Indian J. Dairy Science, 34, 4, 1981

STUDIES ON TRACE MINERALS IN BUFFALOES' MILK. III. ZINC

O.N. MATHUR and N.K. ROY

National Dairy Research Institute, Karnal 132 001

Received on June, 1980

INTRODUCTION

Zinc is unique among the trace elements being consistently present in milk in relatively large amounts (Underwood, 1977). Birckner (1919) first reported that zinc content of cows' milk was 5.5 mg/kg after parturition and 4.0-4.5 mg/kg after three weeks. A review by Archibald (1958) indicated a wide range from 2.1 to 32.1 ppm for 13 reports. Different workers reported the following ranges for zinc in cows' milk (all converted to ppm): 2.0-3.7 (Sato and Murata, 1932), 1.9-5.8 (Archibald, 1944), 3-5 (Berfenstam, 1952), 3.6-4.7 (Falckenthal, 1955), 3.0-4.5 (Taucin and Svilan, 1959; 1962), 3-6 (Jenness, 1966), 3.1-6.3 (Parkash and Jenness, 1967), 3.8-4.2 (Antilla and Antila, 1970), 3.8-4.2 (Kreula and Heiknen, 1970) 0.200-5.520 (Dequidt *et al.* 1974), 3.29-4.02 (Boccia *et al.* 1975) 3.96-5.51 (Tsvetkova, 1976). Findings from others, expressed as average in ppm, are 4.41 ± 0.49 (Mouillet *et al.* 1975), 3.051 (Naplatrova, 1976), 4.05 and 4.48 (Heine *et al.* 1977), 5.5 ± 1.7 (Kureljusic and Vujicic, 1978).

Information on zinc in buffaloes' milk is scanty. Spectrographic analysis (De, 1934-35) revealed that zinc was present in trace in buffaloes' milk. Average of 2.393 mg/kg (Dilanyan and Aslanyam, 1967), 5.751 ± 0.256 ppm, (Abd El-Salam, 1968), and 1.492 ppm, (Ghosh *et al.* 1972) were later reported. This paper lineates further findings on zinc in buffaloes' milk studied by both colorimetric and atomic absorption spectrophotometric methods, on the distribution of the metal in various milk fractions, and on the effect of some process treatments on such distribution.

MATERIALS AND METHODS

Samples and extraction of zinc : Composite samples of milk from Murrah buffaloes of the Institute herd were collected in polythene containers avoiding metallic contamination. Zinc from milk and its fractions was extracted by ashing the samples and dissolving the cooled ash in acid. A 25 ml of the sample was accurately transferred with a pipette into a vitriosil basin (150 ml), and the contents evaporated to dryness on a boiling waterbath. The dried mass was then charred by gently heating the basin over a low bunsen flame, and ashed in a muffle furnace at 550°C for 5-6 hr. The cooled ash was dissolved in 1 : 1 hydrochloric acid (A.R.) by gently boiling for 5 min and the volume made upto 50 ml.

Estimation of zinc : Zinc in the extracted solution was determined colorimetrically by developing the pink colour with dithizone in carbon tetrachloride solution, following the procedure described by Jackson (1967) with minor modifications as detailed hereafter. Ten ml aliquot of the zinc extract was pipetted into a 125 ml separating funnel and mixed with 50 ml of ammonium citrate buffer (0.4M, pH 8.5) and 3 ml of 0.2% (W/V) sodium diethyl dithiocarbamate solution. The final pH was adjusted between 8.5 and 8.8 by NH_4OH or HCl, using phenolphthalein indicator. Subsequently, 10 ml of 2% (W/V) dithizone solution in carbon tetrachloride was added, and the contents shaken well for 5 min. The organic phase was transferred to another separating funnel, mixed with 25 ml of 0.01 N NH_4OH solution and shaken for 3 min to eliminate the excess dithizone into the aqueous phase. Then 5 ml of the organic phase was accurately transferred and diluted to 25 ml with carbon tetrachloride. Absorbance of this solution was measured in a Klett-Summerson photoelectric colorimeter using green filter, and concentration of zinc found out by using standard curve. Standard zinc solutions were prepared by dissolving accurately weighed amounts of pure metal in known volumes of dilute hydrochloric acid to give concentrations of 0, 1, 2, 3, 4 and 5 ppm Zn. The standard curve was plotted from observed absorbances through the regression equation

$$Y = 79.2\,X$$

where Y and X are respectively Klett readings and ppm of Zn. The variation in regression for this equation (R^2) was 96.8%, significant at $P < 0.01$.

Zinc in milk fractions : Distribution of zinc in whole milk and its fractions, namely, skim milk, cream, rennet curd and whey, was studied using 12 samples. Whole milk heated to about 45°C was separated into skim milk and cream using a hand operated cream separater. Preparation of the milk fractions by renneting was according to earlier communication on boron (Mathur and Roy, 1980).

Process treatments : Influence of heating whole milk on distribution of zinc in milk fractions was studied with 5 samples, which was subjected to three process treatments of pasteurization, sterilization and cool-ageing as described earlier (Mathur and Roy, 1980).

