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Effect of Feeding a Combination of Zinc, Manganese and Copper Methionine Chelates of Early Lactation High Producing Dairy Cow

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ABSTRACT

The objective of the study was to compare the effect of feeding mixed chelated minerals (Mn, Cu and Zn) methionine on dairy cow productive performance and milk yield and its components. The trial was conducted with dairy cows across various stage of lactation. The experimental treatments include chelated minerals (15 mg Zn as Zn Met, 20 mg Mn as Mn Met, 10 mg Cu as Cu Met). Inorganic mixture contains (15 mg Zn as $ZnSO_4$, 20 mg Mn as $MnSO_4$, 10 mg Cu as $CuSO_4$) in sulphate forms. The experiment was commenced in the dry period of cows, 6 weeks before calving, and after calving the first three months of lactation was taken into consideration. Milk samples were collected from each cow evening and morning for estimation of milk yield production. The inorganic metals caused a significant decline ($P < 0.05$) in digestibility coefficients, nutritive value, nitrogen utilization, cell wall constituents, total VFA's, rumen volume, microbial and nitrogen synthesis compared to the organic metals. The treated group (chelated minerals) improved the milk yield, and the milk fat percentage of animals across various stages of lactation as compared to inorganic minerals treated group of animals, and no significant differences were observed among groups concerning the entire blood constituent.

Keywords: Milk Yield; Chelated Minerals; Dairy Cows; Milk Production

1. Introduction

The benefits of supplementation organic trace minerals in dairy diets have been demonstrated in research and in the field. Traditionally inorganic forms of trace minerals rapidly dissociation in the rumen and are free to interact with antagonists, resulting in the loss of the trace minerals prior to absorption by the animal [1,2]. Chelated organic trace minerals are bound to organic ligands through coordinate covalent bonds. The bonds between the ligand and the mineral can prevent the minerals from interacting with antagonists and improve the bioavailability of the mineral [2,3]. The basic reason for the use of organic forms of trace minerals is the increased bioavailability of organic vs inorganic sources of the minerals.

In addition to vitamin, protein, and energy requirements, the dairy cows also needs certain trace elements including zinc, copper, and manganese, plus the microelements magnesium and potassium for lactation and reproduction concurrent with growth and maintenance of body tissues [4,5]. During periods of dietary mineral insufficiencies, essential minerals will be cannibalized from body tissues to support milk production, which ultimately affects the quality and quantity of milk as well as reproduction [6,7]. [8] Demonstrated that supplemental metal amino acid chelates (AACs) were more bioavailable than inorganic metal salts (IOMs) and resulted in improved reproductive performance. Thirty days before expected parturition they supplemented the feed of dairy cows with AACs and IOMs and continued supplementation into lactation until the animals in the study became pregnant with a second calf. Cows receiving the AAC supplement conceived 48 days earlier than heifers receiving the IOM supplement (90 days versus 138 days) ($p < 0.05$) and experienced 45% less early embryonic mortality (EEM) ($p < 0.05$) [8]. Other researchers have also reported that AAC supplementation enhanced reproductive performance, but their studies have tended to focus on multiparous cows [9,10].

A few studies have been undertaken in sheep. Those of Spears [11,12] corroborated each other in that each found that absorption was essentially identical for inorganic and chelated zinc, being about 40%, but that the chelated

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form of zinc was retained better ($p < 0.05$) than the inorganic form of zinc.

2. Materials and Methods

2.1. Animal and Experimental Design

Twenty cross-Friesian dairy cows with an average initial weight of 515 kg were used in the study. Care and handling of the animals and sampling procedures described herein were approved by the El-Nubaria Research Station of Animal Production Research Institute, Alexandria, Egypt.

The experiment was commenced in the dry period of cows, 6 weeks before calving, and after calving the first three months of lactation was taken into consideration. The cows were divided by an analogue method into two experimental groups (10 animals in each), and the number of lactation, milk yield for previous lactation, percentage proportion of HF genes and calving date were considered.

