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RESEARCH ARTICLE

EFFECT OF ZINC METHIONINE SUPPLEMENTATION ON MILK PRODUCTION AS AFFECTED BY SUBCLINICAL MASTITIS IN AN EXTENSIVE BREEDING OF DAIRY COWS IN MOUNT LEBANON

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ABSTRACT

The experiment was conducted in an extensive breeding of dairy cows in Ghineh-Mount Lebanon in order to identify the effect of zinc methionine supplementation on milk production affected by subclinical mastitis. The animals were divided into 2 groups; the first group was non supplemented (control ZM-, n=7), while the second was supplemented with 4 g the first month (June) before doubling the dose to 8 g of Zn-methionine/head/day the next month (July) (experimental ZM+, n=7). Milk samples were collected once a week from 40 teats selected based on CMT score during the two months June and July. The methods employed in this study included California mastitis test, bacteriological culture and milk composition analysis for milk fat, protein and lactose content. Milk yield and milk composition decreased significantly ($P < 0.05$) with increasing of total aerobic bacterial count TABC. Zinc methionine ZM supplementation for subclinical mastitis cows resulted in significant decrease in total aerobic bacterial count comparing to time effect (from 1.16 ± 0.20, day1 to 0.70 ± 0.13 x105cfu/ml, day 57; $P < 0.001$). The obtained results showed a significant increase in milk production in the supplemented group ZM+(16,00 ± 0.80, day1 to 18.86 ± 0.71 l/day, day 57; $P < 0.001$). Milk lactose and protein content were also significantly increased especially after one month of treatment in ZM+ group (from 4.10 ± 0.06 day 1, to 4.40 ± 0.04 % day 57 for lactose content and 2.70 ± 0.08 day 1, to 3.20 ± 0.05 % day 57 for protein content; $p<0.001$). Whereas, no significant difference was noted in milk fat content between groups ($P> 0.05$). This study showed the positive effect of supplementation of zinc methionine on TABC, milk yield and milk composition. This shows the importance of chelated minerals supplementation in the ration, in order to prevent the progression of subclinical mastitis to clinical cases that cause economical loses in dairy cows and affects the composition of the milk and consequently will be refused by the industries.

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INTRODUCTION

Milk composition and microbiological characteristics are important factors for the dairy farmer (raw milk quality), dairy industry (technological process and quality of dairy products), and consumer (nutritional quality and safety). Milk composition varies according to factors such as breed, age, mammary gland health, lactation stage, nutritional management, and season (Dobranié *et al.,* 2008). The lack of hygienic practices in the breeding, is one of the main factors favoring the appearance of several diseases, being able to modify the organoleptic parameters of the milk. Among these diseases, mastitis is an important disease of dairy animals because it reduces the welfare of animals, the quantity and quality of milk. Mastitis is one of the most common diseases in dairy cows and results in great economic losses in the dairy industry (Halasa *et al*., 2007). Mastitis is defined as a mammary gland inflammation that is generally caused by bacterial infections (Santos *et al.,* 2003). This disease negatively affects the physical-chemical characteristics, composition, and yield of milk (Cunha *et al*., 2008). Increased somatic cell count SCC in milk affected by subclinical mastitis is associated with reductions in casein, milk fat, and lactose; increased enzymatic activity; and reduced quality and yield of dairy products and consist of a high risk for milk hygiene as it may even contain pathogenic organisms (Ballou *et al.,* 1995). In recent years, and since the use of antibiotics has not been the ideal solution, chelated micronutrient supplementation has become more and more practiced by farmers for lack of these minerals in the diet and their important roles in the body.

