Developmental Effects of Dietary Nucleotides in Second-Generation Weaned Rats

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ABSTRACT The aim of the present study was to evaluate dietary effects of dietary nucleotides (NTs) on developmental parameters in second-generation Sprague-Dawley rats. Experimental design was set up as six groups, including one control and five nucleotide administered groups, doses ranged from 0.01% to 1.28% nucleotides. First-generation (F0) rats were divided into two terms: (1) fed for 90-day study; (2) mated for offspring (F1). After weaning, each group of second-generation F1 rats was fed the control diet. Evaluating parameters were weight gain and food utilization ratios of rats, hematological and biochemical parameters, and organ pathology. The results showed that there was a greater weight gain and food utilization ratio in weaned rats (F0 and F1). Furthermore, weight gain and food utilization ratios were increased in the first 2 weeks of F1 male and the first week of F1 female rats. There were no significant changes in blood indicators of NT groups with the exception of decrease in aspartate aminotransferase and serum uric acid levels. Overall, it was demonstrated that NT supplements could promote the early growth and development at a 0.01% dose. Although NTs may have cumulative reproductive effects, they were safe even at a high-dose supplementation. Additionally, NT supplementation could promote restoration and optimize liver function.

KEY WORDS: • *developmental* • *dietary nucleotides* • *safety* • *Sprague-Dawley rat*

INTRODUCTION

NUCLEOTIDES AND THEIR metabolic products play key roles in most of the biological processes; they are base units of nucleic acids, coenzyme components, and biological regulators involved in transferring chemical energy or in biosynthetic pathways. There is increasing evidence that dietary nucleotides (NTs) affect different aspects of body function and health, including lipid metabolism,¹ immune function,^{2–5} intestinal microbiota,⁶ liver function and morphology⁷ as well as growth performance.⁸ Although nucleotides can be synthesized *de novo*, several tissues with a rapid turnover rate, such as the intestine, may benefit from an exogenous supply to save energy. Nucleotides become conditionally essential nutrients, during disease states, periods of limited nutrient intake or rapid growth, especially during development.⁹

Numerous studies have shown that the nucleotide-fortified formula is safe and beneficial.^{6,10–14} At the same time, several studies have shown that adding NTs in infant formula is safe and confers benefits to the infant health.^{14,15} It has been suggested that the similar incidence of adverse events, between the human milk-fed and the formula-fed groups, provides presumptive evidence of the safety of NTs

for preterm infants. In our previous multigeneration, assessment of NTs reported that no reproductive and developmental toxicity were observed. In addition, it indicated that NTs may play roles in the promotion of early growth and development after weaning.¹⁶ However, it is not clear whether the developmental effect resulted from the cumulative effect of parental rats.

Based on these previous studies, we were interested in whether NTs affect development after weaning and whether the effects of NTs accumulate across generations. The totality of this study comprised two 90-day studies across two successive generations.

MATERIALS AND METHODS

Test substance

NTs provided by the Zhen-Ao Biotechnology Ltd. Co. (Dalian, China) were derived from brewer's yeast RNA with a purity of more than 99%. The NT mixture contained adenosine monophosphate (AMP), guanosine monophosphate (GMP), uridine monophosphate (UMP), and cytidine monophosphate (CMP) with the following ratio 5'AMP:5'CMP:5'GMPNa₂:5'UMPNa₂ being 22.8:26.6:30.2:20.4. The experimental diet was based on AIN93G formulation.¹⁷ The dosages were 0.01%, 0.04%, 0.16%, 0.64%, or 1.28%. Dietary ingredients (Table 1) were thoroughly mixed in a mixer, pelletized, and air-dried at room temperature.