2.2. Experimental Diets

Table 1 shows the chemical composition of the diets during the experimental.

All cows were fed the same diets during the experiment: maize silage, concentrate fed mixture (CFM), rice straw (RS) and mineral-vitamin mixture. The amount of fodders was different in dry and lactation period. The daily rations of feed covered cows' maintenance requirement and requirement for the production of 10 kg milk. The cows producing more than 10 kg milk/day were additionally fed 1 kg of concentrate per each 2 kg of surplus milk. The concentrate was produced in a local feed mill belonging to an Experimental Station in Pawlowice according to our formula. The basal components were maize grains (40%), soybean meal (17%), wheat bran (25%), cottonseed meal (8%), molasses (6.5%), salt (1.5%) and limestone (2%). The feeding groups were diversified with respect to the supplementary mineral mixtures which contained Zn, Cu, and Mn in sulphate or methionine forms given individually to the animals at a dose of 2 g/day/head: Group I—control Mineral-vitamin mixture contains (15 mg Zn as $ZnSo_4$, 20 mg Mn as $MnSO_4$, 10 mg Cu as $CuSO_4$) in sulphate forms and 2500 IU of vitamin A, 900 IU of vitamin D_3, and 5 IU of vitamin E. Group II—experimental Mineral-vitamin mixture contains (15 mg Zn as ZnMet, 20 mg Mn as MnMet, 10 mg Cu as Cu Met) in organic forms and 2500 IU of vitamin A, 900 IU of vitamin D_3, and 5 IU of vitamin E.

2.3. Milk Collection and Analysis

Milk samples were collected from each cow evening and morning for estimation of milk yield production by [13]. A sample of milk (100 ml) was taken from two consecutive milking. Milk samples were chemically analyzed for total solid (TS), protein, fat and ash according to [13] while lactose was calculated by differences.

Table 1. Chemical composition and fiber fractions of concentrate fed mixture (CFM), rice straw (RS) and corn silage (CS) (% on DM basis).

Item	CFM	RS	Corn silage
DM	89.62	90.48	30.83
OM	94.83	87.95	91.54
CP	15.88	3.81	8.14
CF	7.84	38.22	24.83
EE	2.93	0.94	1.43
NFE	68.18	44.98	57.14
Ash	5.17	12.05	8.46
NDF	23.65	67.86	52.75
ADF	15.87	49.88	39.54
ADL	5.32	19.75	12.77
Hemicellulose	7.78	17.98	13.21
Cellulose	10.55	30.13	26.77

2.4. Feces Collection and Analysis

For the digestibility trials three adult male Barki sheep weighing approximately 52.50 ± 2.00 kg BW, were housed in metabolic cages for each trial. Sheep were kept on the diets for a preliminary period of 21 days, and during the next 7-day total feces and urine were collected. Subsamples (20%) of feces and urine were taken once daily and were frozen until analyses.

Fecal samples were dried at 60˚C for 72 h. Feed and fecal samples were ground through 1 mm screen on a Wiley mill grinder and the sample (50 gm/sample/treatment/sheep) were composed for analysis. The samples of feed and feces were analyzed for crude protein (CP), crude fiber (CF), Ether extract (EE) and ash, while the urine sample output for each sheep was analyzed for nitrogen (N) according to [14]. Values of the total digestible nutrients (TDN) were calculated according to the classic formula of [15] on a dry matter basis (DM).

2.5. Rumen Liquid Collection and Analysis

Rumen liquid samples taken at 0, 3 and 6 h post feeding from three fistulated adult female Barki sheep weighing approximately 47.50 ± 0.50 kg BW for each treatment, were analyzed immediately for pH using Orian 680 digital pH meter. Samples were strained through four layers of chesses cloth. For each sampling time, rumen fluid samples were preserved for ammonia nitrogen (NH_3-N) determination by adding concentrated H_2SO_4 (3 drop per 5ml). The concentration of NH_3-N was determined by using magnesium oxide (MgO) as described by [13]. Total volatile fatty acid (VFA's) concentration was estimated by using steam distillation methods [15].