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Among these micronutrients, zinc is essential for the health and performance of dairy cows (Andrieu, 2008). A mineral complex is a mixture of mineral and organic compound. Chelation refers to a bond formed between a metal ion (mineral) and ligand (protein or amino acid).Because of their stability, most chelated minerals are not modified in the digestive tract and are completely absorbed by their amino acids. In vitro studies have shown that organic micronutrients are better absorbed by intestinal tissues than inorganic trace elements (Predieri *et al*., 2005). Moreover, in vitro studies of intestinal tissues have demonstrated increased absorption of organic minerals compared to inorganic minerals (Wright *et al*., 2008). Chelated trace elements may have better use and more bioavailability higher than inorganic sources (Abdallah *et al*., 2009). Based on this assumption, chelated trace elements can be added at a much lower concentration in the diet than inorganic trace elements, without having a negative effect on performance and potentially reducing mineral excretion. The use of chelated trace elements can improve the intestinal absorption of minerals and can reduce the interference of agents that form insoluble complexes with ionic trace elements. Several studies have shown that supplementation of the chelated trace element zinc-methionine reduces the somatic cell count in milk of dairy cows (Kellogg *et al*., 1990; 2004). The question arose as to the possibility of obtaining in Lebanon the same results concerning the effect of the addition of this chelated substance in the diet of dairy cows affected by subclinical mastitis. On this, the following question arises: Will we obtain the same results in Lebanon, or will our farming methods play a positive role and lead to other conclusions? Therefore, it would be interesting to study the relationship between the addition of this product to the rations of extensive breeding of Holstein dairy cows in the Mount Lebanon region and their productive performances, and to establish the variation of milk quality. affected or not by subclinical mastitis depending on the treatment and the period of administration. The choice of the chelated trace mineral to be added to the cow's ration was based on its availability on the market and its affordability. Thus we limited our choice to the study of the effect of zinc methionine. Therefore, we seek through our study to determine the influence of this product added to food rations in Lebanese conditions by taking as a sample a typical traditional dairy cattle breeding to draw appropriate conclusions that could be of interest to farmers and farmers. consumers at the same time.

MATERIALS AND METHODS

Animals and their management

The experiment was carried out in an extensive breeding of dairy cows in Ghineh, Mount Lebanon, (1000 m altitude), during the months of June and July 2017. The average temperature in summer could reach 30 ° C, and fall to 5 ° C in winter. The cows were of Friesian Holstein breed; their average milk yield was 18 kg / head/ day. They were individually fed a basal diet (barley, wheat bran, corn, soybean meal, salt, vitamins and hay) appropriate for physiological state formulated to meet or exceed NRC (2001) recommendations twice daily from day 1 to day 60 of the experiment, during the milking process that was done manually and took place early in the morning (06:00) and in the late afternoon (16:00) in two equal portions. The general condition of the cows was fairly average with a general body condition score of 2/5, with some problems of mastitis and lameness detected in a few individuals.14 cows in the early and mid-lactation period, between 2 and 5 years old, with different number of lactations, were included in the study. They were randomly assigned to two homogeneous groups: untreated control group of cows (ZM^-;n=7) having the basal diet and supplemented experimental group of cows (ZM^+; n=7) receiving in their diet a supplement of zinc in the chelated organic form "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 and late afternoon feeding to cows of experimental group at a rate of 4 g/head/day using a ground corn as carrier for the first month and 8 g/head/day for the second month of the trial. Forages were given first, in order to ensure a good and long rumination, and was then followed by the concentrates in which the supplement ZM was incorporated. Water was available for ad libitum consumption throughout the experimental period. Milk samples for compositional analyses were collected approximately once per week from the morning milking during the period of the trial.

Sample collection and laboratory analyses

Samples were collected weekly during the lactation trial and stored at 4°C for subsequent analysis. They were taken from each udder half for analysis of composition, and bacteriology. 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, and were excluded if any sign was observed (Motta, 2008). Then the teats were disinfected using cotton wool soaked in alcohol 70%. The initial two-to-three squirts of milk stripped from the udder half were discarded and the next ones were collected onto a paddle in order to perform the California Mastitis Test (Blowey and Edmondson, 2010) and CMT score was recorded immediately prior to the sample draw at the beginning of the trial. Then, individual milk samples per animal were aseptically collected in sterilized tubes, kept in icebox and transported to milk analysis laboratory of the Lebanese university, faculty of agriculture-Dekwaneh-Lebanon, and then stored in refrigerator at 4°C before being analyzed within 24 hrs of sampling. For analysis of milk composition, a sampleofapproximately100 ml was collected in order to analyze fat using Gerber (ISO 2446/IDF 105 and Fernando, 1978), and total protein using Kjeldahl Methods (ISO 8968 /IDF 20-1; 20-3; Graff in and Horwitz, 1988). As well as samples was compared using an Ultrasonic Milk Analyzer machine (Lactoscan, Navo Zagora, Bulgaria)for analysis of lactose, protein and lipid content for more accurate results. All measurements were made in duplicate. Moreover, 50 ml of milk sample collected aseptically from each mammary gland before milking in a sterile plastic container and examined on the same day for the bacteriological isolates of the bacterial load in raw milk samples as total aerobic bacterial count TABC using standardized protocols based on the NMC guidelines for bacteriological culture (NMC, 1999), which was carried out by the Standard Plate Count (SPC) of raw milk that gives an indication of the total number of aerobic bacteria present in the milk at the time of pickup. Where milk samples were plated in a semi-solid nutrient media (nutrient agar system remains the gold standard) and then incubated for 48 hrs at 37°C to encourage bacterial growth. Single bacteria (or clusters) grew to become visible colonies that were then counted. All plate counts were expressed as the number of colony forming units per milliliter (CFU/ml) of milk. Newer films based tests have