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TABLE 1. INGREDIENT COMPOSITION OF THE EXPERIMENTAL DIETS FED TO RATS

Components	Control	0.01% NTs	0.04% NTs	0.16% NTs	0.64% NTs	1.28% NTs
Cornstarch	397.486	397.446	397.327	396.850	394.942	392.398
Casein ($\geq 85\%$ protein)	200.000	199.980	199.920	199.680	198.720	197.440
Dextrinized cornstarch (90–94% tetrasaccharides)	132.000	131.987	131.947	131.789	131.155	130.310
Sucrose	100.000	99.990	99.960	99.840	99.360	98.720
Soybean oil (no additives)	70.000	69.993	69.972	69.888	69.552	69.104
Fiber	50.000	49.995	49.980	49.920	49.680	49.360
Mineral mixture	35.000	34.997	34.986	34.944	34.776	34.552
Vitamin mixture	10.000	9.999	9.996	9.984	9.936	9.872
L-Cystein	3.000	3.000	2.999	2.995	2.981	2.962
Choline bitartrate (41.1% choline)	2.500	2.500	2.499	2.496	2.484	2.468
TBHQ	0.014	0.014	0.014	0.014	0.014	0.014
Nucleotides mixture ^a	0.000	0.100	0.400	1.600	6.400	12.800

Ingredient composition was based on AIN-93G rodent diet.¹⁷ Values are reported in g/kg diet.

^aComposition of nucleotide mixture: 22.8% adenosine monophosphate, 26.6% cytidine monophosphate, 30.2% guanosine monophosphate (2Na), 20.4% uridine monophosphate (2Na). The purity of each nucleotide was more than 99%.

NTs, dietary nucleotides; TBHQ, *tert*-butylhydroquinone.

Experimental animal and housing conditions

A total of 280 male and female Sprague-Dawley rats (4 weeks old), weighing 35–55 g, were obtained from the Animal Service of Health Science Center, Peking University, Beijing. Rats were housed two per plastic cage with free access to chow and tap water in a specific pathogen-free filter-protected air-conditioned room with controlled temperature (25–28°C), relative humidity (60% $\pm$ 5%), and 12-h light/12-h dark cycles (light on 7:30 a.m.–7:30 p.m.). All animals were handled in accordance with the guidelines of the National Institutes of Health (NIH Publication No. 85–23 revised 1985) and the guidelines of the Peking University Animal Research Committee (www.lab.pku.edu.cn).

Experimental design

After a 1-week acclimation period, rats were randomly assigned to one of the six groups (40 animals per sex per group): one control group and five experimental groups. Control rats were fed with the AIN93G rodent diet (Vital River Ltd. Co., Beijing, China). Rats in the five experimental groups were fed with 0.01%, 0.04%, 0.16%, 0.64%, or 1.28% (wt/wt) NTs in the diet for 90 days, respectively (Vital River).

The first generation (F0) was fed the diets (control or NTs) for 90 days before mating through scheduled sacrifice. The F0 were divided into two groups: (1) 90-day subchronic toxicology F0 rats (8/group/sex) that were not mated and (2) mated (32/group/sex) used in the reproduction phase of the study.

For the mating portion of the reproduction feeding study, an F0 female was paired with one F0 male from the same treatment group in a cage for a maximum of 14 days. Detection of mating was confirmed by evidence of sperm in the vaginal smear or by the appearance of the vaginal copulatory plug at 7:00 a.m. the next day. After confirmation of mating, the female was placed in an individual cage, en-

suring that a total of five female rats had become pregnant. The day parturition was initiated was designated as day 0 of lactation for the dams and postnatal day (PND) 0 for pups (F1). All pups, on PND 4, were examined externally for abnormalities and culled to yield 4 pups/sex/litter. Culled pups were weighed and discarded. The dam and litter remained together and remained on the control or NT diets until weaning on LD 21. After being weaned, F1 rats were fed with a control diet. Rats (8/group/sex), for a 90-day subchronic toxicology, were selected at random.

After all the 90-day subchronic toxicology rats (F0 and F1) were fed for 90 days, they were anesthetized by CO₂ inhalation and then humanely killed. The remaining rats were used for other experiments.

