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Effect of Calcium of Lactose Free Millet Milk on Growth and Development in Albino Weanling Rats

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Abstract

Background:Calcium, the very important mineralhelps in growth and development ofinfants.Calcium helps in building strong bones, teeth,proper functioning of nerves and muscle, blood clot and in activating the enzymes that convert food into energy. Infants and Children are growing new bone all the time, they need continuous supply of calcium to support the healthy growth.Milk is the only food for infants which is richest source of calcium. Some infants are sensitive to lactose in milk because they have Lactose Intolerance. The present study aimsto develop an alternate milk forlactose intolerance infants with finger millet and pearl millet.Method:Traditional methods were used to process the milletswhich help in retaining and increasing the nutritional content in millets.The millet milk was analyzed for calcium content using the ICPMS.The millet milk was supplemented for 6 weeks to albino rats in comparison with cow milk. The tibia weight and length were measured and calcium content in tibia was analyzed. Result:The calcium content of the millet milk was 80mg/100mlwhere as in cow milk it was 120mg/100ml.The mean calcium content of the tibia inalbino rats was15.35±3.50mg/dl fed with millet milkand20.40±3.74mg/dl in rats fed with cow milk.Conclusion: The developed millet milk contain good amount of calcium on par with cow's milk, it can be used as substitute milk for lactose intolerant infants.

Keywords:Bone calcium, Cows milk, Finger millet, Lactose intolerance, Millet milk, Pearl millet, Tibia,

1.1. Introduction

Millets are traditionally processed using either malting or fermentation. Malting of millets helps in improving the digestibility with beneficial effects of lowering the antinutrients along with improving sensory and nutritional quality. Millets are rich in calcium [1]. Calcium is an important mineral which helps in strengthening of bones till the age of 20-25 and in later age it helps in maintaining bone density.The calcium requirement is high during the growth years of infancy and childhood [2].It plays an important role in the formation process of new bone and maintenance of existing bone by collaborating with other factors such as phosphorous, vitamin- D and calcium–binding proteins [3].Optimizing calcium and bone status during infancy can have immediate homeostasis and preventing disturbances in bone mineralization and can provide long term benefits by helping infants genetic

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potential. Over 99% of total calcium in human body is stored in bone and teeth.Lactose intolerance is a common gastric disorder which is caused due to malabsorption of lactose or insufficient amount of lactase enzyme to digest milk products[4]. Lactose intolerant infants often become calcium deficient. Hence the developed millet milk can be used as substitute milk in place of cow milk which helps to overcome calcium deficiency.

2. Methodology

2.1. Animal Care

In this experiment 12 weanling male Albino rats (3 weeks old: n =12) were housed in polypropylene laboratory rat cages (1 per cage) [3].The rats were kept in cages for three days to adjust to the new environment. The temperature and relative humidity were 23 ± 2^0 C and 40-70%, respectively was maintained throughout the experiment period[5].The12h day and night cycle was maintained.

2.2. Diet

Therats were on stock diet prior to the experiment. Initially they were fed on stock diet for three days. The day before the experiment, initially the rats were weighed, the average weight of rats was 43 to 45g. According to weight of rats they were grouped into two experimental group and control group each with six rats. The initial mean weight of each individual rat from each group was recorded which was 43.33±2.58g.

The rats were fed daily,with stock diet 7am in the morning and 5pm in the evening, the left over feed was weighed and recorded and then fed with the fresh diet. The milk feedwas given in the mornings. Weekly consumption of milk was recorded. Care was taken for cleaningand sterilizingthe water bottles and food cups daily before administering the diet.The experiment was done for 6 weeks.

Table 1:The composition of Stock diet, cow milk for control and millet milk for experiment

S.no	Nutrients	Stock diet	Cow's milk	Millet milk
1	Carbohydrate (g)	44.9	4.4	17.7
2	Protein (g)	7.8	3.2	3.2
3	Fat (g)	0.6	4.1	0.4
4	Energy (kcal)	216.4	67	87.12
5	Calcium (mg)	50	120	80
6	Iron (mg)	2.0	0.2	1.0

Nutrient composition of Stock Diet and Millet Milk was Analysed by using Standard Procedures from AOAC and NIN manual. Nutrient composition of Cows milk is taken from Nutritive value for Indian Foods, Gopalan.

2.3. Parameters

The weight and heights of the rats were recorded weekly.To measure weight, laboratory animal weighing balance was used for measuring the accurate weight of the rats. The animals were weighed and height was measured once a week throughout the experimental period and the changes in body weights and heights are shown in the table 2.