allowed for automation of this procedure. Milk was collected daily from each cow of each group, and the total of the week of each group was calculated then the result was reported as mean value of head per day (in l/head/day).

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 yield, milk fat, protein and lactose percent, and total bacterial count). The statistical model included the factorial effect of the experimental factors tested over time. Statistical significance was considered at $P<0.05$.

RESULTS

Lactose content

The graph of figure 1 represents the evolution of the lactose content as a function of treatment and time. The results show significant difference as function of treatment ($p <0.001$), time ($p <0.001$) and time x treatment effect ($p <0.001$) between groups. However, according to this graph, it is first noted that the lactose content of the experimental group (ZM +), which has been supplemented in the diet, is higher than that of the control group (ZM-) all along the experience, which already shows a positive effect of Zn-methionine supplementation on milk. In addition, this percentage of lactose increases significantly after one month of supplementation, and this during the month of July, corresponding to the date when we doubled the amount of Zn-methionine, to pass from 4 to 8 g / head / day. Specifically, a significance from day 35 appeared between the 2 groups (3.80 ± 0.04 % for ZM- vs. 4.40 ± 0.04 % for ZM+, $P <0.001$) followed by day 42 with (4.00 ± 0.03 % ZM- vs. 4.30 ± 0.04% ZM+, $P <0.001$); and day 50 with (4.00 ± 0.04% ZM- vs. 4.50 ± 0.04% ZM+, $P <0.001$) and finally day 57 with (3.90 ± 0.04 % ZM- vs 4.40 ± 0.04% ZM+, $P <0.001$). Moreover, milk lactose content was also significantly increased with time effect especially after one month of treatment in ZM^+ group (from 4.10 ± 0.06 day 1, to 4.40 ± 0.04 % day 57; $p<0.001$).

Fat content

The graph in Figure 2 shows the evolution of fat content as a function of treatment and time. The results obtained show no significant difference of fat content between groups (5.40 ± 0.31 % ZM- vs. 5.44 ± 0.31 % ZM+; $p> 0.05$) with treatment effect, neither with time effect, nor with time x treatment effect. Previously, with the exception of the two dates of the experiment (day 1 and day 29), the fat content of the experimental group ZM+ appears to be slightly higher than that of the control group ZM- ($p> 0.05$). In our study, we observed that in the experimental group ZM+, the fat content increased slightly, reaching a maximum of 5.6 ± 0.29 % on day 57. While in the other control group ZM-, the rate fluctuated between 5.23± 0., 39 and 5.5± 0.,43 % during the study.

Protein content

The graph in Figure 3 shows the evolution of the protein content as a function of treatment and time. The variation of the protein content was significant between groups compared to the treatment effect (2.71± 0.03 % ZM- vs 2.93± 0.03 % ZM+, $P <0.001$). Also, a significant difference was observed between groups with time effect especially during the second half of the study (2.70 ± 0.04 vs 3.00 ± 0.04, day35; 2.90±0.05 vs 3.20 ± 0.05, day 42; 2.60 ± 0.05 vs 3.10± 0.05, day 50and 2.80 ± 0.05 vs 3.20 ± 0.05,day 57 for ZM- vs ZM+ respectively; $P <0.001$). In the same way, these values were also significant compared to the time x treatment effect ($P <0.001$). Therefore, according to the Libnor and European standards, the protein content of raw cow's milk must be between 3.00 and 4.00 %. In our experiment, the protein level was lower than normal at the beginning of the experiment in both groups. This rate remained slightly below 3.00 % throughout the experiment for ZM- group. On the other hand, the group ZM +, had a rate of 2.70 ±0.08% on day 1 which increased as of the day 29 to reach a maximum value of 3.2±0.05% on day 57 (figure 3), which is a value acceptable by the dairy industries and according to Lebanese and European standards.