Cell walls were analyzed for neutral detergent fiber NDF, acid detergent fiber ADF and acid detergent lignin ADL using Tecator Fibretic system. Hemicellulose and cellulose were determined by difference between NDF and ADF, and ADF and ADL, respectively according to [16].

2.6. Blood Collection and Analysis

Blood samples were collected twice (once before the experimental beginning and the other once at the end of the experimental period, from all cows. Blood samples were obtained from the external jugular vein of the animals in the morning before access to feed and water. Plasma or serum were obtained by centrifugation of blood and were stored at –20˚C until used for analysis. Serum total protein (TP) was measured as described by the Biuret method according to [17]. Albumin (A) concentration was determined according to the method of [18]. Kidney function was evaluated by measuring blood urea using the colorimetric methods of [19] using commercial kits. Liver function was assessed by measuring the activities of aspartates aminotransferase (AST) and alanine aminotransferase (ALT) by method of [20].

2.7. Statistical Analysis

Means were calculated for all variables by cow within period. Data were analyzed using the mixed procedure of SAS (SAS, 1999). Period and cow were considered random effects; diet and cannulation effects were considered fixed. Estimation method was restricted maximum likelihood and the degrees of freedom method was Kenward-Roger [21]. Differences were tested using the PDIFF option in SAS [21]. Differences were declared significant at a $p < 0.05$; and trends were discussed at a $p < 0.15$, unless stated otherwise.

2.8. Aim of the Work

Comparing the effect of feeding chelated minerals (Mn, Cu and Zn methionine complexes) and mixture of inorganic metals (Mn, Cu and Zn sulphate) on dairy cow productive, performance of milk and its components.

3. Results and Discussion

3.1. Digestibility Coefficients, Nutritive Values and Nitrogen Utilization

The data on intake, digestibility of different nutrients during metabolism trial and data of daily nitrogen intake, excretion and retention are presented in **Table 2**. The DMI was comparable ($p < 0.05$) at 63.19 and 65.74 in inorganic and organic metals respectively. Digestibility of (DM, OM, CP, EE, NDF, hemicelluloses and cellulose) and total digestible nutrients (TDN and DCP) were significantly ($p < 0.05$) higher in organic metal as compared to inorganic metal groups. supplementation of organic metal had a great effect on DMI as compared to inorganic metals (**Table 2**) contrary to our observation [22] reported a linear decrease in the DMI with the increasing concentration 20, 100 and 200 mg/kg DMI of Zn in the diet (having 70 mg/kg) in beef steers. But it might be due to very high intake of organic Zn (being 90, 170 and 270 mg/kg) DM, respectively, in these animals. However, [23] did not find any effect on DMI in group calves, when level of organic Zn was increased from 26.21 to 85.67 mg/kg DM in their diet. Supplementation of Zn methionine to a basal diet containing more than 25 mg Zn/kg DM had no effect on feed intake ewes. Feed intake was not significantly affected by Zn source or level during the experiment [24]. The feed intake tended to drop less in zinc methionine fed calves challenged with infectious Bovine Rhinotracheits (IBR) compared to ZnO fed calves. The calves fed 30 ppm Zinc methionine had lower ($p < 0.05$) feed intake compared to calves receiving 90 ppm zinc. While in recovery from depressed feed intake due to IBR fever was slower for ZnO fed calves compared to calves fed zinc methionine. The differences among groups were significant [25]. Reported that newly arrived feedyard calves fed Fourplex (a feed additive from Zinpro Corporation that contains copper lysine, zinc methionine, manganese methionine and cobalt glucoheptonate) required fewer ($p < 0.05$) medication days, but Fourplex did not improve average daily gain or feed intake, or reduce mortality [26,27]. They reported that copper lysine and copper sulfate performed the same [28]. Reported no effect of zinc methionine added to a basal diet when lamb average daily gain was measured. During gestation, zinc methionine fed ewes had higher ($p < 0.05$) feed intake, but no such effect was seen in lactation. Sheep fed zinc methionine ration as 15 mg Zn showed higher ($p < 0.05$) apparent digestibility DM, OM, CP, CF, EE, NFE, NDF, ADF and nutritive values TDN and DCP than those fed other rations. These results are consistent with zinc methionine being absorbed more efficiently than $ZnSO_4$ when supplemented at high concentration. Increased uptake of Zn from zinc methionine could be

Table 2. Feed intake, digestion coefficients and nutritive values of experimental rations fed to sheep (mean ± SE).

