OBLIGATORY AND INTEGUMENTAL NITROGEN LOSSES — CHILDREN

15. OBLIGATORY NITROGEN LOSSES AND FACTORIAL CALCULATIONS OF PROTEIN REQUIREMENTS OF PRE-SCHOOL CHILDREN

Benjamín Torún and Fernando E. Viteri
Institute of Nutrition of Central America and Panama (INCAP),
Guatemala City, Guatemala

Objectives

1. Measurement of obligatory faecal and urinary nitrogen excretion in children two years old or a little older.

2. Calculation of such children's protein requirements following the factorial nitrogen approach.

Experimental Details (all values given as mean ± S.D.)

1. Subjects

a. Five children, all males, of mixed Maya and Caucasian descent (Ladino).

b. Chronological age: 24 ± 5 months (range: 17 to 31).
Height-age: 16 ± 5 months (range: 10 to 23).

c. All had been treated for severe, oedematous protein-energy malnutrition (kwashiorkor and marasmic-kwashiorkor). They had recovered fully at least one month before beginning the studies, based on clinical, anthropometric, and biochemical criteria (plasma proteins, non-essential/essential amino acid ratio, haematological indices, urinary creatinine excretion, and creatinine-height index [CHI]).

d. Weight: 10.66 ± 1.14 kg (range: 8.82 to 11.96).
Height: 79.8 ± 4.9 cm (range: 72.9 to 86.4).
Weight-for-height, percentage of expected: 98 ± 1 per cent (range: 96 to 100 per cent).
CHI: 0.95 ± 0.07 (range: 0.89 to 1.04).

e. Intestinal parasites: Two children had asymptomatic giardiasis. One of them also had a light infestation with *Trichuris trichiura* (one or two eggs per microscopic slide preparation). They were not treated before the study.

f. All children were healthy throughout the study.

2. Study Environment
INCAP's Clinical Centre in Guatemala City, 1,500 m above sea level.
Temperature: 18° to 24° C. Relative humidity: 40 to 50 per cent.

3. Physical Activity
Since no child had diarrhoea and defecation habits were known by the nurses, the children were confined to metabolic beds only part of the day. During most of the day they moved freely in the Clinical Centre and outdoor playing grounds wearing urine-collection bags, except for those children who were toilet-trained. They participated in games that involved climbing ramps, walking uphill, and tossing balls.

4. Duration of the Study
Four children were studied simultaneously for nine days. A fifth child was studied five months later for seven days.

5. Diet
a. A nitrogen-free, liquid formula was prepared with the following ingredients (g/kg/day): cornstarch 2.5; sugar 15.2; cottonseed oil 3.3; mineral mixture (NaCl, KCl, Na_2HPO_4, $CaCO_3$, mg SO_4) 0.6; water to make a final weight of 80 g/kg/day.

b. The liquid formulas were cooked for 10 to 20 minutes. They were free of fibre, and vegetable oil provided 30 per cent of the energy.

c. The diet, which provided 100 kcal/kg/day, was divided into five isoenergetic meals and fed at three-hour intervals. It was supplemented with vitamins, iron, iodine, zinc, and manganese. Additional water was offered *ad libitum.*

d. Prior to the study, the children consumed milk-based liquid formulas for several days.

6. **Indicators and Measurements**

a. Urine was collected at 23- to 25-hour intervals, and the volume was corrected mathematically to correspond to a 24-hour period. Faeces were not collected during the first day of the study. Beginning on the second day, carmine red and brilliant blue were given alternately as markers with breakfast every two days. Faeces were collected between markers as 48-hour pools. They were dried at 80° C and homogenized.

b. Urinary and faecal aliquots were digested, and their nitrogen contents were determined by a micro-Kjeldahl technique using selenium (Se) as a catalyst. Diet aliquots were analysed in the same way to ensure the absence of nitrogen. Tryptophan standards were analysed simultaneously.

c. Body weight was measured daily before breakfast.

