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EFFECT OF FEEDING DIETS SUPPLEMENTED WITH ZINC, COPPER AND SELENIM PRE OR POSTPARTUM ON MILK YIELD AND SOMATIC CELL COUNT OF FRIESIAN COWS

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SUMMARY

The objective of this study was to evaluate the effects of adding Zn, Cu and Sein the ration of dairy cows on milk yield, somatic cell count (SCC) and blood parameters. A total of 39 Friesian cows between the 1st and 3rd parity and average live body weight (LBW) of 532.7±23.5 kg were divided into three similar groups (n=13 each) Mutiparous cows (n=10 in each group) were divided according to their BW, parity and milk production of the previous season, while primiparous cows (n=3 in each group) were allotted based only on their BW. Cows of the 1st group (G1) were fed concentrate feed mixture (CFM), rice straw and corn silage (control). Cows of the 2nd group (G2) received 60 mg Zn, 20 mg Cu and 0.3 mg Se /kg diet at 30 day pre-partum to calving, while those of the 3rd group (G3) received the same dose of (G2) but from day one to 60 days of postpartum in the ration. Throughout the experimental period, cows were machine milked and daily milk yield was individually recorded for the 1st four months. Milk composition, blood samples and lymphocytes, monocytes and granulocytes (immune response) were determined. Milk samples for SCC determination were collected biweekly from 10 days after lactation until 90 days of lactation. Results revealed that Zn, Cu and Se diet improved ($P<0.05$) daily milk production of G2 and G3 by 22.7 and 12.0%, respectively compared with G1. G3 had higher ($P<0.05$) percentages of fat, protein and lactose as compared to G1. G2 had higher ($P<0.05$) percentages of fat and lactose compared to G1, while protein percentage was similar to G1. Diet supplemented with Zn, Cu and Se (G2 and G3) showed lower ($P<0.05$) somatic cell count in milk as compared to G1. Increase SCC lead to decrease milk yield, fat, protein and lactose percentages. Cows of treatments G2 and G3 were higher in red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin (Hb) concentration and haematocrit percentage (HCT, %) than those of the control group. Cows treatment were higher significantly lymphocytes and significantly lower moncytes than control group. All protein fraction and Se concentrations were higher in G3 and G2 than in control group (G1), however, significantly higher in G3 than in G2. But, plasma Zn and Cu concentration were higher significantly ($P<0.05$) in G2 and G3 than the control group (G1).

Keywords: Friesian, lactation, somatic cell count, milk yield and trace minerals.

INTRODUCTION

Trace elements such as Manganese (Mn), Copper (Cu), Iron (Fe), Iodine (I), Selenium (Se) and Zinc (Zn), are essential in animal nutrition and are needed in very small amounts for essential metabolic reactions in the body. Their deficiencies are often associated with alterations in many metabolic processes and cause various kinds of diseases. Deficiency of these trace elements causes severe economic loss due to increased susceptibility to oxidative stress, growth retardation in young animals, anemia (Bureau *et al*., 2008), decrease in feed efficiency and fertility (Grenier *et al*., 2003), enhance the virulence of the infectious agent (Failla, 2008) and decrease immune system function (Knutson and Wessling-Resnick, 2003).

Copper has a basic role in the metabolism and transition of Iron in the body. It is an essential component of several enzymes such as Ceruloplasmin, Cytochrome Coxidase, Lysil oxidase, Superoxid dismutase, and Tyrosinase, that are required to maintain host homeostasis (Swenson and Reece, 2004). Deficiency has been linked to a variety of clinical signs, including anemia, pale coat, spontaneous fractures, poor capillary integrity, myocardial degeneration, hypomyelinization of the spinal cord, impaired reproductive performance, and decreased resistance to infectious disease (Heidarpour Bami *et al*., 2008).

Appropriate trace mineral supplementation is essential for maintaining optimum level of growth and performance of the animal (Šrejberová *et al*., 2008).

The level of mastitis infection in a dairy herd has a significant impact on herd profitability. Losses due to mastitis include decreased milk production, increased treatment costs, discarded milk quality, premature culling, death, decreased genetic potential, decreased reproductive performance, load rejection due to violation of somatic cell counts (SCC) or antibiotic residues and loss of milk quality premiums (Oliver *et el*., 2000 and Ruegg and Reinemann 2002).