Milk yield

The graph in Figure 4 represents the evolution of milk yield as function of treatment and time. The results of milk production show significant values over time ($P <0.05$). While these values were not significant compared to treatment effect (16.30 ± 0.67 vs. 17.18 ± 0.67 l/day, ZM- vs ZM+, $P > 0.05$). Moreover, these results were very significant compared to the time x treatment effect ($P <0.001$). However, the average milk yield of the control group ZM- was 16.40±0.80 l / day at day 1, and remained almost the same until day 57 with some fluctuations. While, in the experimental group ZM+, the milk yield shows a significant increase over time ($P <0.05$), with 16.00 ± 0.80l / day at day 1, and reach 18,86 ±0.71l / day at day 57.

Total Aerobic bacterial count TABC in milk

The graph of Figure 5 represents the evolution of the total aerobic bacterial count TABC of the milk as a function of the treatment and the period of the experiment. Results for total aerobic bacterial count were only significant during the month of July, with time effect ($P <0.001$), and time x treatment effect ($P <0.05$). In contrast, no significant difference was observed with treatment effect (1.34 ± 0.12 vs. 1.03 ± 0.12 x 10^5 CFU/ml, ZM- vs ZM+; $P> 0.05$). In fact, our results show that the control group ZM- had values which vary between 1.20 ± 0.15 and 1.54 ± 0.17 $\times 10^5$ CFU / ml with time effect. These values are reported for average milk quality according to the European standard. Moreover, in the experimental group ZM+, a decrease in the total number of aerobic bacteria is clear, especially in the second half of the study from day 29 with 1.07 ± 0.16 $\times 10^5$ CFU / ml, to reach values below 1.00 x10^5CFU / ml (0.70 ± 0.13x 10^5CFU/ ml) which correspond to standard values of normal quality milk according to Libnor and European standards. According to time effect, a significant difference ($P <0.05$) is shown between the 2 groups especially from day 35 until the end of the study.

DISCUSSION

The results of the present study indicate that subclinical mastitis negatively affects and Zn-methionine positively affects milk composition and milk yield, as shown for lactose, protein content, TABC and milk yield. The overall means were as follows for the control group ZM-comparing to the

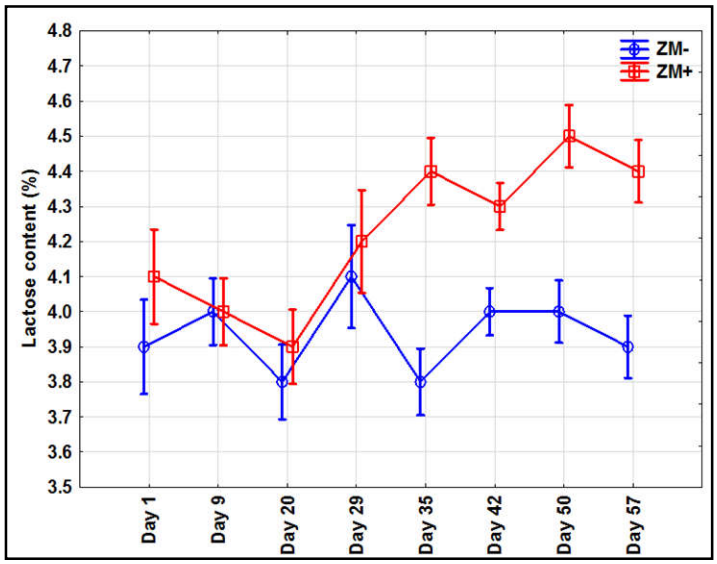

Figure 1. Means and their 95% confidence intervals (middle markers and vertical bars respectively) of the lactose content as a function of treatment (○ for ZM- vs □ for ZM +) and time