Summary of the Main Results

1. Obligatory Nitrogen Losses

The results are summarized in table 1 and figure 1. The mean and S.D. for the combined data of days five to nine were 34.0 ± 5.3, 19.5 ± 6.9, and 53.7 ± 8.1 mg N/kg/day for urinary, faecal, and both urinary and faecal nitrogen, respectively.
If the study had been done in only six days and the mean values of days five and six used, the corresponding results would have been 33.2 ± 5.9, 19.9 ± 6.8, and 53.0 ± 7.7 mg N/kg/day for urinary, faecal, and both urinary and faecal nitrogen, respectively.

2. Factorial Calculations

Assuming that integumental nitrogen losses were of the order of 5 mg N/kg/day on a protein-free diet, total obligatory nitrogen losses would be 59 mg/kg/day, or 40 per cent less than the current FAO/WHO estimates. Adding 15 mg N/kg/day for growth of children of the same height-age and multiplying by 1.3, as suggested by FAO/WHO (WHO Tech. Rep. Ser. No. 522, 1973), results in an estimated mean requirement of 96.2 mg N/kg/day, equivalent to 0.60 g of milk or egg protein/kg/day. This value coincides with the mean requirement of 0.61 g/kg/day calculated by us using multiple-level nitrogen balance techniques (see summary of study by Torún, Cabrera-Santiago, and Viteri, this volume).

Conclusions

1. The obligatory faecal and urinary nitrogen excretion on a protein-free, low-residue diet can be assessed during the last two of six experimental days.

2. Urinary and faecal nitrogen are 34 ± 5 and 20 ± 7 mg N/kg/day, respectively.

TABLE 1. Obligatory Nitrogen Losses (mg/kg/day)

Child	Days on a nitrogen-free diet								
	1	2	3	4	5	6	7	8	9
Urinary nitrogen	58.9	28.9	41.0	37.4	25.5	33.6	27.4	31.3	44.2
C.R.	58.9	28.9	41.0	37.4	25.5	33.6	27.4	31.3	30.2
W.M.	81.3	52.4	29.8	39.0	31.2	33.4	35.2	34.0	30.2
I.G.	85.4	39.0	37.1	33.6	31.1	39.8	34.3	39.4	33.3
H.A.	89.4	68.6	60.8	43.4	40.8	38.4	39.8	31.1	40.3
A.A.	–	58.2	36.9	39.9	22.8	35.5	29.2	–	–
Mean	78.8	49.4	41.1	38.6	30.2	36.1	33.2	33.9	37.0
S.D.	11.8	15.8	11.8	3.6	6.9	2.8	4.9	3.4	6.0
Faecal nitrogen									
C.R.[a]		19.8		26.3		27.9		28.2	
W.M.[a,b]		19.0		23.1		20.1		13.9	
I.G.		9.7		7.4		15.2		9.7	
H.A.		29.7		12.1		27.2		22.1	
A.A.		12.9		22.0		17.9		–	
Mean		18.2		18.1		21.6		18.5	
S.D.		7.7		8.0		5.7		8.3	
Total (urinary + faecal nitrogen)									
Mean	–	67.6	59.3	56.7	48.4	57.7	54.8	52.4	55.5
S.D.	–	20.5	18.4	9.4	6.8	5.8	7.3	5.2	14.4

a *Giardia lamblia* in stools.
b *Trichuris trichiura* in stools. Both parasites diagnosed in faecal specimens collected four to nine weeks before the study.