RESULTS AND DISCUSSION

Zinc in whole milk : The concentration of zinc in 17 samples of buffaloes' whole milk averaged to 4.68±0.297 (standard error) ppm in the range of 1.95 to 6.80 ppm. Samples were analysed, *ab initio* in duplicate. The 't' value (Snedecor and Cochran, 1968) for these pairs of analyses was 0.136, which was statistically not-significant and indicated reliability of the analytical procedure adopted.

Nine additional samples of buffaloes' whole milk were analysed for zinc simultaneously by the present colorimetric method and an atomic absorption spectrophotometric method (AAS). The procedure and results for AAS study were reported earlier (Mathur and Roy, 1977). A comparison of these two sets of results are shown in Table 1. The average zinc concentration in whole milk was 6.506±0.3352 ppm by the AAS and 6.22±0.3689 ppm by colorimetry. The difference between the two sets of results was statistically not-significant, and confirmed reliability of the colorimetric procedure followed.

TABLE 1

Estimation of zinc by atomic absorption spectrophotometry (AAS) and colorimetry

Sample No.	Concentration of zinc (ppm)	
	AAS	Colorimetry
1	7.146	7.00
2	5.926	5.40
3	5.073	5.00
4	5.195	5.00
5	7.878	7.40
6	8.366	8.00
7	6.414	6.40
8	5.926	5.40
9	6.634	6.40
Average	6.506	6.22
Standard error	±0.3352	±0.3689

t = 2.097 for 8 degrees of freedom (Not significant)
Analysis by paired "t" test (Snedecor and Cochran, 1968)

The present findings of 4.68±0.297 ppm zinc from 17 samples of Indian buffaloes' milk is higher than 1.492 ppm reported from a limited number of samples by Ghosh *et al.* (1972), or than 2.393 mg/kg for milk from Russian buffaloes (Dilanyan and Aslanyan, 1967). But it is lower than 5.751±0.256 ppm for Egyptian buffaloes' milk (Abd-El-Salam, 1968).

Zinc in buffaloes' milk observed now is slightly higher than that in cows' milk reported by several workers, as evident from the results cited earlier. However, some recent findings show comparable results, namely, 4.41±0.40 (Mouillet *et al.* 1975), 5.5±1.7 (Kureljusic and Vujicic, 1978).

Zinc distribution : Distribution of zinc in whole milk, skim milk and rennet whey studied with 12 samples is shown in Table 2. Concentrations of zinc in cream and rennet curd, calculated by difference are also included therein. Out of the average 4.85±0.326 ppm zinc in whole milk 4.40±0.311 ppm went into skim milk, while the rest 0.45±0.047 ppm was associated with the cream, which represented 90.4 and 9.6 respectively of the total, averaged from results for 12 individual samples (Table 2).

TABLE 2

Distribution of zinc in buffaloes' whole milk and various milk fractions[a].

A. Concentration of zinc in milk and milk fractions.

Sample (code)	Concentration		
	Average±S.E. (ppm)	Range (ppm)	Average (per cent)
Whole milk (WM)	4.85±0.326	1.95—6.70	——
Skim milk (SM)	4.40±0.311	1.65—6.20	90.40 of WM
Cream[b]	0.45±0.047	0.20—0.70	9.60 of WM
Rennet whey	0.83±0.099	0.40—1.50	20.35 of SM
Rennet curd[b]	3.40±0.291	1.00—4.78	79.64 of SM

B. Correlation coefficient for distribution of zinc in milk fractions

Between Samples	Correlation coeffcient
Whole milk and skim milk	0.989†
Whole milk and rennet whey	0.111 NS
Skim milk and rennet whey	0.067 NS
Whole milk and rennet curd[b]	0.741†
Skim milk and rennet curd[b]	0.762†

NS—Not significant
† —Significant at $P < 0.01$
a —Results pertain to 12 herd milk samples
b —Calculated by difference.

Such observation agreed fairly with that for cows' milk reported by Parkash and Jenness (1967), but contradicted those by Herald *et al.* (1957), Imamura *et al.* (1962) and Capella *et al.* (1974) according to which higher concentrations of zinc were encountered in the fat-globules and fatglobule membranes. Results from the present findings further revealed that about 80% of zinc from skim milk (Table 2), which was equivalent to 72% of that from whole milk, was bound to the rennetted curd. The soluble fraction of zinc in buffaloes' milk was about 20% of skim milk, or 18% of whole milk. Thus, in buffaloes' milk 72% of zinc was associated with casein and 18% present in the soluble form as compared with 88% and 12% respectively in cows' milk (Jenness, 1966; Parkash and Jenness, 1967).

The regression equations, Nos. 1, 2 and 3, calculated from 12 individual results described such distribution of zinc in fractions from buffaloes' milk.

$$Y = 0.94X - 0.16 \quad (1)$$

(Y and X stand for zinc in ppm in skim milk and whole milk respectively).

$$Y = 0.034X + 0.67 \quad (2)$$

(Y and X represent zinc in ppm in rennet whey and whole milk respectively).

$$Y = 0.69X + 0.34 \quad (3)$$

(Y and X express zinc in ppm in rennet curd and skim milk respectively).

