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Effect of oral zinc supplementation on hematology, serum biochemistry, performance, and health in neonatal dairy calves

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Abstract There has been a dogma that milk cannot provide adequate zinc requirements of calves in the first days of their life. This study was performed to determine whether zinc supplementation of the diet of neonatal dairy calves could promote a healthy hematology, biochemistry, growth rate and health. Thirty three Holstein calves were divided equally into three groups (control, test group 1, and test group 2). The dietary, environmental, and husbandry factors were similar in all groups. Zinc (50 ppm) as $ZnSO_4$ was added to each milk meal of test group 1 and 100 ppm $ZnSO_4$ was added to each milk meal of test group 2 for 14 days. No zinc sulfate was added to the diet of the control group. Blood samples were collected from the jugular vein 24 h–48 h after birth and at the end of the first, third and sixth weeks of life for measuring hematological and biochemical parameters including aspartate transaminase (AST), alkaline phosphatase (ALP), iron (Fe), calcium (Ca), phosphorus (P), albumin (Alb), total protein (TP), globulin (Glb), and glucose (Glu). Daily weight gain, total weight gain, and days of treatment during the study were also recorded. Statistical analysis indicated that the levels of total protein and albumin at 6 weeks in test group 2 were significantly higher than test group 1. Calcium at 6 weeks in test group 2 was significantly higher than test group 1 and controls. Glucose at 6 weeks in controls was significantly higher than test group 1. AST in test group 1 and globulin in test group 2 at the first sampling time were significantly higher than controls. No significant differences were observed among groups for other parameters.

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Introduction

Zinc is involved in many metabolic processes. It is essential for the function of more than 200 enzymes involved in DNA synthesis, mitosis and cell division, protein synthesis and carbohydrate metabolism (Prasad 1995). Zinc can function as a structural component of enzymes away from the active site, as a proton donor at the active site and as a bridging atom between the substrate and the enzyme (Keen and Graham 1989). Among the most important mammalian Zn-enzymes are carboxypeptidases, alkaline phosphatase, alcohol dehydrogenase, carbonic anhydrase and superoxide dismutase (Keen and Graham 1989; Coleman 1998).

Several hundreds of Zn-containing nucleoproteins are probably involved in gene expression of various proteins. Also, it is a component of many transcription factors and proteins that control cell cycles. Zinc is also thought to have a critical role in the stabilization of biomembranes (Keen and Graham 1989). Consequently, zinc deficiency can lead to a wide range of morbidities. Impaired growth is one of the most consistent signs of malnutrition. Zinc deficiency can also cause cell-mediated immune disorders, probably by adversely affecting lymphocyte proliferation.

Thymulin, a thymic hormone involved in T-lymphocyte maturation, is known to be zinc dependent and is adversely affected by Zn deficiency. Zn deficiency is known to decrease interlukin-2 production by helper T-lymphocytes and abnormalities in T-lymphocyte subpopulations have been observed in Zn deficient humans. Other effects of Zn deficiency include skin change, poor appetite, mental lethargy, delayed wound healing and neurosensory disorders (Prasad 1995). Lack of gonadal development and skeletal disorders during growth periods are other signs of zinc deficiency (Underwood

and Suttle 1999). Deficiency of zinc can cause reduction in production of cattle, e.g., growth, reproduction or lactation (Graham 1991). Zinc is a very important element in the reproductive cycle in many species. In humans, it is necessary for the formation and maturation of spermatozoa, for ovulation and for fertilization (Favier 1992).

Zinc requirements of domestic animals are influenced by age, growth rate, and other various physiological states such as gestation and lactation stage. Zinc absorption is influenced by age, availability of dietary source, criteria of adequacy and the zinc content of dietary ration. The net requirement of calves with 0.5 kg daily weight gain is approximately 16 mg/day of zinc (net requirement consists of maintenance: 0.1 mg Zn/kg; growth: 24 mg Zn/kg) (Underwood and Suttle 1999). Milk contains approximately 4 ppm of zinc (NRC 2001). Generally, calves are fed 4 l of milk during the first 3 months of life and milk is the only source of Zn for 1 or 2 weeks after birth. Furthermore, calves have only a 50% absorption of dietary zinc. Zinc deficiency, then, is a significant possibility in this period. The objective of this study was to investigate the effects of zinc supplementation on erythrogram, non-specific immunity, serum biochemistry, performance and health in neonatal dairy calves.