Table 2: The effect of Millet milk on growth parameters- Body weight and height

Week	Mean weekly weight of control rats (g)	Mean weekly weight of experimental rats (g)	Mean weekly length of control rats (cm)	Mean weekly length of experimental rats (cm)
1	43.33±2.58	45±0	23.25±0.75	23.33±0.40
2	60.16±10.68	59.33±5.50	24.33±1.21	23.96±0.20
3	79.5±15.63	63.33±7.81	25.75±1.54	24.66±0.25
4	82±14.91	68.166±8.30	26.83±1.43	25.83±0.98

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5	87.6±17.10	71.08±9.35	29.41±2.04	26.25±0.61
6	99.16±19.25	74.75±10.3	27.5±1.71	26.83±0.62
Mean±SD	77.54±17.91	66.214± 11.01	26.31±2.34	25.13±1.38
t-value		0.01^{**NS}		0.03^{**NS}

Including Standard error of the Mean, P < 0.05 Significant, NS-Not Significant**

2.4. Collection of Tibia bone

The rats were sacrificed at the termination of experiment. The tibia bone was collected, sterilized for further analysis. Bone weight and length were recorded and are shown in the table 3. Allthe tibias wereindividually placed in glass petridishes and were subjected to place in ahot air oven at 100^0 C for 1 hour [6].

2.5. Reagents and Chemicals

Analytical reagents – grade chemicals, concentrated HCl were used in the preparation of solution. 10% chloroform was used for anaesthesia. Ethanol was used for work table sterilization and disinfection. Distill water for cleaning and sterilizing the collected samples. All the glassware were cleaned and soaked in dilute sulfuric acid and were rinsed with distilled water and dried in hot air oven prior to use. The reagents were purchased from SSR scientifics.

2.6. Bone ash and sample preparation

The bones were placed in a silica crucible individually and subjected to ashing in a muffle furnace at 600^0C for 6 hours. Allowed it to cool for 8 hours. Then digestion process is carried out for bone calcium analysis. The ash is moistened with a small amount of distill water and 5ml of conc.HCl is added to it. The mixture is evaporated to dryness on a boiling water bath and filtered into 100ml volumetric flask using whatmann no.40 filter paper, after cooling the volume is made upto 100ml. The resulting solutions were used to carry out in ICP-OES [7].

2.7. Ethical Statement

The ethical permission was taken from Committee fro the purpose of Control and Supervision of experiments on animals (CPCSEA). The ethical number is 41/2012-13/(i)/00/CPCSEA/IAEC/SVU/KTR/-BS,08.07.2012

2.8. Statistical Analysis

When comparison was made between the samples SPSS statistical package was used. Data are given as means±SD and t-test values. All the subjects were compared using control and experiment feed.

3. Results and discussion

The effect of millet milk on the body weight and height of the rat is shown in table 2. There was a steady increase in body weight of animals from day 1 to day 45. The body weight has increased from 45g to 75gin both the groups by the end of the experiment. Rats on cow milk hasgained weight 77.54±17.91 as compared to rats on millet milk was 66.214±11.01.

3.1. Body Weight and length

Weight is one of the main growth parameter which shows the absolute effect of the acceptability of the food intake [8]. Weight is the most widely used and simplest, reproducible anthropometric measurements for the evaluation of nutritional status. Till 60days of age the growth and development is rapid and gradually slows down [9].

The data in the Table No. 2 interprets the weekly body weight and length changes of rats. The mean weight of the rats of the both groups increased gradually from first week to sixth week. There was no significant difference in weight and length between the groups which indicate that the millet milk provided adequate nutrition which was similar to cow milk.

3.2. Bone weight and length

One of the best indices for the assessment of bone development is bone weight. Measuring bone parameters after prolonged treatment is the most reliable way of estimating the long-term effects of qualitatively and quantitatively of the calcium intakes [10].The data obtained on mean bone weights of albino rats fed with different milks were presented in the Table 3. The mean bone weight of control rats was (0.60±0.10 g) and experimental rats were (0.41±0.10 g).The mean bone length of control rat was 1.96±0.38 cm and experimental group was (1.69±0.18 cm). There was no significant difference in between control and experiment rats. Bone is a mineral reservoir for calcium and phosphorous. Itis a living and constantly remodeling tissue [11].

Table 3: The Effect of millet milk calcium on bone weight, bone length and calcium levels in the bone mineral of albino rats

Parameters	Bone weight (g)		Bone length (cm)		Calcium levels (mg/dl)	
	Control	Experiment	Control	Experiment	Control	Experiment
1	0.606	0.550	2.50	1.49	15.78	13.93
2	0.592	0.290	2.40	1.64	17.76	17.68
3	0.420	0.505	1.82	2.03	19.02	14.57
4	0.660	0.290	1.59	1.57	20.84	11.74
5	0.665	0.420	1.70	1.72	22.84	21.92
6	0.725	0.440	1.76	1.71	26.21	12.26
Mean±SD	0.60±0.10	0.41±0.10	1.96±0.38	1.69±0.18	20.40±3.74	15.35±3.50
t-value	0.040^{**}		0.239^{NS}		0.071^{NS}	

Including Standard error of the Mean, P < 0.05 Significant, NS-Not Significant**

3.3. Bone Calcium

The data in the Table No.3 interprets the bone calcium levels of controlandexperimental rats. The mean bone calcium levels of the control ratswere 20.40±3.74 mg/dl and the experimental rats was 15.35±3.50 mg/dl. There was no significant difference of bone calcium levels between both control and experimental rats. The calcium levels are almost equal to the control rats which showsmillet milk is as good as cow milk for feeding the infants with lactose intolerance [12-13].

