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Influence of fortified iron and zinc dahi on blood iron, serum zinc concentration and blood glucose levels in experimental animals

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Abstract
Iron is an essential micro-mineral in human nutrition. It is a mineral found in every cell of the body to make part of blood cells. The total content of iron in an adult body is 3-5 g. About 70% of this occurs in the erythrocytes of blood as a constituent of hemoglobin. At least 5% of body iron is present in myoglobin of muscle. Heme is the most predominant iron containing substance. It is a constituent of several proteins/enzymes-hemoglobin, myoglobin, cytochromes, xanthine oxidase, catalase and peroxidase.

Zinc is needed for the activation of over 300 enzymes in the body. This is important, since many of these enzymes' functions as regulators of blood sugar level. Zinc plays a key role in the synthesis, storage and secretion of insulin. It seems to stimulate insulin action and insulin receptor tyrosine kinase activity. Zinc also plays a vital role in overcoming insulin resistance. In the current study, an attempt was made to fortify with iron and zinc by using ferrous lactate, zinc sulphate @ 40 mg/kg milk. This level showed satisfactory organoleptic, physico-chemical viz., acidity, total solids, fat and protein; and microbiological characteristics such as Total Viable Count, coliform count and yeast & mold counts. Animal experimentation by feeding rats with micro-mineral fortified dahi revealed increased gain in body weight, Feed and Protein Efficiency Ratio values (FER & PER). Blood iron and Blood serum zinc concentrations showed tremendous increase in rats fed with fortified dahi than in animals fed with unfortified dahi. Comparatively, lower blood glucose levels were observed in experimental animals fed with fortified dahi than animals fed with control dahi. From the present study, it can be concluded that, iron fortified dahi showed increase in blood iron then the unfortified dahi and the zinc fortified dahi doesn't caused sharp fluctuations in blood glucose level thus stabilized blood glucose levels and hence, the zinc fortified fermented milk products can safely be consumed by early diabetic people to control blood glucose levels.

Keywords: Dahi, Serum zinc, blood glucose level, Micro-mineral, hemoglobin

Introduction
Fermented milk products reportedly have therapeutic, anticholesterolemic, anticarcinogenic and anticariogenic properties beyond their basic nutritive value. Dahi is the most important fermented milk product used in India from time immemorial. The popularity of dahi is not only due to its refreshing taste and palatability but also due to its scientifically proven role as a nutritious fermented milk product. In Indian system of medicine (Ayurveda), dahi has been strongly recommended for curing ailments like dyspepsia, dysentery and other gastrointestinal disorders. This product is also believed to improve appetite and vitality.

Iron as an essential trace element participates as catalyst in several metabolic reactions. As a component of hemoglobin, myoglobin, cytochrome and other proteins, iron plays an important role in the transport, storage and utilization of oxygen. It is also co-factor of many enzymes (Bates and Prentice, 1996) [12].

Micro mineral especially zinc play a pivotal role in various physiological processes. It is the most important mineral for body metabolism, part of many enzymes like retinal dehydrogenase and alkaline phosphatase (Samy, 2010) [11]. Zinc is an essential nutrient for health and very important for growth, normal functioning of immune system and other physiological processes. It is also a component of the hormone insulin. Its deficiency results in growth retardation, delayed wound healing and hypogonadism.

Materials and Methods
The procedure for preparation of dahi by Khedkar *et al* (2014) [13] was adopted with suitable modifications. Milk obtained from Student Experimental Dairy Plant (SEDP), of Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU), of Hebbal, Bengaluru-24.