Graphical presentation for the distribution of zinc in different milk fractions are given in the Figure.

The correlation coefficients for distribution of zinc between whole milk and skim milk, whole milk and rennet curd, and skim milk and rennet curd were 0.989, 0.741 and 0.762

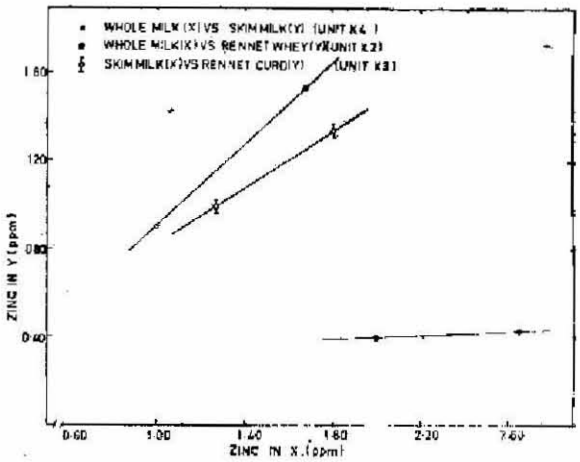

Fig.

Distribution of zinc between the fraction of buffaloes' milk.

respectively, all significant at $P < 0,01$ (Table 2). Comparable data for cow's milk could not be found from available literature.

Effects of process treatments : Results for the influence of processing whole milk by pasteurization (72±0.1°C, 15 s), sterilization, (121.5°C, 15 min) and subsequent cool-ageing (4-6°C, 24 hr) on the distribution of zinc in 5 samples of whole milk, 5.12±0.124 ppm, was taken uniform for the treated samples also, since treatments could not change the total zinc in milk, except through experimental error. Zinc in skim milk and rennet whey obtained from the untreated whole milk was respectively 4.60±0.105 and 0.62±0.147 ppm. Pasteurization and sterilization changed the former respectively to 4.69±0.111 and 4.52±0.139 ppm. Further, due to cool-ageing of the heated milk the concentrations changed to 4.72±0.199 and 4.40±0.152 ppm in pasteurised cool-aged and sterilized cool-aged samples, respectively. Zinc in rennet whey changed slightly from initial 0.62±0.97 ppm in raw milks' fraction to 0.49± 0.078 and 0.48±0.017 ppm respectively in pasteurized and sterilized milks' fractions. Cool-ageing also induced a slight lowering in zinc contents of rennet whey (Table 3). A

TABLE 3

Effect of processing conditions of milk on the distribution of zinc in buffaloes' milk and its fractions.

Sample No.	Treatment	Concentration of zinc (ppm) (Average ± standard error) a Sample		
		Whole milk	Skim milk	Rennet whey
1	Raw	5.12±0.124	4.60±0.105	0.62±0.097
2	Pasteurised	5.12±0.124	4.69±0.111	0.49±0.078
3	Pasteurised and cool-aged for 24hr	5.12±0.124	4.72±0.199	0.41±0.090
4	Sterilized	5.12±0.124	4.52±0.139	0.48±0.107
5	Sterilized and cool-aged for 24 hr	5.12±0.124	4.40±0.152	0.45±0.098

a — Results are for 5 herd milk samples simultaneously analysed in 3 milk systems and for 5 treatments.

TABLE 4

Analysis of variance for influence of different treatments of milk on the concentration of zinc in buffaloes' milk and its fractions.

Sr. No.	Source of Variation	Degree of freedom	Mean sum of squares	Variance ratio (F)
1.	Between samples	4	0.4550	9.40†
2.	Between treatments	4	0.0375	0.77 NS
3.	Between fractions of milk	2	160.0400	3306.61†
4.	Interactions between treatment × fractions of milk	8	0.0388	0.80 NS
5.	Error	56	0.0484	—

Analysis is for the data for 5 herd milk samples averages for which are reported in Table 3.
† Significant ($P < 0.01$)
NS Not-Significant

similar trend for cow's milk heated at 90°C for 30 min, namely, decrease in soluble zinc and increase in the casein associated metal, was also reported (Parkash and Jennes, 1967). However, these changes in zinc in buffaloes' milk fractions due to process treatments of the whole milk were statistically not significant (Table 4).

SUMMARY

Zinc concentration in 17 samples of buffaloes' whole milk averaged to 4.68±0.297 ppm and varied from 1.95 to 6.80 ppm. Variation of zinc content in different samples was significant at $P < 0.01$.

From an average of 4.85±0.326 ppm zinc in 12 samples of whole milk approximately 90% was found in skim milk leaving about 10% in cream. Further partitioning of skim milk showed that 80% of its zinc content was associated with rennet curd. Calculated on the basis of concentration of whole milk about 18% zinc was in the soluble form 72% in association with casein and the rest 10% in lipid phase. Such partitioning of zinc between whole milk and skim milk, whole milk and rennet curd, and skim milk and rennet curd was significant at $P < 0.01$.

Processing of whole milk by pasteurization, sterilization and cool-ageing although slightly lowered the soluble fraction of zinc, and also changed zinc in skim milk non-uniformly, such changes were not significant.

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