Materials and methods

The study was conducted in a dairy herd with approximately 600 calves per year at Mashhad suburb (northeast of Iran). This herd consisted of pure bred Holstein cattle and was totally confined in free-stall housing without access to pasture. Dry cows were fed with alfalfa (1.25 kg) hay (20.08 kg), a proprietary concentrate containing barley, cotton seed, bran, beet root and 1% DM supplement and corn silage (11.4 kg).

Cows were dried 2 months before expected time of parturition and transferred to a single occupation separate stall. As the time of parturition approached, the cows were moved to straw bedded maternity pens. Prompt assistance was given to cows with dystocia. Following parturition, the umbilicus of each calf was treated with pavidone iodine and the calf was weighed and transferred to a separate individual pen. Within the first 6 h of life, 2.5 kg of dam's colostrums was fed by nipple bottle and colostrum feeding was continued every 12 h for 48 h. Subsequently, herd milk replaced other feeds and was allowed twice daily (2 kg every 12 h) until 30 days of life together with calf starter (Table 2) including concentrate (90% DM) and high quality alfalfa (10% DM) with water available ad libitum. After this time, calves were fed milk replacer (Table 1) twice daily (2 kg every 12 h) until 90 days of life. The calves were weaned at 90 days of life. The Heifer calves were mainly used as herd replacements. Thirty-three calves were used in the present study from July 1 to July 17. The animals were divided equally into three groups (control, test 1 and test 2). For test 1 group 50 ppm and for test 2 group 100 ppm of zinc as zinc sulfate (Merck Co., Germany) was added to each milk meal for 14 days. All other aspects of the diet were identical for all groups including the controls. Ten milliliters of jugular blood were taken from all calves 24 h–48 h after birth and at the end of the first, third, and sixth weeks of life. Two milliliters of blood was anticoagulated with EDTA for hematological measurement, and plain tubes supplied serum for measurement of some biochemical parameters. The serum was separated after centrifugation at 1,800 *g* for 10 min and stored at −18 °C until required for analysis.

Anti-coagulated blood was analyzed shortly after collection for: number of red blood cell (RBC), hemoglobin (Hb), hematocrit (PCV), total leukocyte count (TLC), platelet (Plt), MCH, MCV and MCHC by an automatic hematology cell counter (Nihon kohden, Celltac α, Tokyo, Japan). Differential leukocyte counts were performed on routinely prepared Giemsa stained blood films using the cross-sectional technique (Jain 1986).

Stored serum samples were analyzed for total protein (TP), albumin (Alb), glucose (Glu), alkaline phosphatase (ALP), aspartate transaminase (AST), calcium (Ca), phosphorus (P), and iron (Fe) by an auto analyzer

Table 1 Ingredient composition of milk replacer (DM%)

Ingredients	Percent
Milk product	80
Vegetable fat	15
Premix of vitamins, mineral, etc.[a]	5

[a] Each kilogram milk replacer contains: vitamin A (55000 IU); vitamin D3 (4500 IU); vitamin E (80 mg); vitamin C (120 mg); vitamin B1 (16 mg); vitamin B2 (10 mg); vitamin B6 (8 mg); vitamin B12 (40 mcg); vitamin K (6 mg); niacin (50 mg); panthothenic acid (25 mg); choline chloride (1,900 mg); probiotic (1.4×10^6 CFU); iron (110 mg); copper (17 mg); manganese (26 mg); zinc (150 mg); cobalt (2 mg); iodine (0.6 mg) and selenium (0.3 mg)

Table 2 Ingredient composition of concentrate mix fed to calves (DM%)

Ingredients	Percent
Corn	50
Barley	15
Soybean meal	22
Beet pup	3
Wheat bran	3
Molasses	5.5
DCP	0.2
Limestone	0.9
Supplement[a]	0.4