For standardization of cow milk (fat 3.5% and SNF 8.5%), Nandini cream and spray dried Nandini skim milk powder were used. The standardized milk was preheated to 60^0C and homogenized (at 2500 psi and 500 psi). Then milk was heated to 95 ^{o}C for 15 min. During heating milk was stirred continuously with the help of stirrer to avoid formation of cream layer, cooled to 37 ^{o}C, divided into two equal portions: The first portion served as control. The second portion was fortified separately with zinc sulphate (Food grade: Merck Chemicals. Germany) at a level of 20, 30 and 40mg zinc/ kg milk. Milk was inoculated with desirable proportion of starter culture (*Streptococcus lactis, streptococcus diacetylactis* along with the species of *Lactobacillus* such as *Lactobacillus bulgaricus* and *Streptococcus thermophiles* in the ratio of 1:1) at the level of 1%.Milk along with culture added was filled into clean polystyrene cups. The samples were incubated at 30±1°C until curd formation/coagulation (17hr). Thus, obtained dahi samples were stored at 5±1 °C until they are used for experiments.

PH of the sample was measured using a digital pH meter (Chemi line Pvt. Ltd.) at 25 °C. Acidity, protein, fat, total solids, ash and moisture content of dahi was estimated as per the standard procedure IS: SP 18 (Part XI) 1981. MSNF content in milk was computed by using ISI Lactometer by using the following formula:

$$SNF\ (\%) = 0.25\ CLR + 0.2F + 0.35$$

Where,
CLR-Corrected Lactometer Reading
F-Fat (content in per cent)

Prepared dahi samples were given to a panel of five judges for sensory evaluation. Each judge was supplied with standard score card of a total of 25 points for assessing the degree of firmness, whey separation, body and texture, flavour.

A total of 18 adult male albino rats weighing about 100-110 g were used in experiment. Animals were kept under normal laboratory conditions for 1 week before the initiation of the experiment. Rats were allowed free access of water and fed on uniformly diet. Six rats served as a normal control another six rats subjected to the dahi (without treatment with mineral) and other six rats were subjected to the fortification with mineral treatment. Fortified dahi similarly along with control sample transformed into pellets those pellets are fed to experimental animals (Dhar, 1959) [1].

For blood collection, overnight fasted rats were first anesthetized by exposing them to ether vapour in a glass jar for a brief period of time. On 28th day, blood was collected in experimental animals in clean, sterilized and labelled test tubes, by retro-orbital plexus puncture method using microhaematocrit capillary tubes. Blood glucose levels were measured immediately after the blood collection by using Glucochek glucometer (Aspen diagnostic (P) Ltd. Delhi, India).

Immediately after collection of blood, the tubes were held in a slanting position to facilitate serum separation at room temperature for 1-2 hrs, then centrifuged at 2000 rpm for 10 min and the clear nonhaemolysed serum was then transferred into a clean, sterilized vial. The separated serum was used for analysis of minerals. Iron and Zinc in blood serum was estimated in Erba Versatile Biochemical Analyzer. Using commercially available ready to use kits (Lab-Care Diagnostics).

The protein efficiency ratio (PER) of both control and experimental diets fed rat were determined as per the procedure of IS: 7481 (1974). The food value and growth rate as effected by food intake was determined as feed efficiency ratio (FER) and expressed as gain in weight in grams per unit of food consumed.

The results (average of 3 trials) are analyzed statistically for test of significance by using ANOVA as per SPSS10.0 software package and MS Excel 2007.

Results and Discussion

Influence of different levels of starter culture on sensory attributes of dahi

The effect of different levels of starter culture on sensory attributes of dahi (table 1) containing different levels of starter culture was subjected for organoleptic evaluation by a panel of Judges. Sensory scores for the dahi were graded organoleptically on a 25-point hedonic scale.

From the table it can be observed that the score for dahi made from 1% starter culture bagged highest score. Increased levels of addition of starter culture resulted in increased sourness of the samples and reduced the sensory score. Statistically it is proved that significant difference exists between samples and control as well as between the samples. Increase in sourness due to increased production of lactic acid which is due to utilization of principal milk sugar lactose by more number of starter organisms at a lesser time.