Animal observations

Animals were observed daily and weekly for adverse clinical signs. A detailed physical examination was conducted at least once during the pretreatment period, once weekly during the test substance administration and on the day of necropsy. The animals were removed from their home cages and placed in a standard arena for observations. Clinical signs that were monitored include, but were not limited to, changes in skin, fur, eyes, and mucous membranes, occurrence of secretions and excretions and autonomic activity. Individual body weight and food consumption for males and nonmated females were recorded individually on a weekly basis until sacrifice. The diet utilization ratio was calculated using the following formula: diet utilization ratio = (weight gain [g]/food consumption [g]) $\times$ 100%.

Hematology, serum biochemical evaluation

Blood samples were collected from the femoral artery for clinical pathology evaluations (hematology, serum chemistry) from rats (8 rats/group/sex) immediately before the

scheduled necropsy on day 90. All animals were fasted overnight before blood collection.

The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, serum uric acid (UA), blood urea nitrogen, creatinine, total cholesterol, triglyceride, high-density lipoprotein cholesterol, and glucose in serum were determined using an automatic biochemistry analyzer (Hitachi, Japan).

The following hematology parameters were analyzed using MLA Electra 1400 C™ automated coagulation analyzer: white blood cell, lymphocyte (LY), monocyte (MO), neutrophilic granulocyte (GR), LY count, MO count, GR count, red blood cell, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin (MCH), MCH concentration, red cell distribution width, blood platelet, mean platelet volume, plateletcrit, platelet distribution width, hemoglobin, eosinophil count, eosinophil ratio, basophil count, and basophil ratio.

Organ pathology

All animals were euthanized by CO₂ inhalation followed by exsanguination. Animals were necropsied and the external surface, all orifices and the cranial, thoracic, abdominal and pelvic cavities, including viscera, were examined. Weights were recorded for the adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries with oviducts, spleen, testes, thymus, and thyroids/parathyroids from all animals. Approximately 40 tissues were collected from each animal in the study, but were examined microscopically for the male and female and control male

and female groups. Pathological examinations are mainly focused on the kidney.

Statistical analysis

Statistical analyses were performed using SPSS software (version 19.0; SPSS, Inc.). Variances in the measurement data were checked for homogeneity by the Bartlett's test. When the data were homogeneous, the one-way analysis of variance test and LSD methods were used. The Tamhane's T2 test is used after data are transformed to analyze data among multiple groups if variances are unequal. All reported *P* values were two sided. A value of *P* < .05 was considered significant.

RESULTS

General information, body weight, and food consumption

There were no treatment-related adverse clinical observations or effects in either male or female F0 and F1 rats (data not shown).

As shown in Tables 2 and 3, weight gain and food utilization of F0 male rats in the NT group, compared with control, increased significantly in the first 4 weeks after weaning; whereas there were no differences from week 5 to 90 days. Moreover, no differences were found between the control and NT-treated females in the 1st and 2nd week. Compared with the controls, in F1 90 days after weaning, the body weight gain and food utilization of the 0.64% and 1.28% NT group were significantly higher within 2 weeks in male rats, but it only appeared in the 1st week in females.