The objective of this study was to evaluate the effects of adding Zn, Cu and Se in the ration of dairy cows on milk yield, SCC and blood parameters, as wall as milk quality and immune response in Friesian cows.

MATERIALS AND METHODS

The present study was carried out at Sakha Animal Production Research Station, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt during the period from July to November 2010.

Animals and management:

A total of 39 healthy Friesian cows with an average of 532.7±23.5 kg body weight (BW), between 28 and 56 months of age and 1-3 parities were used in this study. All cows were chosen at late pre-partum period (8 months gestation). At the beginning of the experimental period, the experimental cows were divided into three similar groups, 13 in each. Mutiparous cows (n=10 in each group) were divided according to their BW, parity and milk production of the previous season, while primiparous cows (n=3 in each group) were allotted based only on their BW.

Experimental cows were fed according to their BW and milk production. The 1st group (G1) was served as a control. Cows of the 2nd group (G2) were received 60 mg Zn, 20 mg Cu and 0.3 mg Se /kg diet at 30 day pre-partum to calving, while those of the 3rd group (G3) were received the same dose of (G2) but on 1 to 60 days of postpartum in the ration. All cows were housed separately semi-open yards.

Feeding system:

Experimental cows were fed a diet containing concentrate feed mixture (CFM), rice straw and corn silage according to the recommendation of NRC (2001) for dairy cows based on their live body weight and milk yield. The CFM was composed of 37.5% yellow corn, 20% soybean meal, 15% corn gluten, 22.5% wheat bran, 3% molasses, 0.5% and 1.5% common salt. Chemical analysis of representative monthly samples of foodstuffs was analyzed for CP, CF, EE, NFE and ash on DM basis according to the official methods of the A.O.A.C. (1995). Chemical composition of CFM, rice straw and corn silage as well as calculated chemical composition of the basal diet used in feeding cows in all groups is shown in Table (1).

Table 1. Chemical analysis of different feed stuffs (on dry matter basis) used in feeding cows in all groups

Item	Chemical composition (%)		
	CFM	Rice straw	Corn silage
DM	90.42	88.74	36.14
OM	89.54	82.83	92.4
CP	15.34	1.61	9.35
CF	11.46	37.36	17.15
EE	5.02	1.51	3.04
NFE	57.72	42.35	56.8
Ash	10.46	17.18	7.6

Experimental procedures:

Milk yield and composition:

Cows were machine milked twice daily at 6:00 and 17:00 h. Daily milk yield (morning and evening) was individually recorded for the 1st four months of lactation. Milk samples were biweekly collected to determine milk composition using Milko-Scan (Model 133B). The 4% fat corrected milk (4% FCM) for each cow was calculated from milk yield according to the following formula:

4% FCM = Actual milk yield (kg) x 0.4 +15 x fat yield (kg) Geans equation, (cited by Abou-Raya, 1967).

Somatic cell count (SCC):

After bacteriological plating, SCC were determined for each milk sample with a Fossomatic 90 (A/S N Foss Electric, Hillerod, Denmark) between 24 and 48 h postcollection using the previously described method by (Gonzalo *et al*., 1993).

Blood sampling:

During the experimental period, blood samples were biweekly collected in clean test tubes via the jugular vein from all cows in each group. Blood plasma was separated by centrifugation of the collected blood at 15 g for 10 min, and then plasma was kept frozen at –20 °C until chemical analyses. Concentration of total proteins (Gornall *et al*., 1949) and albumin (Weichselaum, 1946) in blood plasma were determined using commercial kits (Diagnostic System Laboratories, Inc USA). Plasma globulin was calculated by subtracting concentration of albumin from total proteins. Plasma samples were analyzed for Zn, Cu and Se by inductively coupled plasma-mass spectrometry.

Statistical analysis:

The obtained data were statistically analyzed using SAS (1990). The significant differences among treatment groups were tested using Duncan's Multiple Range Test (Duncan, 1955). The statistical model was:

$Y_{ij} = U + A_i + e_{ij}$.