[a] Each kilogram of supplement contains: vitamin A (50,000 IU); vitamin D3 (10,000 IU); vitamin E (0.1 g); calcium (196 g); phosphorus (96 g); sodium (71 g); magnesium (19 g); iron (3 g); copper (0.3 g); manganese (2 g); zinc (3 g); cobalt (0.1 g); iodine (0.1 g); selenium (0.001 g)

(Biotechnica, Targa 3000, Rome, Italy), using commercial kits (Parsazmoon, Tehran, Iran). Globulin was estimated as the arithmetical difference between serum total protein and albumin values. Calves were weighed at birth and at the end of the study (42 days).

Data analysis was done by SPSS computer based software. Parametric one-way ANOVA with Bonferroni multiple range test was used to investigate differences between groups. $P = < 0.05$ was considered as significant.

Results

The results are shown in Tables 3, 4, and 5. Statistical analysis indicates that levels of total protein and albumin levels at 6 weeks in test group 2 were significantly higher than test group 1 ($P < 0.05$). Calcium at 6 weeks in test group 2 was significantly higher than test group 1 or controls ($P < 0.05$). Glucose at 6 weeks in the control group was significantly higher than test group 1 ($P < 0.05$). AST in test group 1 and globulin in test group 2 at the first sampling were significantly higher than the control group ($P < 0.05$). There were no differences for PCV, Hb, RBC indices, platelet and WBC count between groups. Differential counting of WBC showed no differences between groups. Mean of total weight gains were 9.15 kg for the control group, 11 kg for test group 1 and 10.05 kg for test group 2. Mean of daily weight gains were 0.218 kg for the control group, 0.237 kg for test group 1 and 0.243 kg for test group 2. There were no differences among groups for total weight gain or daily weight gain and treatment days.

Table 3 Mean ± SE of RBC parameters and indices at various sampling time for three groups

Parameter	Age			
	24–48 h	Week 1	Week 3	Week 6
PCV (%)				
Control	28.75 ± 1.8	27 ± 1.6	25.75 ± 2	26 ± 1.5
Test 1	28.00 ± 1.5	28.25 ± 1.7	26.5 ± 1.4	24.5 ± 1.2
Test 2	28.75 ± 1.45	28.25 ± 1.3	26.75 ± 1.6	26 ± 1.1
P-value	0.9	0.83	0.91	0.66
Hb (g/l)				
Control	82.5 ± 6.5	77 ± 6	71 ± 6	77 ± 4.5
Test 1	80 ± 4.5	83 ± 6	77 ± 5	77 ± 4
Test 2	82.5 ± 5.4	82 ± 4.5	77 ± 5	78 ± 3
P-value	0.93	0.76	0.65	0.98
RBCs (10^{12}/l)				
Control	7.2 ± 0.37	7.2 ± 0.37	7.4 ± 0.51	8.2 ± 0.41
Test 1	6.8 ± 0.29	7.2 ± 0.32	7.4 ± 0.32	7.8 ± 0.33
Test 2	7.5 ± 0.41	7.3 ± 0.35	7.6 ± 0.43	8.1 ± 0.31
P-value	0.66	0.92	0.91	0.62
MCV (fl)				
Control	40 ± 0.85	37.5 ± 0.55	34.5 ± 0.5	31.75 ± 0.6
Test 1	41 ± 0.75	39 ± 0.7	35.5 ± 0.6	32.75 ± 0.5
Test 2	40 ± 0.75	38.5 ± 0.7	34.75 ± 0.5	32 ± 0.5
P-value	0.71	0.28	0.22	0.3
MCH (pg)				
Control	11.3 ± 0.42	10.8 ± 0.32	9.5 ± 0.22	9.4 ± 0.28
Test 1	11.6 ± 0.34	11.3 ± 0.39	10.4 ± 0.33	9.9 ± 0.26
Test 2	11.5 ± 0.34	11.1 ± 0.26	10 ± 0.21	9.6 ± 0.25
P-value	0.82	0.56	0.06	0.3
MCHC (%)				
Control	28.25 ± 0.54	29 ± 0.8	27.5 ± 0.55	29.75 ± 0.5
Test 1	28.5 ± 0.4	29 ± 0.6	29 ± 0.7	30.5 ± 0.6
Test 2	28.5 ± 0.42	29 ± 0.5	28.75 ± 0.35	31 ± 1
P-value	0.9	0.99	0.16	0.44