TABLE 2. WEIGHT GAIN OF WEANED RATS TREATED WITH DIFFERENT DOSES OF DIETARY NUCLEOTIDES IN THE FIRST 4 WEEKS

Generation, Sex	Weeks	Control	0.01% NTs	0.04% NTs	0.16% NTs	0.64% NTs	1.28% NTs
F0, Male	1st week	24.13 ± 6.50	32.20 ± 5.05**	30.32 ± 7.84**	32.40 ± 5.96**	33.04 ± 5.38**	30.05 ± 5.33**
	2nd week	44.97 ± 4.47	50.07 ± 4.52**	51.20 ± 6.56**	50.81 ± 5.51**	51.89 ± 5.56**	48.40 ± 8.45**
	3rd week	61.81 ± 7.08	66.83 ± 6.58**	66.17 ± 8.63**	66.31 ± 6.18**	65.43 ± 10.69**	66.18 ± 8.09**
	4th week	52.84 ± 13.75	59.63 ± 10.16**	59.79 ± 14.94**	59.66 ± 8.87**	66.01 ± 7.97**	61.73 ± 7.93**
F0, Female	1st week	35.21 ± 5.05	37.53 ± 6.08*	38.28 ± 4.23**	38.45 ± 6.07**	39.17 ± 3.75**	38.59 ± 4.03**
	2nd week	37.67 ± 5.76	43.01 ± 6.29**	41.14 ± 5.30**	41.50 ± 4.25**	43.70 ± 6.57**	42.10 ± 4.46**
	3rd week	36.59 ± 8.18	36.76 ± 7.27	35.23 ± 6.30	36.50 ± 7.38	37.03 ± 6.98	35.17 ± 6.81
	4th week	27.78 ± 8.56	27.61 ± 6.98	28.18 ± 4.73	27.73 ± 5.28	29.76 ± 6.51	27.85 ± 6.55
F1, Male	1st week	37.54 ± 4.56	35.15 ± 5.24	35.28 ± 5.33	36.61 ± 5.45	40.80 ± 6.34*	40.78 ± 5.28*
	2nd week	53.87 ± 5.43	50.61 ± 8.16	52.20 ± 12.28	52.83 ± 6.18	57.29 ± 7.68*	58.30 ± 8.24*
	3rd week	57.07 ± 5.98	59.18 ± 11.01	60.39 ± 8.87	60.58 ± 4.73	57.38 ± 9.12	57.49 ± 6.59
	4th week	46.64 ± 13.98	49.84 ± 13.50	52.14 ± 8.08	50.18 ± 16.44	49.44 ± 10.34	52.56 ± 7.32
F1, Female	1st week	34.43 ± 4.13	33.99 ± 4.84	36.77 ± 5.88	34.19 ± 4.10	37.43 ± 5.98*	36.74 ± 5.57
	2nd week	42.05 ± 11.61	41.36 ± 6.98	44.20 ± 10.23	41.52 ± 5.43	44.43 ± 4.95	41.75 ± 5.69
	3rd week	37.97 ± 13.47	37.63 ± 8.56	33.21 ± 5.20	35.58 ± 5.09	33.81 ± 4.84	32.61 ± 5.66
	4th week	29.57 ± 9.78	26.21 ± 6.37	26.98 ± 5.49	26.68 ± 7.85	31.57 ± 18.16	25.17 ± 9.83

Data are mean ± SD values, reported in g.

Significant difference compared with the control group (by one-way analysis of variance): **P* < .05, ***P* < .01.

NTs, dietary nucleotides; F0, first generation; F1, second generation.