Item	Inorganic metals (Mn, Cu and Zn) sulphate	Organic metals (Mn, Cu and Zn) methionine complexes
DM intake, g/h/d		
CFM	672.15 ± 22.65[a]	656.45 ± 15.77[b]
Corn silage	369.92 ± 19.38[b]	385.33 ± 0.21.84[a]
Rice straw	162.86 ± 0.96[b]	180.96 ± 1.73[a]
Total DMI, g	1204.93 ± 16.59[b]	1222.74 ± 23.14[a]
R:C ratio	44:56	46:54
Digestion coefficients (%)		
DM	63.19 ± 0.38[b]	65.74 ± 0.25 [a]
OM	63.73 ± 0.42[b]	66.20 ± 0.34[a]
CP	57.83 ± 0.28[b]	60.20 ± 0.24[a]
CF	55.47 ± 0.39[b]	59.51 ± 0.31[a]
EE	76.92 ± 0.16[b]	78.24 ± 0.43[a]
NFE	66.69 ± 0.47[b]	68.84 ± 0.22[a]
NDF	62.66 ± 0.48[b]	64.27 ± 0.28[a]
ADF	57.97 ± 0.27[b]	60.28 ± 0.37[a]
ADL	43.27 ± 0.39[b]	47.29 ± 0.24[a]
Hemicellulose	65.27 ± 0.72[b]	67.29 ± 0.25[a]
Cellulose	67.28 ± 0.22[b]	69.23 ± 0.38[a]
Nutritive values (%)		
TDN	61.31 ± 0.46[b]	63.56 ± 0.26[a]
DCP	6.87 ± 0.11[b]	7.05 ± 0.16[a]
Nitrogen utilization (g/h/d)		
N-Intake	22.89 ± 0.14	23.20 ± 0.32
N-Absorbed (NA)	13.24 ± 0.29[b]	13.97 ± 0.15[a]
N-Retention (NR)	4.61 ± 0.17[b]	6.79 ± 0.23[a]
NR % of NI	20.16 ± 0.31[b]	29.27 ± 0.28[a]
NR % of NA	34.85 ± 0.47[b]	48.62 ± 0.36[a]

[ab]Means within rows with different superscript are significantly differ ($p < 0.05$).

explained by zinc methionine interacting less than $ZnSO_4$ with antagonists that form insoluble complexes. Alternatively, Zn from zinc methionine may have been associated with ligands that facilitated Zn uptake in the duodenum. Metal ions may be absorbed as part of metal peptide complex, thereby facilitated absorption of Zn via intestinal transport mechanisms distinct from inorganic zinc [29]. Data of nitrogen of organic metal was the highest (6.799), the mean that treatments improved nitrogen balance. There were reflected in better ($p < 0.05$) N-utilization of the ration fed to sheep. It may be possible for metal ions to be transported into the intestinal mucosa as part of metal peptide complexes via mechanism distinct from ionic Zn [30]. [31] reported increased N-retention with the increasing dietary concentration of Zn from 40 to 70 mg/kg DM in dry cows. But contrary to our finding [32-35], did not find any effect of Zn supplementation on N metabolism. It appears that there is a three should level of Zn needed in the diet for optimum N metabolism and supplementation above which has no further impact. The basal diet in the present experiment contained 15 mg as zinc methionine, 20 mg manganese as manganese methionine, 10 mg copper as copper methionine in organic forms, which might have been sufficient for optimum nitrogen metabolism in the cattle.