Where:

Y_{ij} = Observed traits

U = Overall mean

A_i = Experimental group 1-3 (1= G1, 2= G2 and 3=G3)

e_{ij} = Random error

RESULTS AND DISCUSSION

Data in Table 2 show that pre-partum trace mineral supplementation of Ze, Cu and Se (G2) increased ($P<0.05$) daily actual and fat corrected milk yield during the 1st three months of lactation as compared to G1 (Table 2). However, the differences between G1 and G3 or G2 and G3 were not significant. Also, pre-partum trace mineral supplementation (G2) or post-partum periods (G3) improved fat, protein and lactose percentages as compared to G1, but the differences were only significant between G2 and G1, except protein was not significant. This may indicate that trace mineral supplementation during pre-partum has a positive reflection on the yield of fat and protein (Table 2). On the other hand, trace mineral supplementation reduced ($P<0.05$) somatic cell count in milk of G2 and G3 as compared to G1.

Table 2. Yield and composition of milk and somatic cell count as affected by trace mineral supplementation, throughout the first 120 days of lactation

Item	Experimental group			±MSE
	G1	G2	G3	
Average daily milk yield (kg/day):				
Actual milk yield	12.97^b	15.92^a	14.51^{ab}	0.8
4% fat corrected milk	11.16^b	14.51^a	13.14^{ab}	0.7
Milk composition (%):				
Fat	3.07^b	3.41^a	3.37^{ab}	0.10
Protein	2.78^b	3.32^a	3.13^a	0.08
Lactose	3.96^b	4.48^a	4.13^{ab}	0.09
Total solids	10.43	10.77	10.40	0.85
Solids not fat	7.37	7.26	6.93	0.87
Somatic cell count (10^3/ml):	455.5^a	291.6^b	318.7^b	13.94

a and b: Means within the same row with different superscripts are significantly different ($P<0.05$).

G1= Control, G2= Cows received 60 mg Zn, 20 mg Cu and 0.3 mg Se /kg diet 30 day pre-partum. G3= Cows received 60 mg Zn, 20 mg Cu and 0.3 mg Se /kg diet 1 - 60 days of postpartum.

The present results come in line with the findings of Kincaid and Socha (2004), who reported an increase in milk yield between week 5 and 10 postpartum in high-yielding dairy cows when supplemented with complexed minerals during postpartum.

Moreover, these results are in agreement with Nocek *et al*. (2006), who reported an increased milk production in animals receiving diets containing complexed minerals and a mixture of inorganic minerals. In the studies of Nocek *et al*. (2006) using diet supplemented with a mixture of complexed minerals and supplemented in excess of NRC (2001) requirements, and do not permit the improvement in performance to be identified to one specific mineral. Other studies have observed that feeding organic minerals such as zinc methionine (Kellogg *et al*., 2004) can increase milk production. In contrast, Campbell *et al*. (1999) reported no effect on milk yield by feeding minerals in an organic form.

However, Neathery *et al*. (1973) reported no differences in milk composition when supplementing varying levels of Zn in the diet during postpartum, agreeing with the results from the current study. There was also no effect of Zn form on milk fat or protein, results agreeing with work carried out by Nocek *et al*. (2006). The concentration of Zn in milk is maintained over different concentrations of dietary Zn (Krebs, 1998). It is thought that this is because the mammary gland's import and export process of Zn has a strict regulation to maintain an adequate supply of Zn to the neonate (Kelleher and Lonnerdal, 2003).

Somatic cell count was decreased in cows treated with Zn, Cu and Se than control group. There are a variety of trials that have demonstrated a link between selenium supplementation and udder health (Weiss, 2002). Malbe *et al.* (1995) demonstrated a reduction in somatic cell count when they supplemented selenium. Erskine *et al* (1989) showed that cows supplemented with 2mg of supplemental selenium per day showed greater resistance to mastitis compared to the control cows on a diet with 0.04 ppm selenium. Kommisrud *et al.* (2005) concluded in their study on the association of blood selenium and health and fertility traits that there was a positive association between increased blood selenium concentration pre-partum and decreased incidence of mastitis. Whitaker *et al*. (1997) found a reduction in somatic cell count when zinc was supplemented in the region of 360 mg/head/day. Young *et al*. (1960) estimated the correlation of SCC and clinical mastitis to be 0.80 or 0.98 from two methods. The results agreed with (Harmon and Torre, 1997) who, supplemented selenium, zinc, and copper in dairy cattle diets and it has been shown to be important for improving udder health and reducing SCC in milk. Smith *et al*. (1985) found that supplemented heifers with subcutaneous injection of Se at 21 days prepartum showed 57% reduction in clinical mastitis in early lactation and 32% reduction throughout lactation, and significantly lower somatic cell counts.