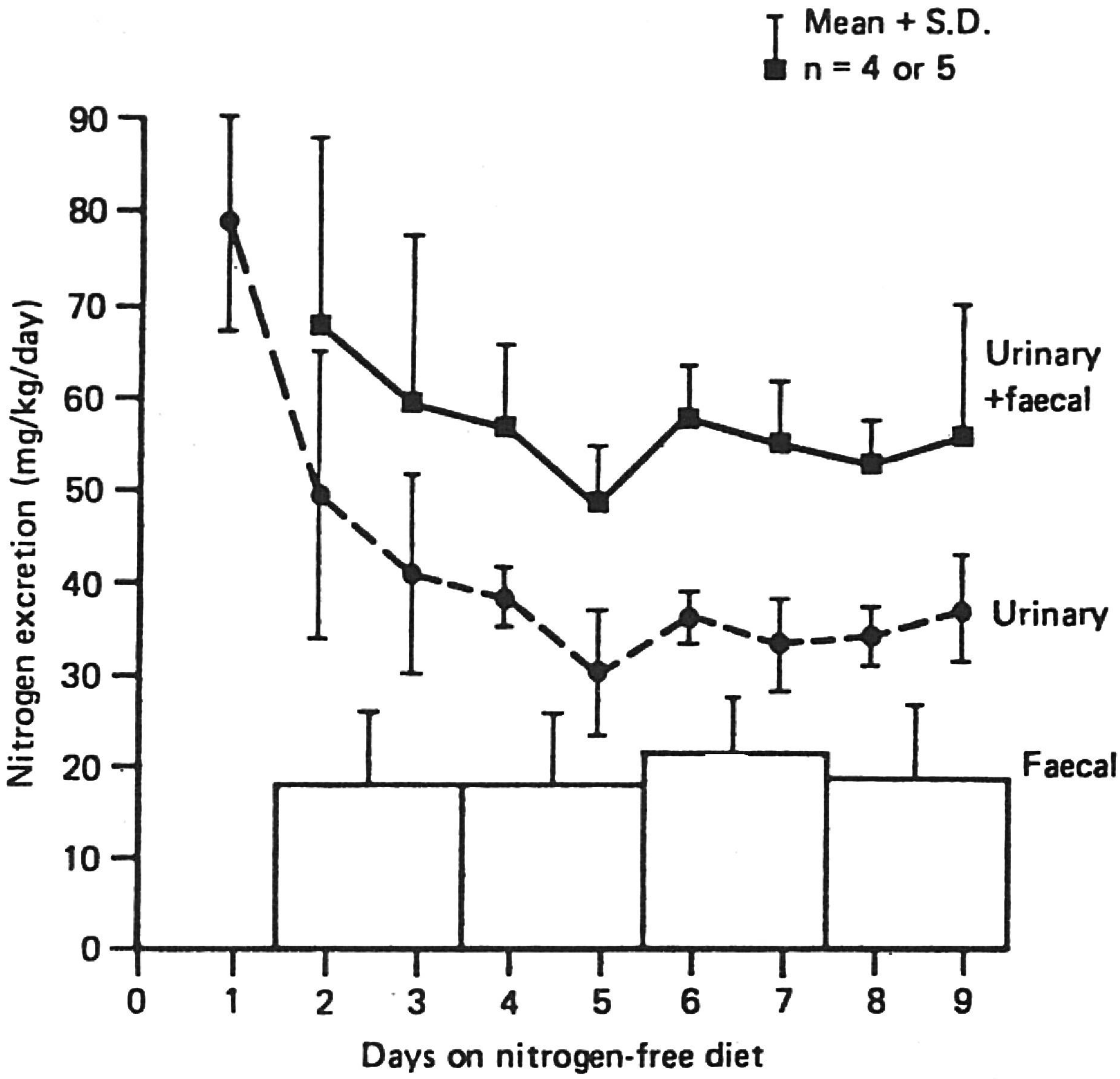

FIG. 1. Urinary and Faecal Nitrogen Excretion on a Nitrogen-Free Diet

3. The mild parasitic infestations in two of the five children did not increase faecal nitrogen.

4. Factorial calculations using the empirical correction factor of 30 per cent support the conclusions of our studies with milk protein.

16. INTEGUMENTAL NITROGEN LOSSES OF PRE-SCHOOL CHILDREN WITH DIFFERENT LEVELS AND SOURCES OF DIETARY PROTEIN INTAKE

Fernando E. Viteri and Cristina Martínez
Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala

Objectives

1. To determine the nitrogen losses by different routes in pre-school children ingesting different nitrogen levels.

2. To determine whether the nitrogen source (whole egg or rice-soy-milk) affects nitrogen losses by different routes.

Experimental Details

1. Subjects

a. Four boys of mixed Maya Indian and Caucasian descent (Ladino).