3.2. Rumen Liquid Collection and Analysis

Resulted of **Table 3** indicated that rumen liquor pH values did not significantly differ among treatments. Organic metals sources tested in the present study showed different degrees acidity. When saturated solutions were prepared in deionized water, the pH of the solutions decreased. The consistency of pH readings can be used as one criterion to test product uniformity from batch to batch [36]. The amount of organic metals that could be dissolved in deionized water varied than inorganic metals in the present experiment indicating different degrees of solubility. The NH_3-N were significantly ($p < 0.05$) higher in inorganic metals than organic metals. Sheep in organic metals treatment had higher ($p < 0.05$) total VFA concentrations than those in the inorganic metals. Alternatively metals from organic source may have been taken up by ruminal microorganism to a greater extent, and this

Table 3. Rumen liquor parameters of sheep fed the experimental diets (mean ± SE).

Item	Inorganic metals (Mn, Cu and Zn) sulphate	Organic metals (Mn, Cu and Zn) methionine complexes
PH	6.57 ± 0.11	6.45 ± 0.05
NH_3-N (mg/100ml)	14.16 ± 0.16[b]	13.32 ± 0.23[a]
Total VFA’s ml equv/100ml	8.28 ± 0.27[b]	10.98 ± 0.11[a]
Rumen volumes (L)	3.17 ± 0.17[b]	3.88 ± 0.13[a]
Rates of outflow (% hr)	6.07 ± 0.13[b]	5.38 ± 0.09[c]
Microbial protein synthesis (g/h/d)	26.75 ± 0.45[b]	29.11 ± 0.62[a]

[ab]Means within rows with different superscripts are significantly different ($p < 0.05$).

could explain the lower ruminal soluble metal concentration in steers fed inorganic metals. Steers supplemented with zinc proteinate [37] or a zinc polysaccharide [38] also had higher ruminal soluble zinc concentration than those receiving inorganic zinc oxide. The higher total VFA concentration observed in steers supplemented with zinc methionine or zinc glycine compared to animals fed the $ZnSO_4$ treatments could relate to a slower rate of feed consumption or reduced rate of ruminal digestion. Extremely high concentrations (1142 mgZn/kg) of $ZnSO_4$ have been shown to affect ruminal protozoa numbers and degradation of feed protein [39]. high dietary concentrations (250 - 1142 mgZn/kg) of organic Zn have also increased molar proportion of propionate [40]. The effect of more physiological additions of Zn on ruminal fermentation has received little attention data of rumen volumes, rates of outflows and microbial protein synthesis are presented in **Table 3**. The differences among groups were significant. The organic metals rations were higher ($p < 0.05$) values of rumen volumes and microbial protein synthesis than inorganic metals, while the both of organic metals were the lower ($p < 0.05$) values of rates of out flow than inorganic metals. Ruminal microbial protein synthesis depends on supply of adequate amounts and type of carbohydrates (CHO) as an energy source for the synthesis of peptide bonds [41]. Synthetic amino acids or amino acids precursor (Methyl Hydroxy Analogue: MHA) are also used as ligands in chelating trace minerals. The definitive advantage of MHA is that it is non-degraded in rumen as there is no nitrogen atom in its chemical structure and hence rumen microbes do not recognize it as a source for microbial protein synthesis escapes the rumen degradation. Moreover, the molecular size of MHA chelated is below 400 Dalton which facilitated its efficient absorption through intestine.

