

Use of nucleotides in weanling rats with diarrhea induced by a lactose overload: effect on the evolution of diarrhea and weight and on the histopathology of intestine, liver and spleen

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Abstract

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Until recently, dietary sources of nucleotides were thought not to be essential for good nutrition. Certain states with higher metabolic demands may require larger amounts that cannot be provided by endogenous production. The objective of the present study was to determine the action of nucleotides on the recovery from lactose-induced diarrhea in weaned rats. Thirty-six weanling Fisher rats were divided into two groups. Group 1 received a standard diet and group 2 received a diet containing lactose in place of starch. On the 10th day, six animals per group were sacrificed for histopathological evaluation. The remaining animals were divided into two other subgroups, each with 6 animals, receiving a control diet, a control diet with nucleotides (0.05% adenosine monophosphate, 0.05% guanosine monophosphate, 0.05% cytidine monophosphate, 0.05% uridine monophosphate and 0.05% inosine monophosphate), a diet with lactose, and a diet with lactose and nucleotides. On the 32nd day of the experiment all animals were sacrificed. Animals with diarrhea weighed less than animals without diarrhea. The introduction of nucleotides did not lead to weight gain. Mean diet consumption was lower in the group that continued to ingest lactose, with the group receiving lactose plus nucleotides showing a lower mean consumption. Animals receiving lactose had inflammatory reaction and deposits of periodic acid-Schiff-positive material in intestinal, hepatic and splenic tissues. The introduction of nucleotides led to an improvement of the intestinal inflammatory reaction. In lactose-induced diarrhea, when the stimulus is maintained - lactose overload - the nucleotides have a limited action on the weight gain and on recovery of intestinal morphology, although they have a protective effect on hepatic injury and improve the inflammatory response.

Key words

- Nucleotides
- Diarrhea
- Weight
- Weanling rats

Introduction

Until recently, dietary sources of nucleotides were thought not to be essential for good nutrition. Endogenous synthesis was thought to be able to meet the requirements of a normal metabolism. However, several studies have demonstrated that certain states with higher metabolic demands, such as periods of rapid growth and the presence of organic disease, may require larger amounts that cannot be provided by endogenous production. Thus, nucleotides have been considered to be conditionally essential nutritional elements. Many studies have demonstrated the beneficial effect of supplementation, especially for infant nutrition when the nutritional requirements imposed by growth are high (1).

Nucleotides are phosphoric nucleoside esters. Nucleosides are the basic units of the nucleic acids DNA and RNA, consisting of a nitrogen base (purines: adenine, guanine and hypoxanthine; pyrimidines: uracil, cytosine and thymine) plus one pentose (ribose or deoxyribose). Pyrimidine rings are formed in the organism from aspartate, glutamine and CO₂, while purine rings also utilize glycine and tetrahydrofolate derivatives (1). When phosphorylated, a nucleoside forms a nucleotide. The nucleotides of primary interest are adenosine, guanosine, inosine, cytidine and uridine monophosphates.

Dietary nucleotides are ingested as nucleoproteins. Digestion is initiated by stomach proteases, with later exposure to pancreatic nucleases and phosphoesterases, with the production of nucleotides and nucleosides in the intestinal lumen. There is evidence suggesting that the mixture of nucleosides and nitrogen bases may be the final product absorbed by the enterocytes (2). Absorption mechanisms include facilitated diffusion, active transport (3), cotransport with sodium (4), and incorporation into tissues.

Dietary nucleotides are especially important in the development and proliferation of

tissues with rapid cell turnover such as the intestine, bone marrow and cells of the immunological system. The capacity for endogenous synthesis has not yet been established and may not be sufficient to respond to increased needs during periods of rapid growth and after aggressions to the organism such as disease states or trauma (5).

In animals with diarrhea induced by lactose, the introduction of nucleotides into the diet resulted in the recovery of intestinal morphology (5), improved inflammatory response and higher disaccharidase activity (6). Maltase activity is higher in animals fed nucleotides although a similar effect has not been observed for lactase or saccharase (2). Ortega et al. (7) demonstrated that nucleotide-free diets produced a lower enzymatic activity in the top of the intestinal villi of rats. Adjei et al. (8) demonstrated in mice that a mixture of nucleotides is more effective than each nucleotide separately in leading to the recovery of the mucosa and in providing protection against bacterial translocation in animals submitted to a protein-free diet. Tanaka et al. (9) demonstrated in tissue culture that an exogenous nucleotide (AMP) plays an important role in the control of intestinal cell turnover.

