZM$^+$CMT$^+$ group (*P<0.001)*. However, during the first five weeks of trial (from day 1 until day 31), milk fat percent was not differing in ZM$^+$CMT$^+$ while a significant increase (4.55%, *P<0.05)* in milk fat after six weeks of the beginning of the trial till the end was detected (from day 35 until day 59). While in ZM$^-$CMT$^+$ group, a slightly significant decrease in milk fat percent was observed from day 1 to the end of the trial (from 5.05 ± 0.14 to 4.88 ± 0.10 %,*P<0.05*). Adding too, no significant difference was observed (*P>0.05)* in milk fat in ZM$^-$CMT$^-$ group while, surprisingly, an increase in fat percent for group ZM$^+$CMT$^-$ was detected on day 39 (6.70 ± 0.10 %) after that date, the value returns as it was previously.

Moreover, significant results with SCM × time effect (*P*<0.05), also with treatment × time effect and treatment x time × SCM interactions (*P*<0.001) were observed for fat percent (table 1). Effects of ZM treatment, time, and SCM (CMT$^{\pm}$) on milk SCC variable are shown in Figure 3. Milk SCC significantly differed between ZM$^+$ group and ZM$^-$ group (339.82 ± 4.48 for ZM$^+$ vs 324.63 ± 4.48 x10^3 cells/ml for ZM$^-$, *p<0.05*). Milk SCC was affected by SCM, where a significant difference (*P<0.001)* among CMT$^+$ compared to CMT$^-$ ewes in the two groups from d1 till the end of the trial was observed; overall, SCC in CMT$^+$ was higher than CMT$^-$ (386 ± 6.33 vs 292.98 ± 6.33 x10^3 cells/ml for CMT$^+$ vs CMT$^-$ in ZM$^+$, *P<0.001* and 368.73 ± 6.33 vs 280.54 ± 6.33 for CMT$^+$ vs CMT$^-$ in ZM$^-$, *P<0.001*). While, no significant difference (*P>0.05*)was observed in the direction of curves in ZM$^-$CMT$^+$ and ZM$^-$CMT$^-$ groups with time effect. Moreover, in ZM$^+$CMT$^+$ group, a significant decrease in SCC was observed from day 1 till the end of the study *(*410.88 ± 6.92 vs 360.88 ± 7.99 x10^3 cells/ml from day1 to day59*, P<0.001)*.Also, ZM$^+$CMT$^+$ showed a 12.16% decrease in SCC, while ZM$^+$CMT$^-$ showedan 8.54% increase in SCC (from 285.38 ± 6.92 vs 309.75 ± 7.99 x10^3 cells/ml from day1 to day59*, P<0.001)*. In contrast, ZM$^-$CMT$^+$ showed no significant change in SCC during the first six weeks of the study. While, a significant increase in SCC was observed from day 31 till the end of the trial in the same group. However, non-significant change in ZM$^-$CMT$^-$ was observed over time (p>0.05). Moreover, significant results with SCM x time effect and treatment × time x SCM interactions were observed for SCC count (*P*<0.001; table 1).

DISCUSSION

In the present study, the average of SCC, fat, and protein was varied significantly (*P<0.05*) between mastitic ewes and normal ewes. The results obtained showed that protein content in mastitic ewes CMT$^+$ was decreased significantly compared to normal ones CMT$^-$. These results were in contrast with (Auldist *et al*., 1995)who reported that the effect of mastitis on milk protein percentage shows that SCM induces an increase in milk protein and this had been attributed to the influx of blood-borne proteins (such as serum albumin, immunoglobulin). On the other hand, Liwiczuk *et al.* (2011)obtained a decrease in the protein components of raw milk with increased somatic cell count. Moreover, Urech *et al.* (1999) declared changes in milk protein fraction as affected by subclinical mastitis, where decreased values are found for total protein. However, according to Bianchi *et al.* (2004) results indicated that both udder inflammation and mammary involution can increase plasmin activity in healthy vs. infected udders, which is responsible for an evident protein breakdown in milk.

Moreover, according to our results, fat contents in mastitic ewes was lower than that of normal ones. Investigation's results of Forsback *et al.* (2009)showed a positive correlation between lipolytic enzyme activity and somatic cell count in milk, leading to decrease in fat content with the increased SCC in milk. Moreover, it has been reported that milk from infected animals with SCM had very high increase in the activity enzyme called lipase that cause milk fat breakdown and release free fatty acids that produce off-flavors in milk and cause great loss to dairy industry. It has been assumed that milk with a high SCC is more susceptible to spontaneous lipolysis. Previously, Hassan (2013)reported a significant difference lower in fat percentage for milk from infected sheep. Our results are in accordance with these findings previously, as reported with the net decrease in the average of milk fat in CMT+ ewes compared to CMT- ewes in both ZM+ and ZM- groups.