The appearance was good for dahi inoculated with 1% starter culture. Increase starter culture addition caused increase in acidity and wheeling off that inturn reduced the score for appearance. The dahi made from 2% starter culture exhibited lack of uniform appearance due to wheezing off. This defect led to reduction in sensory score for appearance.

Body and texture score was highest for dahi made by using 1% starter culture. This is due to the fact that, dahi was practically free from whey separation and body was firm with uniform texture. Increased starter level resulted in loose body due to presence of more whey pockets. Hence, dahi made by using 1% starter culture was employed for further experimental studies.

Effect of different levels of starter culture on physico-chemical properties of dahi

It is evident from the results (table 2) that increase in starter culture addition resulted in reduction in pH (4.2). Reduction in pH is due to increase in lactic acid production from lactose fermentation by more number of organisms of dahi cultures.

Increase in starter culture level caused increase in acidity of experimental samples. The results are in agreement with the findings of Hossain *et al*. (2012) [4] who found that acidity of fruit dahi was significantly increased due to the addition of higher levels of starter. Kale *et al*. (2011) [8] prepared value added stirred dahi using different types of fruits pulp and observed that acidity content of dahi increased due to the addition of fruits pulp. It is evident from the results total solids content of 1, 1.5 and 2% starter culture added dahi samples were statistically non-significant which was seen among the total solids content of different dahi.

The fat content of different dahi samples showed no significant differences. Average protein content of 1, 1.5 and 2% starter culture added samples were showed non – significant difference among different dahi samples. The

protein content of present investigation agrees with the findings of Hossain *et al.* (2012) [4]. The zinc concentration of 1, 1.5 and 2 % starter culture added samples were statistically non-significant which was seen from the zinc concentration of different dahi samples.

Influence of different levels of iron and zinc on sensory attributes of dahi

An ideally Dahi shall possess a pleasant, clean acid flavor. It shall be free from undesirable flavors such as: bitter, rancid, oxidized, stale, yeasty and unclean. The best results for flavour were obtained for iron and zinc fortification at 40 mg/kg level. Because when iron and zinc fortified dahi at 20, 30 mg/kg was subjected for sensory evaluation they have scored less point because of whey separation and high degree of souring. Higher fortification (> 40 mg/kg) yielded dahi with metallic flavour. Hence, for the experimental studies an optimum level of 40 mg of zinc/kg is employed.

For appearance the highest score was obtained for dahi fortified iron and zinc at 40 mg/ kg when compared with zinc fortified as 20 and 30 mg/ kg. These findings were in agreement with those given by El-kholy (2011) [3] and El-Din *et al.* (2012) [2]. Natural dahi shall possess a firm, custard- like body with a smooth homogeneous texture. The best results for body and texture was observed in dahi fortified with iron and zinc at 40 mg/kg level. Lower scores for dahi fortified with iron and zinc at 20 and 30 mg/kg level. Which is due to the whey separation in samples.

Effect of different levels of iron and zinc fortification on Physico-chemical Properties of dahi

Unfortified dahi showed pH, acidity, total solids, fat, SNF, protein contents as 4.4, 0.76 percent lactic acid, 11.77, 3.2, 8.57 and 3.4 percent respectively.

Fortification of dahi with iron and zinc salts at the rate of 20, 30 and 40 mg/kg had no noticeable effect on physico-chemical properties such as pH, acidity, total solids, fat, SNF and protein, i.e., it remains almost similar when compared to unfortified dahi.

Influence of Zinc on blood serum zinc concentration and on blood glucose level in rats fed with experimental diet

A serum zinc concentration was monitored to understand the effect of zinc fortification in dahi (Table 3). Serum zinc concentration was high in rats which were fed with dahi fortified with zinc (288.50 mg/dl) when compared to serum zinc concentration of animals fed with control (221.0 mg/dl), unfortified dahi (254.33). This could be attributed to binding of zinc which is a cation by Caseinophosphopeptides (CPPs) derived from the enzymatic modifications of proteins in dahi as also during gastro-intestinal digestion in experimental animals thus, they are more bio-available.