In children fed formulas supplemented with nucleotides the intestinal flora presented a higher percentage of fecal bifidobacteria and lactobacilli and a lower percentage of Gram-negative bacteria (10). Brunser et al. (11) also demonstrated that feeding infants with diets containing nucleotides resulted in a lower prevalence of diarrhea compared to infants on a standard diet, although the severity of the signs and symptoms did not differ between groups. Thus, it appears that, in addition to promoting a rapid cell regeneration and increasing the production of intestinal disaccharidases, nucleotides also play an important role in the determination of the intestinal bacterial flora, promoting in infants the growth of an intestinal bacterial flora similar to that of infants fed human milk.

Nucleotides also seem to act on liver regeneration after injury (12). Nucleotide-free diets also result in a lower relative liver weight and a higher concentration of hepatic fat (3).

Nucleotides also have an effect on lipid metabolism, promoting increased polyunsaturated fatty acid concentrations in the erythrocytes and plasma of infants (13-16) and increasing the bioavailability of dietary iron (17).

Nucleotide-poor diets have been associated with the reduction of mitosis in lymphocytes, a reduced production of interleukin-2 and impaired humoral immunity (1,18,19). On the other hand, diets supplemented with nucleotides and offered to rats were found to be associated with increased mortality due to graft-versus-host disease, greater rejection of allogenic grafts (20), greater resistance to infection with *Staphylococcus aureus* (21) and *Candida albicans* (22), increased phagocytic capacity of macrophages (23), and reduction of the immunosuppression induced by malnutrition (24).

The mechanism of action of nucleotides on immunity has not been fully clarified. Since intestinal lymphoid tissue can start and regulate the development of T lymphocytes, acting as an organ analogous to the thymus (25), nucleotides may perhaps play their role starting from this important immunological organ.

The activity of CD4 lymphocytes and the activation of spleen macrophages were lower in animals fed nucleotide-free diets than in animals fed a supplemented diet (25). In children, supplementation with nucleotides promoted T natural killer lymphocyte and interleukin-2 activity similar to that found in breast-fed infants (26). A beneficial effect of the supplemented diet on the immunological response to anti-*Haemophilus influenzae* and anti-diphtheria vaccines has also been reported (27). Serum IgG concentrations were higher in premature newborns fed diets containing nucleotides than in newborns fed

standard diets (28).

Human milk contains significant amounts of nucleic acids, nucleotides and related products. Ten to 20% of non-protein nitrogen is represented by free nucleotides, while cow's milk presents much lower quantities. The mean nucleotide concentrations in human milk are (29): uridine, 14 ± 4.2 mg/l; cytidine, 27.3 ± 7.8 mg/l; guanosine, 14.9 ± 6.6 mg/l; adenosine, 11.3 ± 6.9 mg/l. In addition to human milk, viscera, seafood and legumes are natural nucleotide sources.

The recognition of the semi-essential characteristic of these previously unknown components of nutrition, whose need is especially felt in situations of immunological impairment or during rapid growth phases, justified their introduction in some infant formulas in Japan, the United States and, more recently, in Europe and Brazil. However, many aspects of the action of nucleotides still require investigation, such as their role in prophylaxis against infection, in intestinal function and in the recovery of lesions.

Thus, the objective of the present study was to determine the effect of nucleotides on the recovery from lactose-induced diarrhea in weaned rats and to observe the weight evolution and the histology of the intestinal mucosa, liver and spleen of the animals.

Material and Methods

Thirty-six weanling Fisher rats were kindly supplied by the Nutrition Laboratory of Federal University of Ouro Preto. The animals were divided into two initial groups maintained in the animal facilities of the Institute of Biological Sciences. The animals were housed two to a cage in 18 cages, nine per group.

Group 1 animals received a standard diet and group 2 animals received a diet containing lactose in place of starch (Table 1). The animals had free access to food and water. The diet containing lactose caused osmotic diarrhea in the animals of group 2 as early as

on the second day of the experiment.

On the 10th day, six animals per group were sacrificed by ethyl ether inhalation for histopathological evaluation. The remaining animals were divided into two other subgroups respectively receiving a control diet, a control diet with nucleotides, a diet with lactose, and a diet with lactose and nucleotides. The animals were followed up for 22 days. On the 32nd day of the experiment all animals were sacrificed by ethyl ether inhalation.