TABLE 3. FOOD UTILIZATION OF WEANED RATS TREATED WITH DIFFERENT DOSES OF DIETARY NUCLEOTIDES IN THE FIRST 4 WEEKS

Generation, Sex	Weeks	Control	0.01% NTs	0.04% NTs	0.16% NTs	0.64% NTs	1.28% NTs
F0, Male	1st week	47.81 ± 3.78	53.30 ± 1.59**	54.12 ± 4.45**	54.04 ± 2.33**	51.24 ± 1.53**	52.91 ± 1.99**
	2nd week	45.36 ± 1.46	48.95 ± 2.12**	48.70 ± 2.23**	49.68 ± 1.88**	49.00 ± 0.97**	48.91 ± 5.88**
	3rd week	40.06 ± 1.75	44.73 ± 8.64**	45.76 ± 4.20**	44.98 ± 2.91**	45.75 ± 2.83**	45.80 ± 1.21**
	4th week	33.81 ± 4.57	40.95 ± 5.95**	37.99 ± 5.78**	41.30 ± 1.74**	43.00 ± 2.83**	41.52 ± 3.41**
F0, Female	1st week	50.36 ± 0.89	52.76 ± 1.10**	53.81 ± 1.56**	54.70 ± 2.44**	53.77 ± 1.94**	54.95 ± 1.36**
	2nd week	39.12 ± 2.85	43.80 ± 5.28**	43.37 ± 1.46**	45.06 ± 2.07**	44.97 ± 3.94**	44.83 ± 3.27**
	3rd week	26.91 ± 2.22	26.04 ± 2.94	25.52 ± 0.75	26.72 ± 1.36	26.10 ± 1.67	26.30 ± 1.70
	4th week	22.35 ± 3.69	21.37 ± 3.86	23.87 ± 2.09	22.62 ± 2.74	22.77 ± 3.30	22.09 ± 1.34
F1, Male	1st week	52.68 ± 2.28	51.79 ± 2.51	52.21 ± 4.64	50.87 ± 2.04	56.21 ± 3.78*	56.21 ± 3.49*
	2nd week	48.58 ± 2.33	46.31 ± 3.25	50.40 ± 3.35	48.03 ± 2.25	50.40 ± 2.08*	50.79 ± 2.91*
	3rd week	40.06 ± 1.75	42.13 ± 5.59	42.22 ± 5.26	40.96 ± 2.22	40.19 ± 2.49	40.66 ± 3.60
	4th week	33.81 ± 4.57	32.87 ± 6.01	32.29 ± 6.98	33.55 ± 2.25	37.00 ± 2.83	35.45 ± 3.83
F1, Female	1st week	50.79 ± 2.26	50.13 ± 2.82	51.88 ± 3.90	50.10 ± 1.74	53.11 ± 2.97*	53.15 ± 2.64*
	2nd week	43.01 ± 7.41	44.18 ± 2.77	43.34 ± 6.41	42.59 ± 2.06	43.77 ± 3.06	43.71 ± 3.31
	3rd week	30.95 ± 5.21	30.78 ± 5.97	28.90 ± 3.13	32.24 ± 4.52	29.21 ± 3.96	30.02 ± 3.89
	4th week	23.17 ± 4.05	22.49 ± 2.17	23.74 ± 3.110	24.08 ± 1.15	23.91 ± 10.05	23.79 ± 11.09

Data are mean ± SD values, reported as percentages.

Significant difference compared with the control group (by one-way analysis of variance): * $P < .05$, ** $P < .01$.

Additionally, no notable differences were found at other time periods.

Hematology, serum biochemical evaluation

There were no significant differences in clinical pathology parameters, compared with the control, due to NT-treatment in either of the two generations.

Serum biochemistry values were also the same between control and NT-treated rats, with the exception of statistically significant decreases in AST, ALT, and UA levels of F0 and F1 (Table 4). Compared to the control, UA were remarkably decreased in F1 males of all NT-treated groups (0.01% NTs vs. control, $P < .05$; 0.04% NTs vs. control, 0.16% NTs vs. control, and 0.64% NTs vs. control, $P < .01$). In F0 male rats, AST of the NT group was significantly lower compared with the control (0.04% NTs vs. control, 0.16% NTs vs. control, and 0.64% NTs vs. control, $P < .05$; 1.28% NTs vs. control, $P < .01$).

Organ pathology

Organ weights relative to body weight were not different between the male and female control and NT groups in F0 and F1 rats. In addition, the macroscopic appearance of tissues in NT rats were within normal limits of this strain and age and similar to that of controls. Moreover, it suggested that no abnormal tissues could be observed, at terminal necropsy, through microscopically examined paraffin sections and hematoxylin and eosin staining in NT-treated rats (data not shown).

DISCUSSION

NTs, identified as conditionally essential nutrients, have gained increasing popularity in the diet since they seem to

play an important role in human nutrition at different stages of life. Furthermore, infant formulas supplemented with monophosphate nucleotides are already available in both the American and European markets.