Table 4 Mean ± SE of WBC parameters and Plt at various sampling time for three groups

Parameter	Age			
	24 h–48 h	Week 1	Weeks 3	Weeks 6
WBC (10^9/l)				
Control	8.9 ± 0.53	8.9 ± 0.92	9.5 ± 1.03	9.6 ± 0.95
Test 1	10.5 ± 0.92	9.6 ± 0.9	10.3 ± 1.08	8.6 ± 0.86
Test 2	10.2 ± 1.2	11.8 ± 2.23	8.6 ± 0.64	8.7 ± 0.45
P-value	0.43	0.33	0.46	0.58
Neu (10^9/l)				
Control	5.4 ± 0.52	4.4 ± 0.67	3.7 ± 0.93	3.5 ± 0.93
Test 1	6.9 ± 0.81	5 ± 0.85	4.7 ± 0.84	3.2 ± 0.56
Test 2	7 ± 0.1	8.1 ± 1.57	2.8 ± 0.38	3.1 ± 0.65
P-value	0.29	0.52	0.22	0.92
Lym (10^9/l)				
Control	2.9 ± 0.35	4.5 ± 0.56	5.5 ± 0.74	9.6 ± 4.01
Test 1	2.8 ± 0.27	4.7 ± 0.48	6.1 ± 0.36	5.1 ± 0.71
Test 2	2.1 ± 0.41	5.3 ± 0.73	5.2 ± 0.35	6.1 ± 0.33
P-value	0.22	0.6	0.44	0.37
Mono (10^9/l)				
Control	0.49 ± 0.073	0.42 ± 0.090	0.4 ± 0.087	0.25 ± 0.060
Test 1	0.46 ± 0.145	0.68 ± 0.174	0.48 ± 0.108	0.26 ± 0.065
Test 2	0.47 ± 0.125	0.48 ± 0.078	0.45 ± 0.105	0.26 ± 0.065
P-value	0.98	0.29	0.84	0.99
Eos (10^9/l)				
Control	0.015 ± 0.015	0	0	0.019 ± 0.019
Test 1	0.053 ± 0.022	0.071 ± 0.054	0.011 ± 0.011	0.022 ± 0.022
Test 2	0	0	0	0
P-value	0.06	0.2	0.38	0.61
Band (10^9/l)				
Control	0.064 ± 0.046	0	0	0.061 ± 0.075
Test 1	0.292 ± 0.152	0.009 ± 0.011	0.039 ± 0.027	0.005 ± 0.006
Test 2	0.090 ± 0.072	0.095 ± 0.117	0	0.021 ± 0.014
P-value	0.23	0.42	0.19	0.54
Plt (10^9/l)				
Control	3.9 ± 0.35	7 ± 0.6	6.9 ± 0.55	7 ± 0.7
Test 1	5.4 ± 2.4	6.8 ± 0.3	7.2 ± 0.46	6.2 ± 0.4
Test 2	4 ± 0.3	7.4 ± 0.26	7.1 ± 0.32	7.5 ± 0.55
P-value	0.7	0.62	0.83	0.28

Discussion

As shown in Table 3, parameters such as PCV, MCV, MCH, HB, and Neu decreased and some other parameters like RBC, MCHC, Lym and Plt increased according to the expected normal developmental patterns in all groups during study period with no

Table 5 Mean ± SE of biochemical parameters at various sampling time for three groups