3.3. Milk Yields and Milk Composition

Data concerning milk yield and its composition are presented in **Table 4**. The milk yield and fat corrected milk (FCM) were significantly increased ($p < 0.05$) for the organic metals ration compared with inorganic metals ration. The average daily milk yields were increased by 11.10% in organic metals ration than inorganic metals. However, improving nutrients composition, its digestibility and the feeding values of organic metals ration was reflect on the more %FCM produced by dairy cattle fed the ration which had about 12.75% more 4% FCM than the inorganic metals. In addition to about 7% and 7% more protein and fat produced, respectively. Similarly, in the group of animals receiving methochelated bestmingold in mid and late stage of lactation also showed an increase in milk fat percentage by 0.2% which is highly significant compared to that in inorganic mineral treated group of animals (0.08%). It has been suggested that organic minerals have an increased bioavailability, resulted in an increased absorption in the gastrointestinal tract [42]. This may lead to an improvement in performance or health or may reduce the level of mineral supplementation required in the diet [43] who reported an increased milk production in animals receiving diets containing organically complexed minerals and a mixture of inorganic and organically complexed minerals. The above results are thus in agreement to the researchers that feeding of organic minerals improves milk yield and performance of dairy cows. Improvement in milk production and component can be partially attributed to improved udder health and reduction of sometic cell count. From previous study [44] reported that sometic cell count was reduced by addition of Zn methionine complex to the diet, this refer to that Zn methionine plays integral role in immune function by activating T-lymphocyte responsiveness, thus impacting the effectiveness of somatic cells within the mammary gland.

Table 4. Milk yields and milk composition of lactating cows fed on experimental rations (mean ± SE).

Item	Inorganic metals (Mn, Cu and Zn) sulphate	Organic metals (Mn, Cu and Zn) methionine complexes
Milk yields, kg/d	13.78 ± 1.28^b	15.31 ± 1.65^a
4% FCM*	13.01 ± 0.43^b	14.67 ± 0.34^a
Fat	0.50 ± 0.06^b	0.57 ± 0.04^a
Protein	0.44 ± 0.03^b	0.51 ± 0.02^a
Milk composition (%)		
Total solids	11.47 ± 0.54^b	11.85 ± 0.46^a
Solids not fat	7.85 ± 0.25^b	8.12 ± 0.42^a
Fat	3.62 ± 0.28^b	3.73 ± 0.26^a
Protein	3.18 ± 0.16^b	3.31 ± 0.12^a
Lactose	3.54 ± 0.16^b	3.77 ± 0.13^a
Ash	1.13 ± 0.06^a	1.04 ± 0.03^b

[ab]Means within rows with different superscript are significantly differ ($p < 0.05$); *4% FCM was calculated as: 0.4 × milk yield (kg) + 15 × fat yield (kg) (Overmann and Fanmann, 1926).

3.4. Blood Biochemical and Serum Constituents

The average values of some blood constituents in the blood of dairy cattle consuming the different experimental rations are presented in **Table 5**. No significant differences were observed among groups concerning the entire blood constituent. Moreover, they were within the

Table 5. Blood serum parameters of lactating cows fed experimental ration (mean ± SE).

Item	Inorganic metals (Mn, Cu and Zn) sulphate	Organic metals (Mn, Cu and Zn) methionine complexes
Glucose mg/dl	88.78 ± 1.86	90.14 ± 0.95
TP g/dl	8.28 ± 0.23	8.42 ± 0.39
Albumin g/dl	4.88 ± 0.11	4.64 ± 0.22
Globulin g/dl	3.40 ± 0.36	3.78 ± 0.45
Urea mg/dl	39.22 ± 0.95	37.07 ± 1.35
AST U/L	44.75 ± 1.37	44.18 ± 1.33
ALT U/L	13.43 ± 0.67	13.65 ± 0.79

There is no significant differences within either rows.

normal average as described by [45]. [46] compared zinc methionine, zinc sulfate and zinc oxide in 32 yearling cattle and detecting no difference on all parameters measured, which included serum, liver, pancreas, kidney, bone, bone marrow, hair and muscle, metallothionine levels likewise were not different. Working with calves, [47] reported increased ($p < 0.05$) expression of histocompatibility complex class for copper lysine fed animals compared to controls fed copper sulfate. In a companion study, these same workers [48] related that copper lysine increased ($p < 0.05$) plasma copper and monocyte phagocytic activity compared to control, but controls received only half as much copper as the copper lysine group (43 v 104 ppm).

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