In the present study, the results indicated that NTs played an active role in accelerating growth and development, based on the higher weight gain and diet utilization ratio in the first 4 weeks of weaned F0 rats. This suggests that NT consumption could promote growth and development in weaned rats. It has been suggested that NTs could reduce the incidence of diarrhea, possibly by a favorable effect on the gastrointestinal microbiota, in formula-fed infants.^{9,18–20} Studies suggest that nucleotides can enhance the growth of bifidobacteria, which could reduce the growth of pathogenic bacteria and hence the incidence of infectious diarrhea by reducing stool pH.^{21,22} In addition, NTs possess biological activity when using several models of gut integrity and repair under both *in vitro* and *in vivo* (gastric damage) conditions.²³ Similarly, the higher diet utilization ratio can be inferred that NTs may promote development, maturation, and functional optimization of filial rats' intestine, leading to a better digestion, absorption, and utilization of diet.

It must be noted that the weight gain and food utilization ratio were increased in the first 2 weeks of F1 male and the first week of F1 female rats. However, our previous study revealed that the content of four kinds of nucleotides was not changed by NT supplementation, in rats' milk on PND 21. It was suggested that the nucleotide level was higher in the high-dose rats, which may be due to reproductive function accumulation and differentiation. Furthermore, effects of NTs on the growth and development of female rats also existed, despite a lesser effect compared with male rats. This

TABLE 4. ASPARTATE AMINOTRANSFERASE, ALANINE AMINOTRANSFERASE, AND SERUM URIC ACID LEVELS IN RATS TREATED WITH DIFFERENT DOSES OF DIETARY NUCLEOTIDES

	Control	0.01% NTs	0.04% NTs	0.16% NTs	0.64% NTs	1.28% NTs
F0, Male						
AST (U/L)	114.40 ± 11.16	113.90 ± 11.22	103.70 ± 9.96*	103.57 ± 7.03*	103.87 ± 8.93*	102.34 ± 12.57**
ALT (U/L)	39.83 ± 3.98	41.92 ± 5.67	39.77 ± 6.27	34.07 ± 3.90	42.88 ± 6.04	40.61 ± 5.73
UA (μM)	73.34 ± 23.51	62.65 ± 7.69	65.81 ± 7.29	72.83 ± 8.32	72.34 ± 13.70	73.10 ± 19.08
F0, Female						
AST (U/L)	105.23 ± 14.19	104.97 ± 10.41	98.13 ± 12.62	96.6 ± 14.17	99.80 ± 15.72	90.77 ± 16.18
ALT (U/L)	31.46 ± 3.43	30.03 ± 5.29	28.11 ± 4.27	32.27 ± 4.80	29.95 ± 2.96	28.27 ± 4.83
UA (μM)	74.90 ± 16.50	68.89 ± 10.29	73.15 ± 15.27	71.20 ± 10.77	63.99 ± 11.60	76.30 ± 15.81
F1, Male						
AST (U/L)	204.27 ± 30.46	185.71 ± 25.74	182.61 ± 30.07	198.35 ± 24.54	176.91 ± 23.53	227.40 ± 37.07
ALT (U/L)	44.51 ± 7.18	40.42 ± 6.89	36.42 ± 11.14	37.89 ± 6.28	42.12 ± 9.94	44.19 ± 16.06
UA (μM)	96.12 ± 19.58	77.59 ± 19.05*	65.46 ± 19.14**	64.55 ± 20.80**	67.74 ± 12.10**	70.87 ± 7.97**
F1, Female						
AST (U/L)	96.12 ± 19.58	184.50 ± 33.11	168.87 ± 47.73	166.80 ± 28.01	173.25 ± 25.69	163.21 ± 25.86
ALT (U/L)	44.57 ± 16.27	37.27 ± 12.48	29.30 ± 12.40	37.47 ± 11.16	37.04 ± 7.96	28.97 ± 12.02
UA (μM)	92.61 ± 18.19	93.05 ± 22.89	78.20 ± 24.73	68.21 ± 17.03	74.76 ± 16.02	74.66 ± 12.14

Data are mean ± SD values.