The SCC in this study was also affected by SCM, where a significant difference (*P<0.001*) among CMT^+ compared to CMT^- ewes in the two groups from the beginning till the end of the trial was observed. Concerning ZM^+ group, in non mastitic ewes CMT^-, the SCC varied between 285.38 ± 6.92 to 309,75 ± 7.99 x 10^3 cells /ml, while in CMT^+ group, it varied between 360,88 ± 7.99 and 410,88 ± 6.92x 10^3 cells/ ml. According to literature, the SCC concentration in sheep milk, in the absence of mastitis, could vary between 10,000 and 200,000 cells/ml (Paape *et al*., 2007). Moreover, according to Bianchi *et al.* (2004),samples presenting a counting higher than 1,000,000 cells. mL^{-1} were considered mastitis-positive. Further, a SCC standard of 400,000 cells/mL for bulk milk is being adopted in milk quality schemes around the world as a result of the European Union requirements (Auldista and Hubble, 1998). In this study, the maximum SSC for affected ewes averaged only 377.70 ± 4.48 x10^3 cells.ml^{-1}, less than the 750 x10^3 cells.ml^{-1} legal limit in the US and 400 x10^3 cells.ml^{-1} in the European Union (EU) (Paape *et al*., 2007).Sometimes, cut-off values show large difference because these thresholds depend on counting methods. To obtain accurate milk SCC counts, only cell counting procedure specific for DNA should be used. Currently, the Foss and Bentley electronic cell counters are the industry standards for determining SCC.In the European Union (EU) (Council Directive 92/46/EEC, 1992), the legal limit for cows is 400 x10^3 cells.ml^{-1} and there is no legal limit for goats and sheep. However, most of countries use 200 x10^3 cells.ml−1 as a limit for goat and ewe quality milk. Moreover, in the EU, milk collection is rejected if the mean cell counts exceed 400 x10^3 cells.ml^{-1}(Paape *et al*., 2007). Our results for CMT^- ewes in both groups were slightly higher than the threshold of SCC.

Factors other than intramammary infection such as management practices, stage of lactation, parity contribute to an elevation of SCC (Paape *et al*., 2007).This also could be attributed to some environmental factors such as heat stress during late spring and beginning of summer months. Which was the case in our study, where samples were taken between April and June. According to (Pizarro Borges*et al.,* 2008), the composition and SCC of bulk tank good quality milk varied widely according to the environmental factors such as heat stress during late spring and summer months. Moreover, our results obtained concerning treatment with ZM, revealed that ZM decreased SCC and led to an increase in fat and protein milk contents in ZM^+ CMT^+ group after one month of the beginning of the experiment. In our study, the difference in milk protein and fat contents between the 2 mastitic groups was significantly different, where according to our trial, ZM^+CMT^+ showed a significant increase (*P<0,05*) in protein and fat content (1.83% and 4.55% respectively) after one month of the beginning of the trial (from day 35). Spain (1994) reported that, zinc reduces the invasion of pathogens in mammary gland for his role in ceiling keratin production. Where, the keratin coating the teat canal attracts bacteria and prevents the penetration of them into the mammary gland. However, a significant effect(*P<0,05*) of ZM supplemented on milk fat percent was reported in our study even so there was a positive increase of fat percent in ZM^+CMT^+ group. Moreover, it wasnotpossible that when milk production dropped, fat % will increased due to lower milk yield rather than due to treatment ZM, as demonstrated by Oravcová*et al.* (2007)where fat and protein content were almost equally correlated with milk yield.