Parameter	Age			
	24 h–48 h	Week 1	Weeks 3	Weeks 6
Tp (g/l)				
Control	64 ± 2.5	58 ± 1.7	58 ± 1.6	56 ± 1.2[a,b]
Test 1	60 ± 2	59 ± 1.5	56 ± 0.9	54 ± 0.8[a]
Test 2	68 ± 2.5	63 ± 2.1	60 ± 1.6	59 ± 1.5[b]
P-value	0.07	0.1	0.3	0.02
Alb (g/l)				
Control	30 ± 0.7	31 ± 1.2	34 ± 0.7	35 ± 1[a,b]
Test 1	28 ± 1.3	30 ± 0.9	33 ± 0.8	31 ± 0.6[a]
Test 2	30 ± 1.3	32 ± 1.3	35 ± 1	35 ± 1.4[b]
P-value	0.32	0.58	0.17	0.04
Glb (g/l)				
Control	31 ± 1.6[a]	27 ± 1.3	24 ± 1.2	22 ± 1.2
Test 1	34 ± 1.6[a,b]	28 ± 1.8	23 ± 1.1	23 ± 1
Test 2	38 ± 2.5[b]	31 ± 2.2	24 ± 1.6	24 ± 1.3
P-value	0.03	0.26	0.7	0.7
ALP (IU/l)				
Control	476 ± 36	393 ± 32	273 ± 26	576 ± 79
Test 1	591 ± 56	439 ± 33	287 ± 36	486 ± 58
Test 2	593 ± 72	427 ± 37	299 ± 23	429 ± 46
P-value	0.26	0.62	0.82	0.27
AST (IU/l)				
Control	42 ± 2[a]	36 ± 5	42 ± 5	54 ± 5
Test 1	64 ± 9[b]	33 ± 2	41 ± 2	58 ± 7
Test 2	62 ± 5[a,b]	32 ± 2	36 ± 2	51 ± 7
P-value	0.03	0.78	0.52	0.75
Ca (mmol/l)				
Control	2.86 ± 0.074	2.61 ± 0.06	2.49 ± 0.074	2.55 ± 0.062
Test 1	2.93 ± 0.037	2.74 ± 0.074	2.55 ± 0.049	2.61 ± 0.037
Test 2	2.99 ± 0.062	2.8 ± 0.062	2.67 ± 0.49	2.8 ± 0.062
P-value	0.23	0.11	0.09	0.06
P (mmol/l)				
Control	2.45 ± 0.074	2.58 ± 0.064	2.42 ± 0.058	2.35 ± 0.064
Test 1	2.38 ± 0.083	2.55 ± 0.083	2.38 ± 0.064	2.32 ± 0.064
Test 2	2.32 ± 0.087	2.26 ± 0.067	2.42 ± 0.067	2.45 ± 0.08
P-value	0.58	0.56	0.91	0.4
Fe (mmol/l)				
Control	15.22 ± 1.54	12.53 ± 0.82	16.47 ± 1.71	17.73 ± 2.11
Test 1	12.83 ± 1.16	11.82 ± 0.76	16.28 ± 1.47	17.37 ± 1.89
Test 2	16.28 ± 2.5	13.25 ± 1.32	16.47 ± 1.61	18.98 ± 1.83
P-value	0.4	0.64	0.99	0.8
Glu (mmol/l)				
Control	6.21 ± 0.49	5.05 ± 0.27	4.55 ± 0.2	5.32 ± 0.26[a]
Test 1	6.27 ± 0.37	5.1 ± 0.32	4.4 ± 0.17	4.27 ± 0.16[b]
Test 2	6.21 ± 0.29	4.71 ± 0.19	4.71 ± 0.21	5.1 ± 0.22[a,b]
P-value	0.98	0.52	0.07	0.01

[a,b] Shows significant difference between different superscript letters for the same column ($P < 0.05$)

differences between groups for any hematological parameter.