Significant difference compared with the control group (by one-way analysis of variance): * $P < .05$, ** $P < .01$.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; UA, serum uric acid.

may be related to the developmental characteristics and hormone levels of female rats.^{24,25}

Analysis of the whole hematology parameters did not reveal any significant differences in the treatment groups when compared with the control group. Generally, there were no remarkable changes in the serum biochemical data in NT-treated groups in comparison with the control group, with the exception of statistically significant decreases in AST and UA levels. AST and ALT changes are often used as indicators of liver disease. Previous studies have shown that deprivation of NTs for certain periods may adversely affect protein synthesis in the liver and small intestine.⁷ Moreover, NTs may reduce or inhibit the *de novo* synthesis of NTs in the liver through feedback systems. In our study, AST and ALT were decreased without an abnormal liver, which was assumed that NTs could promote restoration and optimize liver function. Furthermore, uric acid, mainly excreted by the kidneys, is a product of purine nucleotide catabolism. Nevertheless, there was no abnormal kidney morphology observed, at final necropsy through microscopically in the two generations of rats supplemented with NTs. The results were the same as those in our previous multigenerational studies.¹⁶ The possible reason is that rats, a kind of rodent, can produce uricase in their bodies, and they may decompose uric acid into allantoin expelled from the kidneys. However, it is not the same purine metabolism as in humans. Because of that, increased UA might be a consequence of excessive purine-NT diet consumption or abnormal purine metabolic disorder. Additionally, estrogen could promote the excretion of uric acid, which may avoid the accumulation of uric acid and weaken the activation of uricase in male rats.^{25,26}

As important human milk constituents, nucleotides and their analogs were included in various forms (for example,

polymeric nucleotides, oligonucleotides, mononucleotides, and nucleotides). The Coordinated International Expert Group of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) recommends the following maximum concentrations for nucleotides added to infant formula: 1.75 mg/100 kcal of CMP, 1.5 mg/100 kcal of UMP, 1.5 mg/100 kcal of AMP, 0.5 mg/100 kcal of GMP, and 1 mg/100 kcal of IMP. Also, the total of all nucleotides must not exceed 5 mg/100 kcal.²⁷ In our experiment, the designation of composition followed guidelines for infant milk formula and the metabolism of purine *in vivo*.^{3,27–29} For assessing potential reproductive and cumulative dietary risks to humans, the rat is considered to be a relevant model. The results showed that nucleic acids in an oral solution had no mutagenic effects on somatic and germ cells.^{30,31} Also, the doses were designed for comparisons with our previous multigenerational study.¹⁶ Furthermore, it suggested that the developmental effect of NTs could be realized at the lowest level (0.01%) of our experiment. The dose is equivalent to 6 g/day/person.

In addition, it can be seen in the study, containing two 90-day studies, that NTs have no adverse effects up to 1.28%.

It must be noted that there were limitations in the experimental design. First, we did not test whether the observed increased weight gain is attributable to increased lean body mass or increased fat deposition. Therefore, we could not speculate on whether the males were getting fatter. Then, results are not comparable between F0 and F1, as a result of the different experimental time. Finally, the developmental effect of NTs did not exhibit dose dependency, so effects might be seen at lower doses than our lowest level. All of these points should be investigated in further research.

In conclusion, it is reported that NT supplementation could promote the early growth and development after weaning. Although NTs may have reproductive accumulation, they were safe even at high-dose supplementation. Additionally, NT supplementation could promote restoration and optimize liver function.

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AUTHOR DISCLOSURE STATEMENT

The authors declare that there are no conflicts of interest.

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