The lowest blood glucose level (82.5 mg/dl) was observed in experimental animals fed with zinc fortified (40 mg/kg) dahi sample when compared to blood glucose level of animals fed with control (92 mg/dl), unfortified dahi (87.5 mg/dl) indicating that the zinc in fortified dahi plays a vital role in stimulating insulin formation, thus such fortified dahi is an ideal food for early diabetics. Because zinc plays physiological role in synthesis, storage and secretion of insulin and it seems to stimulate insulin action and insulin receptor tyrosine kinase activity. Adequate zinc in the body plays at least three roles with respect to glucose utilization involving a hormone. First, zinc binds to insulin so that insulin is adequately stored in the pancreas and released when glucose enters the blood stream. Second, zinc improves cell health, making up a component of the enzymes necessary for insulin to bind to cells so that glucose can enter and be used as fuel. The process of insulin binding to the cell is what is referred to with the term “insulin sensitivity” and means that the cell is receptive to insulin. Once the insulin binds to the cell, it “opens the door” so that the glucose can enter into cell.

Finally zinc is important for preventing insulin resistance, as a high zinc level seems to be connected with decreased insulin resistance. Higher levels may affect the ability of the islet cells of the pancreas to store insulin, zinc appears to have a protective effect against the destruction of the β-cells of pancreas (Ranasinghe *et al.*, 2013) [10]. Which are the production sites for a hormone.

Zinc has been shown to mitigate the harmful effects of diabetes by decreasing glycemic load in blood thus sharp fluctuations in blood glucose are not observed. This effect is responsible for controlling in type 1 and type 2 diabetes. Many of the enzyme systems in which zinc is a necessity for are involved with the metabolism of blood sugar, and therefore make zinc a natural catalyst for insulin secretion. The β-cells that secrete insulin in pancreas are also highly stored with zinc (Jayawardena *et al.*, 2012) [7]. From these discussions it is much clear that the dahi fortified with zinc can safely use in persons with early diabetic mellitus.

Influence of iron on blood iron concentration and on blood glucose level in rats fed with experimental diet

After 28 days of feeding regime, from the rats fed with six different types of diets blood was collected blood iron and blood glucose levels in experimental animals were estimated to see the effect of diets on these parameters, the results are presented in Table 4.

It is evident from the values of the table that, there was variation in blood iron and blood glucose levels in rats fed with control as well test diets, but serum calcium was almost similar in all the groups. In rats fed with control and test diets (D1, D2, D3, D4 and D5), the blood iron concentrations were 35.82, 37.17, 37.92, 46.25, 38.31, 45.78 mg % respectively. It was very interested to know that increase (46.25 mg %) in blood iron concentrations in rats fed with iron fortified diet than in lab animals fed with either control (35.82 mg %) or 82 unfortified milk (37.17 mg %) or dahi (37.92 mg %) or dahi fortified with zinc (38.31 mg %) or dahi fortified with both iron and zinc (45.78 mg %).

The blood glucose level of control sample was 92 mg/dl whereas, it was 90, 87.5, 88, and 84.5 mg/dl for unfortified milk and dahi, dahi fortified with iron, and dahi fortified with both iron and zinc. These results indicate that milk and its fermented milk product, dahi reduces the blood glucose level in rats. And it is very much interested to note that the blood glucose levels were lower in animals fed with mineral fortified samples when compared to control.