The study was conducted according to the regulation of the Brazilian Council for Animal Experimentation (COBEA) and was approved by the Council of the Department of Pediatrics, Medical School of the Federal University of Minas Gerais.

The variables studied were weight and diet consumption. The weight of the animals was determined for each pair using a precision digital scale on days 1, 10, 18, 25 and 32. Mean diet consumption was determined by measurement of the amount offered and the daily leftover food per cage.

Histopathological evaluation

The histopathological study was conducted on intestine, liver and spleen fragments. The material was fixed in an aqueous formalin solution and routinely processed

for paraffin embedding. Histological sections were prepared and stained with hematoxylin-eosin and periodic acid-Schiff (PAS).

The microscopic examination (binocular Olympus, Japan) was performed by a single examiner who was unaware of the experimental procedure applied to the animals. After examination of all the material, the experimental conditions to which each group/animal had been submitted were revealed and a final report was elaborated. The microscopic description was made by comparing the control group with the test group.

Statistical analysis

Weight data are reported as means per animal pair. Mean weight was compared between groups on the different days of the experiment using the Student *t*-test. Mean diet consumption per group was compared by analysis of variance.

Results

Evolution of diarrhea

The animals fed a diet containing lactose presented diarrhea, whereas the animals fed the control diet did not. The introduction of nucleotides into the diet did not reduce the diarrhea of animals which were fed the lactose diet throughout the experiment.

Weight evolution

The animals with diarrhea weighed less than the animals without diarrhea (Table 2). There was a significant difference in weight between the control x lactose group and the lactose x lactose and nucleotides group at all time points evaluated (Table 3). The animals with diarrhea and receiving nucleotides weighed less at the end of the experiment than the animals that did not receive nucleotides ($P < 0.05$). Thus, the addition of nucleotides to a lactose diet resulted in a larger

Table 1 - Diets utilized in the experiment.

Components	Control (g%)	Lactose (g%)	Control + nucleotides (g%)	Lactose + nucleotides (g%)
Casein	17	17	17	17
Salts and vitamins	2.7	2.7	2.7	2.7
Cellulose	5	5	5	5
Sugar	15	15	15	15
Oil	10	10	10	10
Methionine	0.3	0.3	0.3	0.3
Starch	50	0	50	0
Lactose	0	50	0	50
Adenosine monophosphate	0	0	0.05	0.05
Guanosine monophosphate	0	0	0.05	0.05
Cytidine monophosphate	0	0	0.05	0.05
Uridine monophosphate	0	0	0.05	0.05
Inosine monophosphate	0	0	0.05	0.05

weight loss than produced by lactose alone.

Diet consumption

Mean diet consumption during the first 10 days of the experiment was 12.86 ± 0.47 g for the control group and 12.84 ± 0.73 g for the lactose group, with no significant difference between groups ($P = 0.94$). After the animals were divided into the four groups, we observed that mean consumption did not differ between the control group (18.15 ± 0.37 g) and the control group receiving nucleotides (17.36 ± 0.63 g) ($P = 0.07$). However, mean consumption was lower in the group that continued to ingest lactose (13.46 ± 0.93 g), with the group receiving lactose plus nucleotides showing a lower mean consumption (11.56 ± 1.08 g) ($P = 0.008$).

Histopathological analysis

Control animals. Control animals presented no changes and their liver showed weakly positive PAS reactivity.

Animals fed the lactose diet (Figure 1A). More marked lesions were found after the prolonged use of lactose. The intestinal morphology showed exacerbation of the villus pattern and voluminous prismatic enterocytes with a thick brush border, with no significant changes in the large bowel. The lymphoid

component of the lamina propria was expanded.

The liver showed dense PAS-positive deposits, with preserved lobular architecture and with a predominantly periportal distribution.

The spleen showed lymphoid depletion, which was more intense in the animals fed lactose for a longer period of time. Intracellular carbohydrate deposits were observed in voluminous cells of paracortical and medullary location.

Control animals fed nucleotides (Figure 1B). No intestinal changes were observed; the spleen presented slight lymphoid depletion and the liver showed slight intracellular degenerative phenomena of the hydropic degeneration type.

Animals fed lactose and nucleotides (Figure 1C). The intestinal villi showed a pattern similar to that found in the lactose group but with a smaller expansion of the lymphoid

Table 2 - Mean animal weight, per pair, of the groups with and without diarrhea.