b. Chronological age: 18 ± 3 monthts (range: 14 to 24).
Height-age: 14 ± 2 months (range: 11 to 18).

c. All had been treated for severe, oedematous protein-energy malnutrition. They had recovered fully at least two months before beginning the studies, based on clinical, anthropometric, and biochemical criteria (plasma proteins, non-essential/essential amino acid ratio, haematological indices, and creatinine height index).

d. Weight: 10.5 ± 0.8 kg (range: 9.7 to 12.1).
Height: 71 ± 2 cm (range: 69 to 75).
Weight for height: 101 ± 3 per cent (range: 97 to 105).

e. All children were healthy throughout the study, except for one child who developed a mild upper respiratory infection without fever.

f. No other characteristics of the subjects are pertinent to the study.

2. Study Environment
INCAP's Clinical Centre in Guatemala City; 1,500 m above sea level; mean temperature 24.6° C with a maximum of 28.5° and a minimum of 19.0° C; mean relative humidity 66.6 per cent, range from 46 to 96 per cent. All the children remained in the metabolic ward during the study.

3. Physical Activity
The study lasted 40 days with each source of protein and 10 days at the end of a nitrogen-free diet. The children were off the study for three weeks in between nitrogen sources. During the study periods the children were allowed to exercise freely for the first 4 days of each 10 consecutive days. The last 6 days of each 10-day period were nitrogen-balance days, during which the children remained confined to bed but not necessarily lying down. Energy expenditure was measured by insensible water loss determinations during nitrogen balance periods and by using the Newburgh's factor of 2.2125 kcal/g IWL/day.

4. Duration of the Study
The four children were in the study a total of 111 days, distributed as follows:
a. 40 days on a rice-soy-milk formula.
b. 21 days on the rice-soy-milk formula and whole egg.
c. 40 days on the whole egg diet.
d. 10 days on a nitrogen-free diet.

5. Diet
a. Rice-soy-milk: 40 per cent rice flour; 38 per cent full-fat soy flour; 5 per cent skim milk powder; 14.85 per cent sugar; 1.9 per cent mineral mix; 0.1 per cent vitamin mix; and 0.15 per cent artificial flavour.

b. Whole egg protein: lyophilized whole egg homogenate, mineral, and vitamin mix.

c. Nitrogen-free diet: purified corn starch, vegetable oil, sugar, minerals, vitamins, artifical flavour, and water. Twenty per cent of calories came from fat.

The three diets were prepared as liquid formulas that provided 90 kcal/kg body weight/day, 20 per cent of which came from fat. Each protein source (a and b) was fed on four consecutive levels, each for 10 days' duration, starting with a nitrogen intake of 320 mg/kg/day and decreasing to 240, 160, and 80 mg/kg/day (equivalent to 2, 1.5, 1.0, and 0.5 g protein/kg/day). Protein was replaced by cornstarch sugar to maintain constant energy intake.

6. Indicators and Measurements

a. Nitrogen Macro-Kjeldahl for food, urine, and faeces. Micro-Kjeldahl for integumentary losses. Food nitrogen was measured for each ten-day period. Faecal and urinary nitrogen were measured in three-day pools for each child.

Integumentary losses were measured as follows: Before nitrogen balance was started the child was bathed with a non-ionic detergent (nitrogen-free) and dried with nitrogen-free towels by blotting. The bedding, pyjamas, and bibs were all nitrogen-free (pre-washed with 0.5 per cent acetic acid) and were analysed for nitrogen at the end of three days of contact with the children. To this, nitrogen from bath water at the end of three days was added.

Hair and nails were cut to the same length every ten days and analysed with the rest of integumentary nitrogen for that level of intake.

Residual nitrogen in food utensils was 9 mg/day (less than 1 mg/kg/day). Recovery of integumentary nitrogen in pyjamas, bedding, and bibs, tested in "dummy children" with bedding, etc., on which diluted urine was sprinkled repeatedly throughout three days, was 97.3 ± 1.8 per cent (N = 6) (range: 95.8 to 100 per cent).

b. Serum protein and albumin, urea, and ammonia were measured at the end of each ten-day period.

c. Basal oxygen consumption was also measured at the end of ten days.