Table 1: Effect of different levels of starter culture on sensory attributes* of dahi

Level of starter culture (%)	Sensory attributes (Max. score: 25.00)					
	Flavour (10)	Sourness (2)	Appearance (5)	Body &Texture (5)	Closure & Container (3)	Total (25)
Control (0.5)	8.66	1.58	4.41	3.90	2.80	21.35
1.0	9.16	1.50	4.50	4.83	2.85	22.84
1.5	8.50	1.66	4.41	4.50	2.83	21.90
2.0	8.40	1.75	4.33	4.66	2.83	21.97
CD ($p \leq 0.05$)	0.90	0.51	0.86	0.77	0.01	0.90

All values are average of three trials
*Scores obtained based on 25-point hedonic scale

Table 2: Influence of different levels of starter culture on physico-chemical Properties of dahi

Level of starter culture	Physico-chemical properties						
	pH	Acidity % Lactic acid	Total Solids %	Fat %	Protein %	Iron mg/100g	Zinc mg/100g
Control (0.5%)	4.42	0.74	11.8	3.3	3.5	0.10	0.63
1%	4.40	0.76	11.77	3.2	3.4	0.10	0.60
1.5%	4.30	0.78	11.8	3.3	3.6	0.12	0.63
2%	4.20	0.80	11.83	3.3	3.5	0.12	0.60
CD ($p \leq 0.05$)	0.03	0.02	0.15	0.11	0.14	0.06	0.08

All values are average of three trials

Table 3: Effect of Zinc on blood serum zinc concentration and on blood glucose level in rats fed with experimental diet

Type of diet	Serum zinc (mg/dl)	Blood glucose level (mg/dl)
Control	221.00	92.0
D1	245.33	87.5
D2	288.50	82..5

All values are average of six trials at the end of 4th week of feeding period.
Control D1: Unfortified dahi, D2: Dahi fortified with zinc (40 mg/kg)

Table 4: Effect of iron on blood iron and blood glucose level in rats fed with experimental diet

Type of diet	Blood iron (mg/dl)	Blood glucose level (mg/dl)
Control	35.82	92.0
D1	37.17	90.0
D2	37.92	87.5
D3	46.25	88.0
D4	45.78	84.5

All values are average of six trials at the end of 4th week of feeding period.
Control D1: Unfortified milk, D2: unfortified dahi
D3: Dahi fortified with iron (40 mg/kg)
D4: Dahi fortified with iron & zinc (each at 40 mg/kg)

Conclusion

Dahi made by using 1percent starter culture showed uniform body and texture, optimum acidity with no whey separation. The increase in starter culture level resulted in increased acidity reflecting, increased sourness. Hence, dahi made by using 1% starter culture was employed for experimental studies. The sensory score revealed that possibility of making good quality dahi by fortifying milk with food grade ferrous lactate and food grade zinc sulphate and dahi made from such fortified milk respectively at a level of 40 mg/kg milk, the resultant dahi was not differing than the control upon organoleptic properties. Over and above, this level of fortification the dahi samples exhibited metallic flavour. Hence, dahi fortified with ferrous lactate and zinc sulphate at an optimum level of 40 mg/kg is employed for the study. Fortification of dahi with iron and zinc salts at this rate had no noticeable effect on physico-chemical properties. The influence of fortified zinc in dahi was administered to rats and the blood serum zinc concentration was monitored. Serum zinc concentration was significantly higher in dahi fortified with zinc when compared to control and unfortified dahi. Zinc is important to prevent the disorders which occur due to deficiency of zinc. Thus, from the results it can be concluded that fortified zinc is the best choices for fortification in dahi and in infant food formulations to overcome micro mineral related deficiency disorders in infants and growing children.
The effect of fortified iron and zinc in dahi on blood glucose level in rats was measured. Iron fortified dahi also shown low level of blood glucose levels and both iron and zinc fortified dahi are also not showed such fluctuations in blood glucose levels. blood glucose levels were lower in animals fed with mineral fortified samples when compared to control Zinc reduces the glycemic load in blood thus sharp fluctuations in blood glucose levels are not seen. This is due to the fact that zinc plays a key role in the synthesis, storage and secretion of insulin and it seems to stimulate insulin action and insulin receptor tyrosine kinase activity. Zinc plays an important role in overcoming insulin resistance. Thus, zinc in fortified dahi stabilized blood glucose levels as a result; such iron and zinc fortified fermented milk products can safely be consumed by early diabetic people.

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