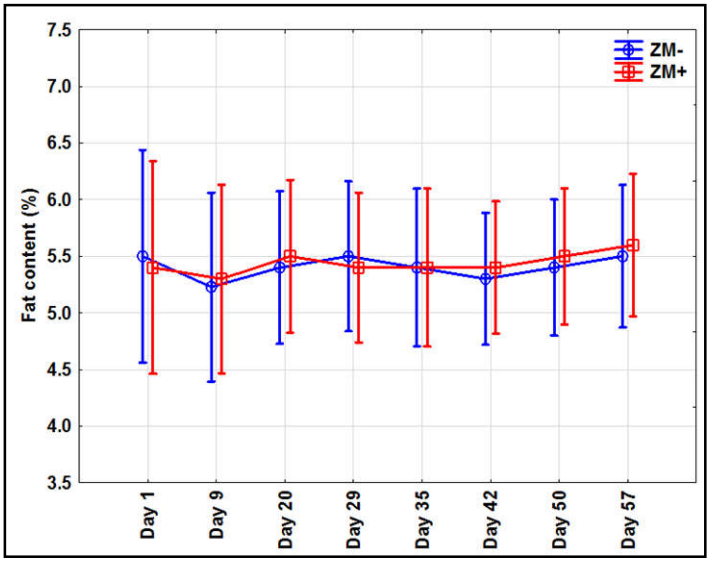

Figure 2. Means and their 95% confidence intervals (middle markers and vertical bars respectively) of the fat content as a function of treatment (○ for ZM- vs □ for ZM +) and time

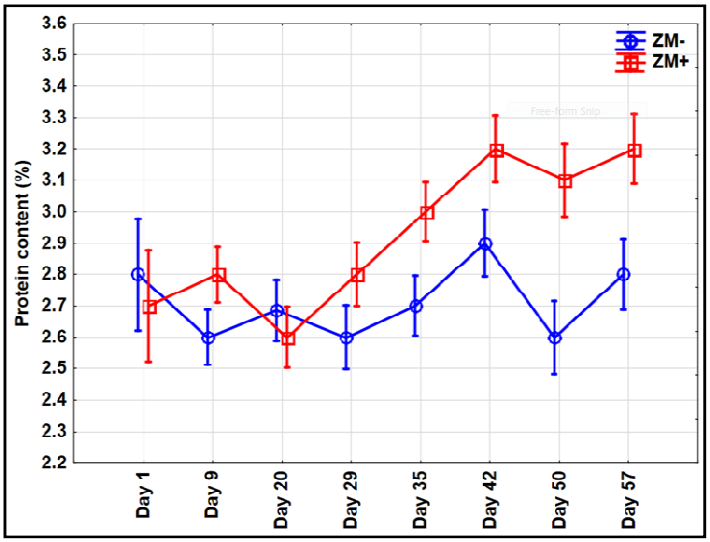

Figure 3. Means and their 95% confidence intervals (middle markers and vertical bars respectively) of the protein content as a function of treatment (○ for ZM- vs □ for ZM +) and time

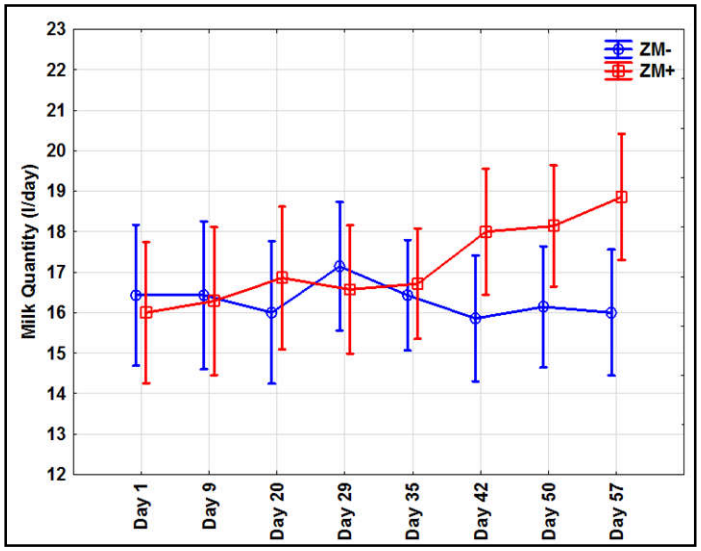

Figure 4. Means and their 95% confidence intervals (middle markers and vertical bars respectively) of the milk yield as a function of treatment (○ for ZM- vs □ for ZM +) and time