Summary of Main Results

1. Total serum proteins, albumin, haemoglobin, and creatinine-height index remained constant, with averages of 7.0, 4.2, 11.9, and 0.94, respectively.

2. Basal oxygen (VO_2) consumption did not change. The averages for different nitrogen intakes, regardless of the source, were 107, 96, 104, and 97 per cent of expected. For nitrogen-free diets, the mean was also 97 per cent (mean ± S.D. : 48.5 ± 6.1 kcal/kg/day). The respiratory quotient was also constant, the means being 0.88, 0.88, 0.82, 0.81, and 0.82 for the different nitrogen intakes. All variation was random.

3. Energy expenditure by insensible water loss determinations came to a mean of 74 kcal/kg/day for diets containing 0.5 g protein/kg/day or higher. For the nitrogen-free diet, it was 58 kcal/kg/day.

4. Weight changes are presented in figure 1 (mean and range). Weight loss in all subjects occurred only with the nitrogen-free diet. Prior to this period there was a consistent increase in skin-fold thickness.

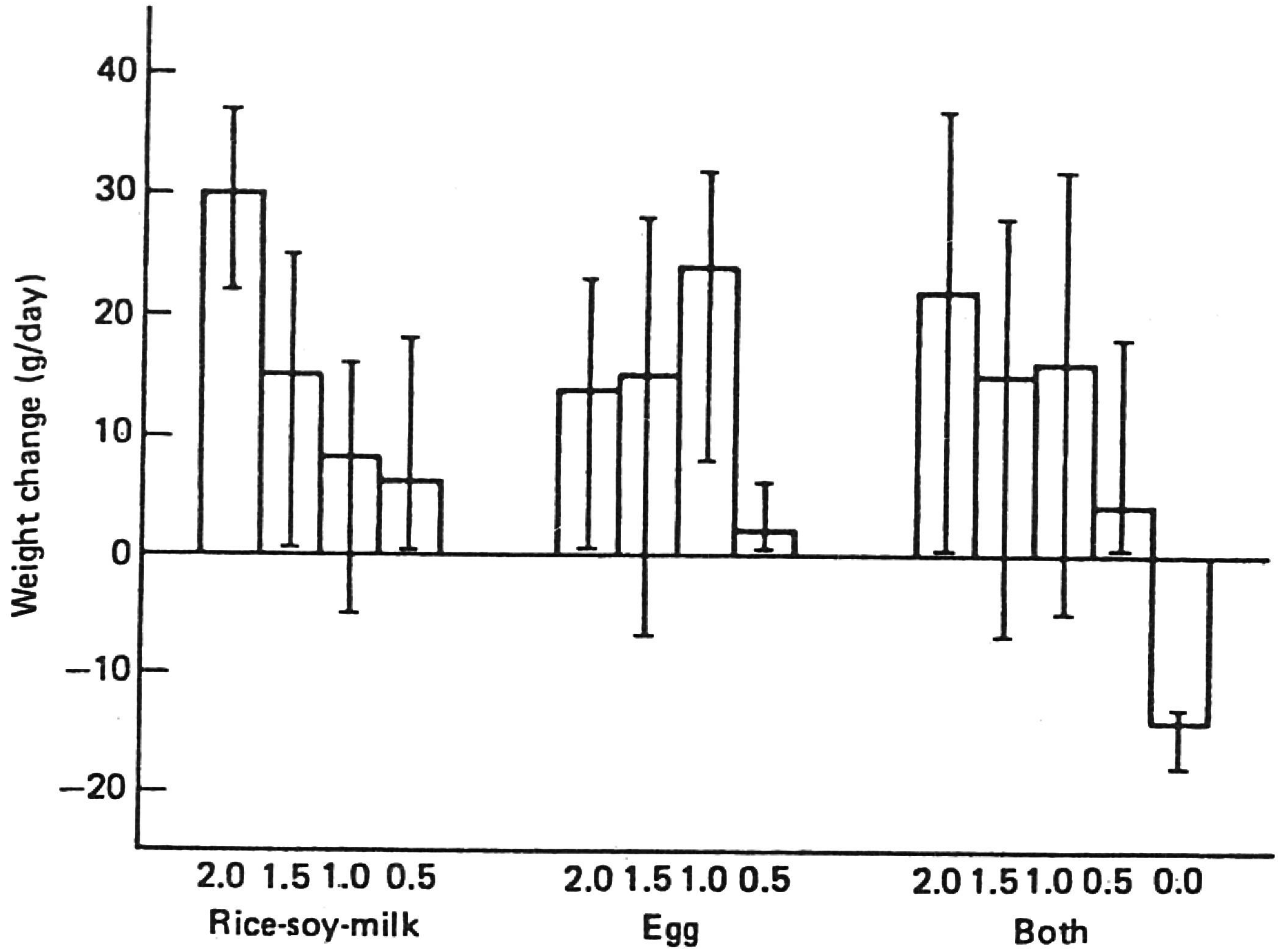

FIG. 1. Mean and Range of Weight Changes in Four Pre-school Children Who Received Different Levels of Protein and 90 kcal/kg/day

5. Obligatory nitrogen losses are:

		mg N/kg/day	mg N/kcal/day
Integumentary	(mean ± S.D.) N = 8	5 ± 1	0.12 ± 0.02
Faecal	(mean ± S.D.) N = 8	19 ± 2	0.38 ± 0.03
Urinary	(mean ± S.D.) N = 8	36 ± 7	0.77 ± 0.17
Total	(mean ± S.D.) N = 8	58 ± 8	1.19 ± 0.21

6. Faecal nitrogen is consistently lower with egg than with the rice-soy-milk (RSM) mixture, by a mean of 4 mg/kg/day. Mean true digestibility for egg was 94 ± 5; for RSM, it was 91 ± 2. Urinary nitrogen was higher for egg protein than for RSM at intakes of 2.0 and 1.5 g protein/kg/day, but at lower values the opposite occurred. Total integumentary nitrogen/kg/day obtained was as follows:

Nitrogen Intake	Egg Protein	RSM
320	10.4 ± 1.3 (9.6)*	8.0 ± 2.4 (7.3)
240	10.2 ± 1.2 (9.3)	6.6 ± 1.5 (5.7)
160	6.1 ± 0.8 (5.3)	6.5 ± 1.7 (5.6)
80	6.1 ± 1.3 (5.4)	5.9 ± 1.3 (5.1)
0	4.7 ± 0.6 (3.8)	4.7 ± 0.6 (3.8)

* (sweat and skin mean)

True nitrogen retention (mg/kg/day) obtained was as follows:

Nitrogen Intake	Egg Protein	RSM
320	58 ± 16	94 ± 12
240	39 ± 15	52 ± 14
160	33 ± 18	22 ± 20
80	+2 ± 11	– 20 ± 15
0	– 58 ± 8	– 58 ± 8

7. Integumentary nitrogen correlated significantly with serum urea nitrogen ($r = 0.56$). Egg protein at different levels of intake, however, by producing greater changes in serum urea than RSM, also produced higher sweat and skin nitrogen losses.

Nitrogen in skin and sweat = 39.9 + 6.65 urea nitrogen (egg) $r = 0.80$
Nitrogen in skin and sweat = 51.3 ± 2.34 urea nitrogen (RSM) $r = 0.41$

Conclusions

1. The nitrogen losses by different routes have been determined in four pre-school children ingesting different nitrogen levels, at constant energy intake. Faecal, urinary, and integumental nitrogen changed proportionally to nitrogen intake. Obligatory faecal and urinary nitrogen agreed with accepted values. Integumental obligatory nitrogen is 4.7 ± 0.6 mg N/kg/day and increases up to 2.5 times at an egg protein intake of 2 g/kg/day.

2. Protein source influences integumental losses as well as faecal nitrogen losses.

Acknowledgements

We are grateful to the staff of the Clinical Centre at INCAP, to Mr. R.D. Mendoza, and to Mrs. J. de Melgar. This study was partially supported by a grant from the World Health Organization.