Data in Table (3) showed a significant ($P<0.05$) improvement of trace mineral supplemented diets on red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin (Hb) concentration and haematocrit percentage (HCT, %) of cows. Treated cows showed higher haematological parameters than those in the control group. Such effect was maximized in G2, except for HCT (Table 3).

Similar results were obtained by Lominadze *et al*. (2004), who found that copper administration can cause significant increase in haemoglobin and serum copper levels.

Trace mineral supplementation (Zn, Cu and Se) significantly ($P<0.05$) improved immune response of treated cows. Treated cows showed significantly higher lymphocytes in G3 and significantly ($P<0.05$) reduced monocytes as compared to control group. Copper is an essential trace mineral which plays an important role in immune response of the animal. Both cell-mediated and humoral immunity were greatly reduced by copper deficiency (Solaiman *et al*., 2007). The supply of Zn improves performance, health, and immune function (Kellogg *et al*., 2004).

Table 3. Haematological parameters and immune response as affected by trace mineral supplementation, throughout the first 120 days of lactation

Item	Experimental groups			±MSE
	G1	G2	G3	
Haematological parameters:				
WBC ($10^3/mm^3$)	10.23^b	12.35^a	11.92^a	0.37
Hb (g/dL)	9.54^b	10.68^a	10.43^a	0.23
RBC ($10^6/mm^3$)	6.11^b	7.12^a	6.79^a	0.22
Haematocrit (HCT, %)	28.3^b	30.42^{ab}	31.5^a	0.82
Immune response:				
Lymphocytes (%)	66.56^b	67.22^b	72.05^a	1.64
Monocytes (%)	12.28^a	11.66^a	8.09^b	0.67
Granulocytes (%)	21.21	21.10	19.46	1.20

$^{a\ and\ b}$: Group means denoted with different superscripts are significantly different at P<0.05.

Data in table (4) show a significant (P<0.05) effect of trace minerals supplemented (Zn, Cu and Se) pre-partum or postpartum on total protein, albumin and globulin and Se concentrations of cows blood plasma as compared to the control group. However, all parameters significantly higher in G3 than in G2, except plasma Zn and Cu concentration. The Zn is an essential trace mineral that is a constituent of enzymes involved in most metabolic pathways, and is important for protein metabolism, cell growth and repair, and immune function. The long-term effects of mild deficiency are unclear, but it has been suggested that they include delayed wound healing, suboptimal immune functioning, increased plasma lipid peroxides and perhaps reduced taste and smell acuity seen in the elderly (Fortes *et al.* 1997).

Table 4. Concentration of proteins, Zn, Cu and Se in plasma as affected by trace mineral supplementation, throughout the first 120 days of lactation post-partum

Item	Experimental group			±MSE
	G1	G2	G3	
Total protein (g/100 ml)	7.46^c	7.96^b	8.28^a	0.03
Albumin (g/100 ml)	3.34^c	3.68^b	3.90^a	0.02
Globulin (g/100 ml)	4.12^c	4.28^b	4.38^a	0.01
Plasma Zn (µmol/L)	14.3^b	15.6^a	15.3^a	0.32
Plasma Cu (µmol/L)	16.3^b	17.8^a	17.4^a	0.27
Plasma Se (µmol/L)	0.86^c	0.97^b	1.12^a	0.01

$^{a\ and\ b}$: Group means denoted with different superscripts are significantly different at P<0.05.

Appropriate trace mineral supplementation is essential for maintaining optimum level of growth and performance of the animal (Šrejberová *et al*., 2008).

Moreover, Se deficiency can adversely affect lymphocyte proliferative responses to mitogens in lambs (Turner *et al*., 1985), and Se supplementation of bovine lymphocytes enhanced IgM production in vitro (Stabel *et al*., 1991).

Correlation coefficients between each of all milk parameters studied are presented in Table (5). Results show that SCC showed the strongest positive correlation with fat percentage (r= 0.38501, P<0.01) and negatively correlated with milk yield (r=0.17008, P<0.05), lactose percentage (r= 0.3845, P<0.001).