Data are reported as mean \pm SD for 18 animals in each group. * $P < 0.05$ compared to the control group (Student t-test).

Day of experiment	Weight (g)	
	Control	Lactose
1	101.76 \pm 8.68	97.42 \pm 9.08
10	158.04 \pm 16.56	116.27 \pm 17.26*

Table 3 - Weight evolution of the animals, per pair, in the groups with and without diarrhea and receiving or not nucleotides.

No statistically significant differences were observed between the control and control + nucleotides groups.

Day	Weight (g)				Statistical differences	
	Control	Control + nucleotides	Lactose	Lactose + nucleotides	Control x lactose	Lactose x lactose + nucleotides
18	228.83 \pm 16.85	210.66 \pm 16.97	171.66 \pm 13.58	140.33 \pm 2.84	$P = 0.0102$	$P = 0.0173$
25	302.66 \pm 18.44	287.00 \pm 10.58	210.33 \pm 14.01	266.33 \pm 8.50	$P = 0.0023$	$P = 0.0096$
32	387.16 \pm 32.38	371.33 \pm 19.66	270.00 \pm 17.43	206.66 \pm 12.06	$P = 0.00526$	$P = 0.00024$

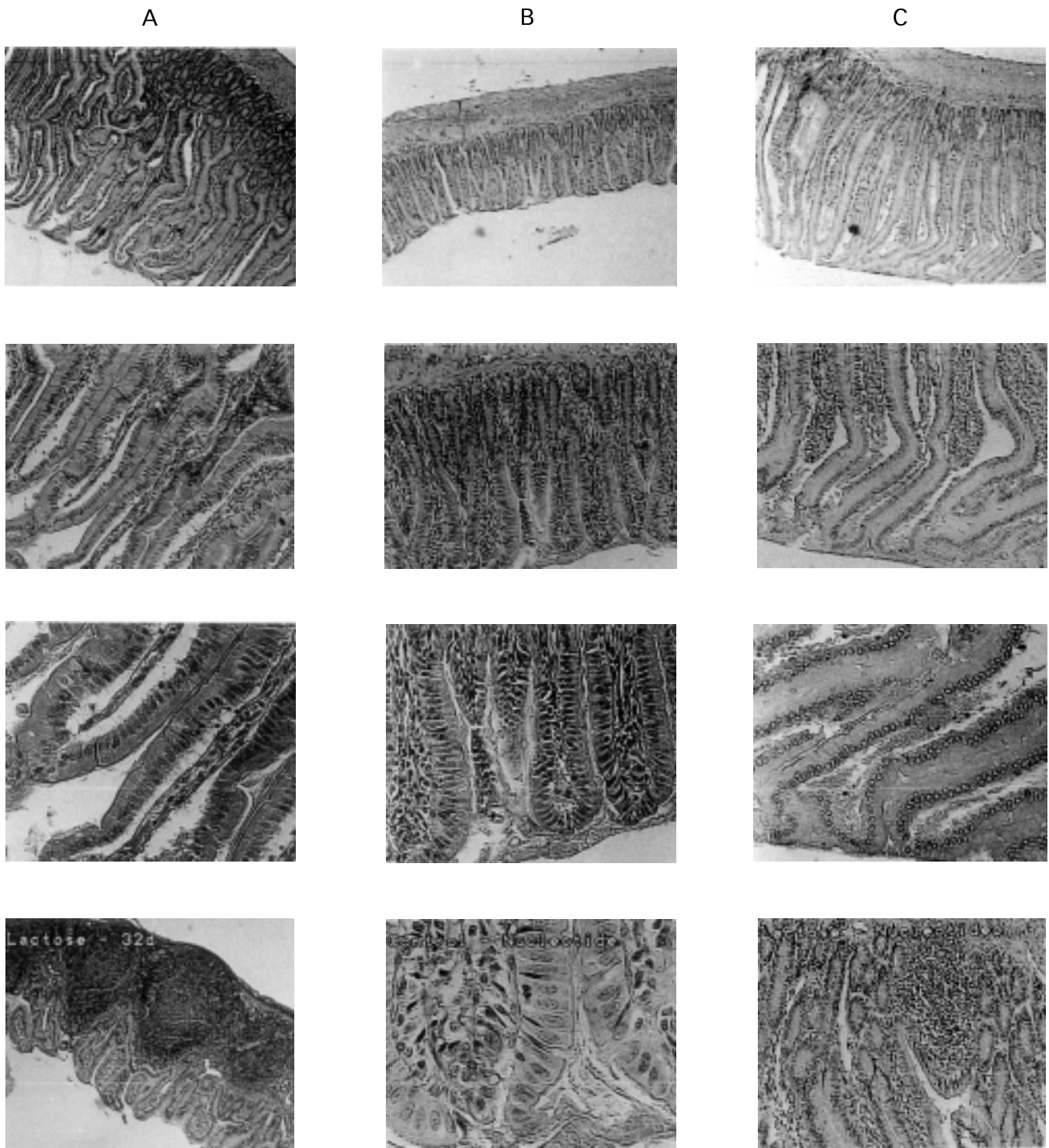


Figure 1 - Histopathological analysis of intestinal morphology in the different groups. A, Animals fed a lactose diet. Intestinal morphology showing exacerbation of the villus pattern and prismatic enterocytes with a thick brush border and expanded lymphoid component. B, Control animals fed nucleotides. No intestinal changes were observed. C, Animals fed lactose and nucleotides. The intestinal villi showed a pattern similar to that found in the lactose group with smaller expansion of the lymphoid component of the lamina propria.

component of the lamina propria. The large bowel was unchanged.

The liver showed deposition of PAS-positive material of less intensity than in the lactose group, with a more disperse distribution throughout the hepatic lobe. The spleen showed changes similar to those found in the lactose group.

Discussion

Evolution of diarrhea and of the weight curve

The model of lactose-induced diarrhea has been used before (5,6). However, no study used the lactose diet throughout the experiment as a way of determining the therapeutic action of nucleosides in diarrheas caused by lactose overload.

The experimental animals submitted to a diet containing lactose in place of starch developed osmotic diarrhea. The consequent nutrient malabsorption caused hair loss and malnutrition, which was confirmed by the difference in weight between groups at the end of the 10th day of the experiment, as also demonstrated by Nuñez et al. (6).

The continuous use of the lactose diet maintained the stimulus of diarrhea and malnutrition regardless of the introduction of nucleotides. The weight reduction in the animals receiving nucleotides may have been the consequence of the intestinal malabsorption resulting from the maintenance of the stimulus causing diarrhea (lactose) combined with the low acceptance of the diet containing nucleotides, which may have turned the ration less palatable.