The effect of calcium in millet milk on growth parameters body weight and length, bone weight and length and calcium levels of bone is shown in Fig.1

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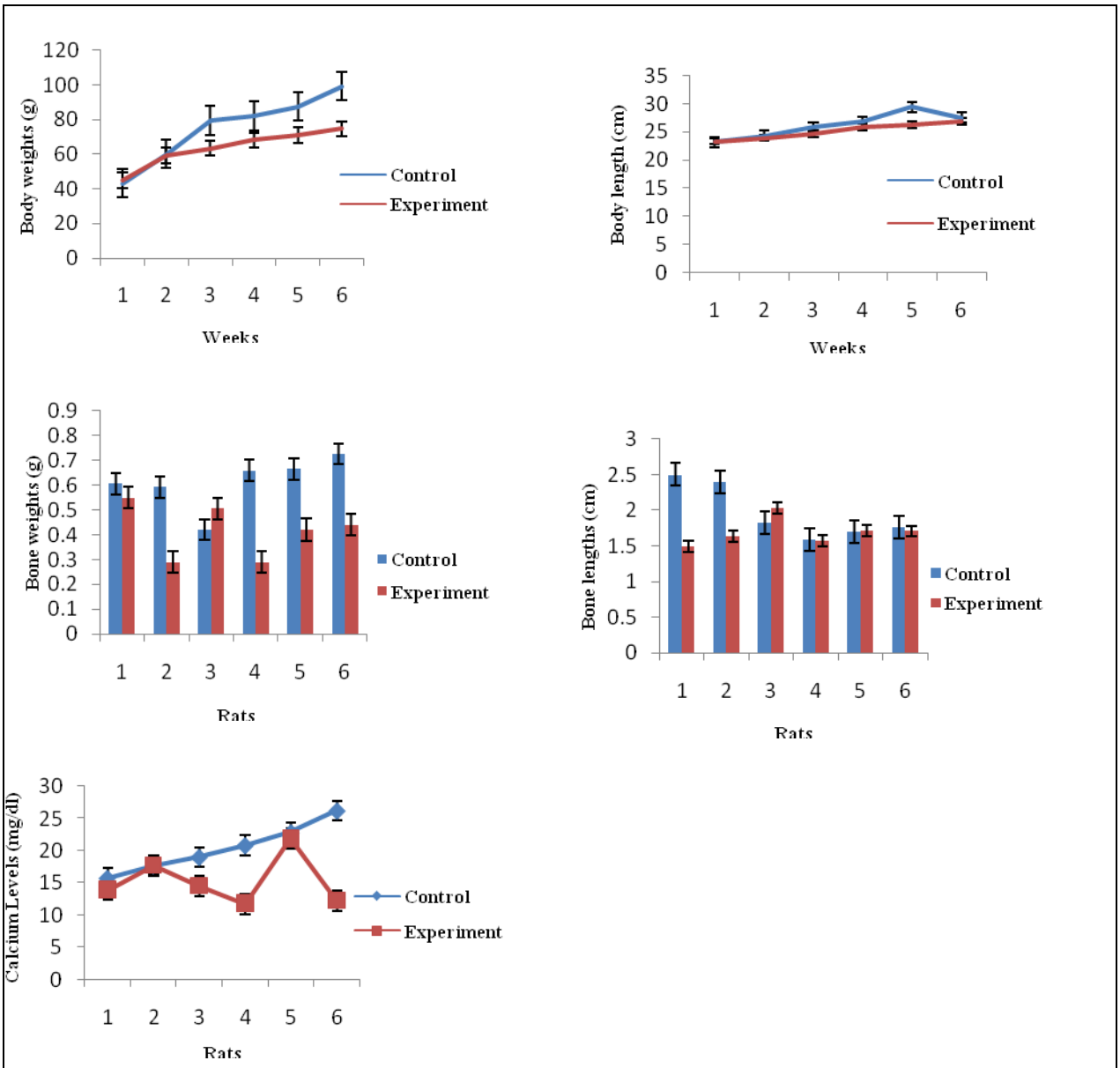

Fig 1: The effect of calcium in millet milk on growth parameters body weight and length, bone weight and length and calcium levels of Bone

4.0. Summary and Conclusion

Lactose intolerance is a common gastric disorder from infants to old age people. The most vulnerable group are infants, as they can only produce low amount of lactase in the beginning of the infancy and gradually increase the production the enzyme, after weaning is introduced, due to primary lactase deficiency the production of lactase will again be decreased. Therefore the lactose free substitutes which can supply calcium and protein are a necessity. During the supplementation to the weanling rats, the investigations have shown that calcium levels in bone tibia of both control and experiment rats, there was no significant difference. The developed millet milk is nutrient rich in

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both protein and calcium and can be used as substitute milk instead of cow milk or human milk during the lactose malabsorption causing gastric problems.

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