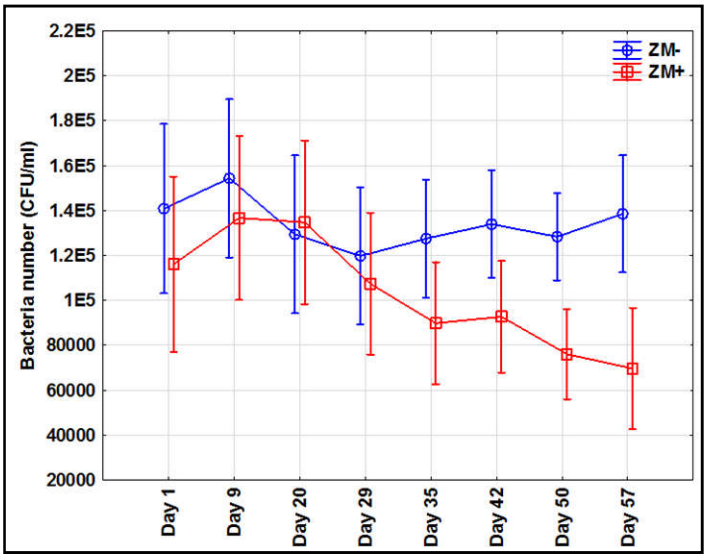

Figure 5. Means and their 95% confidence intervals (middle markers and vertical bars respectively) of the total aerobic bacterial count TABC (CFU/ml) content as a function of treatment (○ for ZM- vs □ for ZM+) and time

experimental group ZM+ (3.94 ± 0.03 vs4.23 ± 0.03 % of lactose, p <0.001;2.71 ± 0.03 vs 2.93 ± 0.03 % of protein, p <0.001 and 16.30 ± 0.67 vs 17.18 ± 0.67 l/day for milk yield for ZM- vs ZM+). The significant difference in lactose content (p <0.001) observed especially after 30 days of the beginning of the trial (day 35), can be demonstrated by the fact that Zn-methionine only begins to affect milk after a minimum of 30 days of assimilation in the body. During this period of adaptation, the organism adapts itself to the change of the ration and thus to the new integrated element, in order to find the means of assimilating it in the smallest time (Kincaid *et al*., 1997). Thus, for control group ZM-, the lactose values vary between 3.8± 0.05 % and 4.1± 0.07%. These values are lower than the standard value set by Libnor and European countries which is 4.00 to 5.00 % in raw cow's milk. While in group ZM+, the lactose content varies from 3.9± 0.05 % to reaches values> 4.00 % (4.50± 0.04 %), and becomes acceptable according to European and Lebanese standards. Previously, it is well accepted that mastitis causes a decrease in milk lactose concentration (Pyorala 2003, Harmon 1994). The decrease in lactose in affected neighborhoods is probably due in part to damage to alveolar epithelial cells.

Given the key role of lactose as an osmotic regulator of milk volume, it is unlikely that the reduced lactose levels are due to a reduction in synthesis and secretion at the cellular level and can only be decreased; there is an increased influx of electrolytes during mastitis (Bruckmaier, 2004). In addition, according to Auldist *et al.* (1995), many mastitis-causing organisms are able to ferment lactose. The lower concentrations of lactose in mammalian milk may be partly due to the activities of these organisms (Auldist *et al.*, 1995). Similarly, by Gaafar *et al.* (2010), which demonstrated a non-significant decrease in lactose content when the number of aerobic bacteriaincreased in subclinically infected cow's milk. This has been well demonstrated in our results obtained with lower lactose content in milk samples that have not been treated (with an average of 3.94 ± 0.03 vs 4.23 ± 0.03 %for ZM- vs ZM+group; p <0.001). The reason for improving the percentage of lactose in milk from treated cows is unclear. A theory possible explanation for increasing the percentage of lactose in the supplemented group is that chelated zinc participates in certain enzymatic cofactors in lactose production. Therefore, it may be inappropriate to dismiss this difference as not a treatment effect. The results of our milk

yield and composition variables were consistent with other published results (Hart *et al.,* 2014; Kessel *et al.,* 2014). Moreover, the effect of mastitis on the characteristics of milk fat has not been studied as extensively as milk proteins. There are contradictory results in the literature dealing with this issue. For example, Auldist and Hubble (1998) as well as Gaafar et *al.* (2010) report a decrease in fat concentration for milk infected by subclinical mastitis, but for other authors, free fatty acid concentrations were higher ($p < 0.05$) in infected quarters compared to normal ones (Ogola *et al*., 2007). In addition, the leaking of lactose from the milk will carry water and the volume of secretion left in the gland will decrease. The fat droplets, however, are large relative to the spaces between the cells and are contained in the cell and therefore their concentration increases. Also, it has been shown that at the genetic level, the longer a cow line gives milk fat, the more susceptible it is to mastitis. According to the European standards of cow raw milk, the fat content must be between 3.50%and 4.20%. Already, and according to our results obtained, the fat content is higher than normal in the control ZM- and experimental ZM+ groups (5.40 ± 0.31 % ZM- vs. 5.44 ± 0.31 % ZM+; $p> 0.05$). The studies that have been done on this subject are rare and contradictory hypotheses are emitted by several authors.