Histopathological analysis

Histopathological analysis of the tissues showed that the animals receiving lactose had significant intestinal, hepatic and splenic changes compared to controls, with an inflammatory reaction and deposits of PAS-positive material.

The introduction of nucleotides with the maintenance of the diarrhea stimulus (lactose) caused a reduction of the inflammatory reaction but had no effect on the changes in the morphology of the intestinal mucosa (exacerbated villus pattern). Previous studies have demonstrated that the intestinal mucosa recovers promptly after the animals with lactose-stimulated diarrhea are returned to a standard diet enriched with nucleotides (5). However, in animals without diarrhea submitted to nucleotide-free diets, the intestinal villi were found to be as much as 25% smaller in the proximal intestinal segment compared to animals receiving a diet containing nucleotides (2).

The hepatic alterations induced by the use of lactose - dense PAS-positive deposits, with preserved lobular architecture and predominantly periportal distribution - were reduced by the introduction of nucleotides, with the liver showing a less intense deposition of PAS-positive material and a more disperse distribution of the latter throughout the lobule. Previous studies have demonstrated the beneficial effect of nucleotides on the recovery from hepatic injury (12) and on fatty deposition in the liver (3).

In the spleen, the lactose diet caused lymphoid depletion, with intracellular carbohydrate deposits in voluminous cells, with paracortical and medullary localization. The introduction of nucleotides did not modify this histopathological picture. The control animals that received nucleotides also showed lymphoid depletion, although of very slight intensity. This phenomenon may perhaps be explained by lymphocyte migration in the presence of aggression.

The introduction of nucleotides into the diet of the animals with diarrhea triggered by lactose did not result in an improved picture. Diarrhea persisted and there was no nutritional recovery. On the contrary, the weight of the animals receiving nucleotide supplementation was significantly lower than the weight of the animals receiving only lactose.

There was improvement of the intestinal inflammatory reaction in the animals with diarrhea and receiving nucleotide supplementation.

The use of nucleotides reduced PAS-positive deposits in the liver of animals with diarrhea, although spleen lymphoid depletion was maintained.

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