Also, our results showed that, the SCC counts were affected by the treatment (ZM). Consequently, ZM^+CMT^+ group induced a highly significant (*P<0.05)* decrease in SCC compared to ZM^-CMT^+ group, where results didn't show any change in SCC during the trial. However, ZM^+CMT^+ ewes showed a 12.16% decrease in SCC, while in ZM^-CMT^+, a non-significant increase was observed after 5 weeks of the beginning of the trial (from day 35). These results were in common with what Salama *et al.* (2003)showed that,milk SCC tended to decrease as a result of Zn-Met supplementation, that could enhance resistance to udder stress in dairy goats. Furthermore, Miller and Madsen (1992) also reported, that zinc is required for maintenance of skin integrity, stabilization of membranes, and activation of the cell-mediated immune system. Together, stress, reduction in immune response, and breakdown in skin integrity may deteriorate the natural defense mechanisms of the mammary gland (Miller, 1970). Adding too, researcher showed that keratin ceiling teat canals of sheep fed with zinc methionine was higher than that of sheep fed an equivalent amount of zinc in the form of oxide, indicating that supplementation of a more bioavailable source of zinc increases keratin production. These results explain particularly the effect of ZM complex in reducing somatic cell count (Kellogg*et al.,* 2004). Moreover, according toKellogg (1990), cows given zinc methionine (ZM) produced more milk with lower somatic cell counts (SCC) than cows given zinc oxide and methionine (ZO+M). Also the decreased of SCC in ZM^+CMT^+ group was in line with that obtained by Dibley (2001) who reported that zinc plays an integral role in immune function by activating T-lymphocytes responsiveness thus, impacting the effectiveness of somatic cells within the mammary gland. Moreover, our results in accordance with Moynahan (1981)who reported, that zinc reduce mastitis and somatic cell count by enhancement of keratin formation, however it is required for incorporation of cysteine into keratin. According to time effect, our results of milk protein and fat percentages in group ZM^+CMT^+ were significant (*P<0,001*), while for SCC counts all experimental groups were also significant(*P<0,05*)with time effect but no significance for control groups (*P>0,05*) was observed. In one hand, it could be attributed to the increase of the amount of ZM from 600 mg to 1200 mg/head/day by the second month of experience in the experimental group ZM^+. In the other hand, this also could be due to the period of time that zinc in organic chelated form requires to be assimilated by the organism of the animal, and then to start affecting positively the milk components. The significant increase in milk protein (1.83%) and fat contents (4.55%) in ZM^+CMT^+, and SCC (8.54%) in

ZM^+ CMT^-, while the decrease in SCC counts (12.6%) in ZM^+CMT^+, were obtained after one month of the beginning of the trial (from day 35 till the end of the trial). According to Pechova *et al.* (2006), a trend toward a positive effect of Zn supplementation on the health of mammary gland of dairy cows was identified in association with the somatic cell count (SCC) which was significantly lower in the experimental group by the end of the month 3. Moreover, Harris (1995)published the results of a 90-day trial in whose the experimental group of cows was supplemented Zn in the bioplex form at the dose of 400mg per animal per day; SCC dropped by 24% in the supplemented group while it increased by 36% in the control group over the same period. Also,Anderson (2005)investigated the effect of supplementing complex zinc (360mg Zn/day per head) from the beginning of the dry period until 3 months' post-partum. Cows supplemented with zinc produce numerically lower SCC, but the difference between groups was not significant. Where, the effect of ZM supplementation by improving udder health and milk composition was also proved by each of Dibley (2001) and Moynahan (1981).

Conclusion

Under intramammary bacterial challenge, results of this study suggested that supplementation over (NRC, 2001) requirements may be beneficial to milking ewes submitted to IMI. The present study showed a proportional function induced by ZM which decrease the disruption of udder health statue by decreasing milk SCC, then improving milk composition (fat and protein) by enhancing teat keratinization statue. However, production variables (milk fat and protein percent) were improved in SCM ewes supplemented with organic trace minerals after one month of adding ZM complex. SCC were also affected by treatment, and it is suggestive that ZM added was more affected in SCM ewes after adding 1200 mg/kg/head on the second month of the trial. To the best of our knowledge, this was the first experiment in Lebanon that evaluated the effect supplementation of blended trace minerals on the health and milk quality of ewes submitted to intramammary bacterial challenge rearing in extensive herd system. The study of more mechanistic effects on use of organic trace minerals, either blended with other minerals or blended with different forms of the compounds, and dietary antioxidants in a long period time would be very helpful to fully understand the physiology of animals under severe stress, here demonstrated as mastitis. Finally, environmental cleanliness and herd health should be closely monitored to avoid or at least minimize the severity of IMI cases. Also, non-infectious factors that contribute to elevations in SCC for ewes need to be considered when establishing legal cell count limits.

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Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this paper. All the contents of this article including results and discussion, conclusion, figures and tables are based on the original research work of the author.

List of abbreviations
SCM: Subclinical mastitis
SCC: somatic cell count
CMT: California mastitis test
IMI: Intramammary infection
Zn-Meth: Zinc Methionine
(CMT^+): positive indication against using California Mastitis Test
(CMT^-): negative indication against using California mastitis Test
(ZM^+): Experimental group with Zinc Methionine
(ZM^-): Control group without Zinc Methionine

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