It has been shown that methionine, an essential amino acid in protein and lipid synthesis, as well as fat content, can increase, especially in high producing dairy cows. While Kellogg *et al.* (2004)does not agree that zinc affects the fat content, where milk composition did not differ between treatment with ZM and control cows. So to reject or confirm the hypothesis that chelated zinc has an effect on fat content, more research needs to be done on this subject. Further, according to Harmon (1994), it has been shown that subclinical mastitis has a negative influence on the quality of raw milk, in particular a decrease in protein (especially casein). As for the mastitis factor, the cow who had mastitis had a lower protein content than a normal cow. Also, Heck *et al.* (2009) has shown that one of the factors that influenced protein content was the season, where the lowest milk protein content was in June and the highest content in December. This made it possible to verify the effect of mastitis, and the seasonal effect on our results as regards the protein content of the non-supplemented control group ZM-, which are lower than normal throughout the period of the experiment (2.71 ± 0.03 % for ZM- below the Lebanese and European standard of 3.00%). The literature seems to be concordant about the modification of protein content. According to Ballantine *et al.* (2002), an increase in the protein content of milk is noted with the addition of zinc methionine. Moreover, Hackbart *et al.* (2010) reported that many factors like stage of lactation, age of the cow, the season and the mastitis can negatively affect milk protein content, while the treatment by replacing a portion of inorganic supplemental trace minerals with an equivalent amount of these organic trace minerals (Zn, Mn, Cu, and Co) tended to increase milk protein percentage. Thus, it may explain the milk protein values in our study that are lower than the standard values in the non-supplemented control group ZM-, whereas an improvement in this level was noted in the supplemented experimental group ZM+, due to the effect of the supplementation of the organic chelated complex Zn-methionine to the diet (2.71 ± 0.03 % ZM- vs 2.93± 0.03 % ZM+, $P <0.001$). However, the significant increase in values of group ZM+ especially at the end of the experimental period (3.20± 0.05% day 42, 3.10± 0.05 % day 50 and 3.20 ± 0.05 % day 57), this may be due to the positive effect of Zn added to the ration and its better assimilation to the organic chelated state by the animal's organism and its effect in keratinization and regulation of protein metabolism. Boland (2003)has shown that keratinization of the teat duct is a physical and chemical barrier to bacterial capture infections and reduced migration to the mammary gland. Previously, we must not forget the effect of subclinical mastitis on milk production. Gaafar *et al.* (2010) demonstrated the effect of increasing the total number of bacteria (from 89 x 10^3 to 178 x 10^3CFU / ml) in dairy cows infected with subclinical mastitis on the decrease in milk production (from 12.63 to 11.50 kg / cow / day, $p <0.05$).The loss of milk yield depends on the severity, the pathogen responsible, the parity of the cow and the stage of lactation at which the mastitis develops (Hackbart *et al*., 2010). Intra-mammary infection, although limited to subclinical levels, has been reported to negatively affect milk production (Lietner *et al.,* 2004). The author hypothesizes that part of the decrease in milk production is due to an increase in the immune system's energy demand for infection, a loss of appetite associated with inflammation and a decrease in food consumption. Similarly, in our study, the milk yield of dairy cows treated with organic chelated complex ZM+ group, increased significantly compared to that of the non-treated control group ZM-with time x treatment effect (from 16.00 ± 0.80 l / day at day 1, to 18,86 ±0.71l / day at day 57 for ZM+ vs 16.43 ± 0.80 l / day at day 1, to 16,00 ±0.71l / day at day 57 for ZM-, $p <0.05$). Results on animal performance versus milk production were also consistent with previous studies that reported an increase in milk yield of lactating dairy cows supplemented with chelated zinc, where essential amino acids such as methionine are often used (Kincaid *et al*., 1997). In another study, supplementation of dairy cow rations with chelated zinc increased milk yield (Kellogg *et al*., 2004). In addition, methionine is known to be a limiting amino acid for milk production, and the results obtained with zinc methionine showed low degradation during ruminal fermentation, that improves the availability of methionine for absorption in the small intestine (Kinal *et al.,* 2005). In our study, improving the availability of a limiting amino acid using chelated zinc appears to improve milk yield as well as lactose and milk protein content in the supplemented group of cows ZM+ compared to the control group ZM-. However, the fat content of the milk was not significantly affected by the chelated mineral ($p > 0.05$).