Rupic et al. (1998) studied RBC parameters and the plasma protein ratios of 42 pigs, which were fed with insufficient zinc diet for 30 days and then divided into three groups. One of them (group T1) received 84.3 mg/kg DM of zinc as $ZnSO_4$ and the other (group T_2) received 40.9 mg/kg DM zinc, as Zn-met, for 105 days. The third group (control) received no extra zinc. During the parakeratosis period (days 28–56), both the basic feed (group C) and the diet enriched with 40.9 mg Zn/kg (as ZnMET) fed to group T2 pigs resulted in a lower red blood cell (RBC) count, Hb concentration and PCV when compared to group T1. Dietary Zn of organic and inorganic origin had no effect on MCV or on the WBC and platelet counts of fattening pigs.

El-Hendy et al. (2001) investigated hematology and serum biochemistary of adequate Zn level (38 mg/kg DM, control) and two low levels of Zn deficiencies (19 mg/kg DM, test 1 and 3.8 mg/kg DM, test 2) in growing male and female rats for 10 days. Hb, total erythrocyte count and PCV decreased and total leukocyte count increase in the two test groups in a dose-dependent manner. In the present study, lack of significant difference among groups for hematological parameters may be due to the transfer of sufficient zinc from their mothers and that the subsequent diet and zinc supplementation was unable to exert a positive effect on hematopoesis.

El-Hendy et al. (2001) reported that in two test groups fed with insufficient zinc diet, globulin and total protein were significantly lower than controls but serum albumin was elevated in those rats fed with the lowest Zn level.

Daghash and Mousa (2002) reported significant increase of total protein and total globulin in buffalo calves after Zn supplementation over 180 days but albumin did not change significantly. In the present study, significant differences of total protein and albumin levels between test group 1 and test group 2 may be due to the positive effect of 100 ppm zinc supplementation on protein synthesis and the negative effect of this supplementary regime on protein degradation.

Colostrum is a much richer source of calcium than milk, hence calcium concentration of plasma increases after colostrum feeding and decreases gradually thereafter. Plasma concentration of calcium is dependant on Ph and concentration of plasma protein and that an increase in plasma protein can lead to an increase in calcium concentration. The high concentration of calcium in test group 2 may be attributed to high concentration of plasma protein at that sampling time. Thilsing-Hansen and Jorgensen (2001) reported that calcium concentration decreased 12 h–24 h after administration of 120 mg/kg body weight of zinc. Daghash and Mousa (2002) reported significant decrease in calcium levels after 180 days Zn supplementation in buffalo calves but inorganic phosphorus was not changed by this supplementation. In our study, there was no significant difference among groups with respect to calcium and phosphorus levels during the study period, thus it is possible that this dose of zinc administration was insufficient to induce an antagonist reaction to calcium and phosphorus concentration. It is also possible that the time of supplementation was not sufficient to induce hypocalcaemia.

Keen and Graham (1989) reported an antagonist reaction between iron and zinc absorbtion during oral administration of iron but in our study, there was no difference between groups for iron levels during the study period. It is possible that this dose of zinc administration was insufficient to induce an antagonist reaction capable of resulting in significant decrease in iron concentration of the test groups.

Zinc has a positive effect on secretion and releasing of insulin hormone and it is effective in maintaining the structure and activity of glycolyse pathway enzymes. El Hendi et al. (2001) showed that plasma glucose concentration in two test groups which were fed with insufficient zinc diet was significantly lower than controls. In our study, there was difference for glucose amounts between test group 1 and test group 2. It is possible that the effect of zinc on glucose metabolism is dose dependent.

The differences seen for globulin and AST at the first sampling time is possibly due to the amount and time of colostrum feeding, as there was no difference in zinc uptake between the groups at first sampling time.

In our study, there was no difference for health and growth rate between groups. Kincaid et al. (1997) found no advantage to the immune function of extra dietary zinc supplementation. Malcolm-Callis et al. (2000) showed neither organic nor inorganic supplementation of zinc affected beef steer performance. On the other hand, a study by Hahn and Baker (1993) on young pigs and a study by Mayland et al. (1980) on calves show zinc supplementation can cause significant increases in weight gain. It seems that this effect may depend on zinc status before supplementation.

Although in our study, some of the biochemical parameters were slightly elevated in test group 2, it seems that excess zinc supplementation during the first 2 weeks of life does not elicit changes in non-zinc deficient calves.

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