Table 5. Pearson correlation coefficients between different milk parameters studied in both treated and control groups

Item	SCC	MY	Fat	Protein	Lactose	TS
MY	-0.17008*					
Fat	0.38501**	-0.3613*				
Protein	-0.0620 NS	0.0418NS	-0.2279***			
Lactose	-0.3845***	-0.1266NS	-0.35657***	0.16264*		
TS	0.1168 NS	0.0823NS	0.8581***	0.0799 NS	-0.1108 NS	
SNF	-0.1054 NS	0.04956	-0.12842 NS	0.5615***	0.4225***	0.399***

NS: Not significant, * Significant at P<0.05, ** Significant at P<0.01, *** Significant at P<0.001 MY = milk yield.

It is of interest to note that the correlation was negative between fat and protein (r=0.2279, P<0.001) and lactose percentage (r=0.35657, P<0.001) and negative with TS (0.8581, P<0.001). The present results indicated a positive correlation between SCC and protein percentage, while SCC negatively correlated (P<0.05) with milk yield and (P<0.001) lactose percentage. The present results indicated positive correlation between protein and lactose percentages and between SNF and each of lactose and TS. Emanuelson (1988) found similar results since the correlations between SCC and milk yield were about the same magnitude as between clinical mastitis and milk production. In contrast to the results of this study, Emanuelson *et al*. (1988) found a genetic correlation between SCC and milk production.

CONCLUSION

The current study concluded that adding Zn, Cu and Se at a level of 60 mg Zn, 20 mg Cu and 0.3 mg Se /kg diet starting 30 days pre-partum improved (22.5%) milk production and composition and decreased somatic cell count (10^3/ml), as wall as improved immune response of treated cows.

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تأثير التغذية علي علائق مضاف إليها النحاس والزنك والسيلينيوم قبل أو بعد الولادة على إنتاج اللبن وتعداد الخلايا الجسدية في الأبقار الفريزيان

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أستخدم في هذه الدراسة ٣٩ بقرة فريزيان متوسط أوزانها ٥٣٢.٧ كجم وفى الموسم من ١-٣ . وقسمت إلى ثلاث مجموعات متماثلة (ن=١٣). قسمت الأبقار إلى ثلاث مجموعات متماثلة في الوزن وموسم الحليب وإنتاج اللبن للموسم السابق ، كانت الأبقار في المجموعة الأولى بدون معامله (كنترول) وأبقار المجموعة الثانية أضيف الى علائقها ٦٠مللیجرام زنك و٢٠مللیجرام نحاس و٠.٣ مللیجرام سیلینیوم / كجم عليقة وذلك قبل الولادة بثلاثين يوما حتى يوم الولادة ، في حين أن المجوعة الثالثة .

أخذت الجرعة نفسها ولكن بعد الولادة لدة ستون يوما. خلال الفترة التجريبية كانت الأبقار تحلب آليا يوميا وإنتاج اللبن يتم تسجيلة علي حدة خلال الأشهر الثلاثة الأولي من الحليب. وتم تقدير مكونات اللبن وعدد الخلايا الجسدية. ويمكن تلخيص النتائج المتحصل عليها فيما يلي:

١- اضافة كل من الزنك والنحاس والسيلينيوم الى علائق الابقار حسنت من إنتاج اللبن فى المجموعتين الثانية والثالثة بمقدار ٢٢.٧ و ١٢ % على الترتيب بالمقارنة بمجموعة الكونترول .

٢- كانت نسبة الدهن والبروتين واللاكتوز فى اللبن فى المجموعة الثالثة أعلى معنويا من المجموعة الاولى بينما لم تختلف نسبة البروتين بين المجموعتين .

٣- اضافة الزنك والنحاس والسيلينيوم الى العلائق ادت الى خفض عدد الخلايا الجسدية معنويا فى المجموعتين الثانية والثالثة بالمقارنة بالمجموعة الاولى .

٤- كان عدد كل من كرات الدم الحمراء والبيضاء وتركيز الهيموجلوبين فى المجموعتين الثانية والثالثة اعلى معنويا من مجموعة المقارنة.

٥- -كانت بروتينات الدم وتركيز السيلينيوم فى المجموعة الثانية والثالثة أعلى من مجموعة المقارنة .

٦-كان تركيز الزنك والنحاس فى بلازما الدم فى المجموعتين الثانية والثالثة أعلى معنويا بالمقارنة بالمجموعة الاولى .