During our study, it has been reported that the number of subclinical mastitis cases had decreased in group of cows supplemented with zinc methionine ZM+ compared to the control group ZM-. These findings may indicate an increase in the immune response capacity that improved mammary gland status, for cows supplemented with ZM. Studies of the effect of organic zinc supplementation on somatic cells and total aerobic bacteria count have shown variable results. The authors (Pechova *et al*., 2006)reported a decrease in somatic cells count SCC and total aerobic bacteria count TABC in dairy cows supplemented with different sources of zinc; the somatic cell count SCC was significantly lower in the experimental group (114 ± 68.7 x 10^3 / ml vs 208.6 ± 148 x 10^3 / ml, $p <0.05$) at the end of the 3rd month according to (Pechova *et al*., 2006). This showed the positive effect of zinc supplementation on somatic cell count SCC. Moreover, Kinal *et al.* (2005) associated TBC reduction with rapid keratin formation in the teat canal provided by organic Zn supplementation. Thus, zinc also plays a role in stimulating T

cells in the immune response. Based on the results of this study, it can be concluded that feeding organic sources of Zn tends to reduce the total aerobic bacterial count TABC after 40 days of supplementation. The season can also have a significant effect on the total number of milk bacteria (Rodriguez *et al*., 2000). The stress of winter cold and the hot summer period significantly affects the total number of bacteria in cow's milk (Dakic *et al.,* 2006). This has been demonstrated from our results obtained in the control group (ZM-), where the TABC began to increase especially at the beginning of the summer period, more precisely at day 29 ($120.00 \pm 15.00 \times 10^3$ cfu / ml) until the end of the trial (to reach $139.00 \pm 13.00 \times 10^3$ cfu / ml at day 57 ($P < 0.05$). Likewise, the TABC tends to decrease with the progression of lactation to the second month and increased significantly ($P < 0.05$) thereafter with months of lactation. These results are consistent with those obtained by Ceron-Munoz *et al.* (2002) who found that the total number of bacteria decreased during the second month of lactation and increased thereafter until the ninth month of lactation. Also, Farghaly (2002) showed that the stage of lactation significantly affected the total number of milk bacteria, since somatic cell count in milk were highest just after calving, and dropped to a minimum between 40 and 80 days postpartum, then gradually increased until the end of lactation. This is in agreement with our results, with the low values of TABC obtained in both groups. Indeed, most cows in both groups were at the beginning or at the first middle of the lactation period.

Conclusion

This study made it possible to highlight the variability of the physico-chemical (protein, fat and lactose content) and bacteriological (TABC) properties as well as the milk production as a function of the intake of Zinc-methionine and the duration of treatment in a traditional extensive breeding of dairy cows in Ghineh region of Mount Lebanon. Based on our results, the addition of organic chelated zinc complex to the dairy cow diet for 2 months showed an improvement in TABC in the milk, as well as the quality (protein and lactose content) and the milk production. On the other hand, no significant difference ($p>0.05$) was observed in the improvement of fat content. In general, this improvement was significant ($p<0.05$) over time in terms of protein and lactose content, milk yield and TABC. Previously, this significance ($p<0.001$) became spectacular after about one month of supplementation (day 35) to the end of the experiment. This is due, on the one hand, to the increase of the amount of ZM supplemented from 4 to 8 g / cow / day on the second month (at day 29), and on the other hand, to the assimilation of this new substance in the ration by the organism. Thus, the digestibility and ingestion of the ration have been improved. Similarly, these results were also significant ($p < 0.05$) with treatment, and time x treatment effect for milk yield, lactose and protein content and TABC. Indeed, the supplemented group ZM+ had TABC significantly lower as of the 5th week of supplementation (day 29) compared to the control group ZM-. Similar to the lactose and protein content, the treated group ZM+ had significantly higher percentages than the control group ZM-. However, the fat content did not vary significantly either with time or with treatment. Based on these results, it can be concluded that the supplementation of Zn methionine has positive effects on the quality, the quantity of milk, as well as the performance of dairy cows. Further studies should be conducted to determine the optimal dose in g per day for each animal that gives the best results without any side effects. Also studies on the addition of zinc-chelated with other amino acids than methionine, and the addition of other trace elements at the same time and their effects on the environment can be interesting topics.

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.

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