The quality of various animal and vegetable proteins with a note on the endogenous and fecal nitrogen excretion of children[1]

RICARDO BRESSANI[2], FERNANDO VITERI[3],
DOROTHY WILSON[4], AND JORGE ALVARADO[5]
Institute of Nutrition of Central America and Panama (INCAP), Guatemala, C. A.

SUMMARY

Results on the biological evaluation of proteins from animal and vegetable origin, in children, are presented in this paper. It also provides information on the endogenous fecal and urinary nitrogen excretion of children aged 25 to 71 months. When the different vegetable proteins were fed at intakes of 250 mg of nitrogen and above, nitrogen retention values were similar to those obtained from milk and egg protein feeding. Better sensitivity in distinguishing between the nutritional quality of proteins was obtained when nitrogen intake ranged from 150 to 250 mg. In all cases, the nitrogen intake to nitrogen balance plots gave a linear relationship at low levels of nitrogen intake. The nitrogen retained tended to reach a plateau as nitrogen intakes increased. Endogenous fecal nitrogen averaged 24 mg, and urinary nitrogen 57 mg/kg/day. The source of protein fed prior to the nitrogen free-feeding periods had no effect on

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2 Chief, Division of Agricultural and Food Science, Institute of Nutrition of Central America and Panama.

3 Chief, Biomedical Division of INCAP.

4 At the time this work was carried out, Medical Officer of the Division of Clinical Research, INCAP. At present Dr. Wilson is Nutrition Adviser, Zone I, Pan American Health Organization, with headquarters in Caracas, Venezuela.

5 When this study was performed, Dr. Alvarado was Associate Chief of the Biomedical Division of INCAP.

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endogenous nitrogen excretion. Fecal nitrogen after correction for endogenous fecal values reoresents about 20% of the nitrogen intake when the protein was of vegetable origin, and 10% when derived from an animal source. As nitrogen intake decreased the biological value in all protein sources increased. The proteins of better quality had the lowest biological value at highest nitrogen intake levels, and the value increased more as intake decreased. Egg protein had the righest nutritive value, followed by milk. The vegetable protein-rich foods had a similar protein value.

INTRODUCTION

The development of news sources of protein and of protein-rich food mixtures for the supplementary feeding of children and for the prevention of protein malnutrition has created the need for suitable tests of their protein quality, safety and suitability for human consumption.

One of the tests often used to evaluate the protein quality of foods is the nitrogen balance method (1, 2). Like all protein evaluation methods, it is affected by many variables (1). However, if these are properly controlled, the nitrogen balance method gives reliable results. One of the factors affecting nitrogen balance is the level of nitrogen intake. It becomes very important when the nutritive value of different proteins is compared. When testing protein quality, the protein must be fed at a level that is not far above maintenance requirements, thus allowing reasonable nitrogen retention and growth. If intake is high, the efficiency of nitrogen utilization decreases and differences in biological value tend to disappear.

The work under discussion was carried out during the last six years and was designed to evaluate several protein foods of animal and vegetable origin, as well as to obtain basic information on several constants needed to estimate protein requirements. The data presented herein also reveal several relationships between nitrogen intake, nitrogen balance, apparent and true protein digestibility and biological value.

MATERIAL AND METHODS

Proteins of both animal and vegetable origin were used. Skim or whole milk and whole egg-powder were the animal protein sources fed, while INCAP Vegetable Protein Mixtures 9, 14 and 15 supplied the vegetable proteins studied. These were previously tested in experimental animals as described elsewhere (3-5). An additional source of proteins was also studied, and the results of its evaluation have already been reported (6). However, for a more efficient over-all interpretation of the results presented in this paper, some of the values obtained in such experiments are included herein. Mixture 9 consists of a blend of cottonseed protein concentrate and corn; Mixture 14 is a combination of soybean protein concentrate and corn, while Mixture 15 is formed by equal parts of cottonseed and soybean protein concentrates and corn. All mixtures contain approximately 27% protein.

Each protein source was fed to four male children in apparently good health who had been admitted to the INCAP Metabolic Unit with varying degrees of protein-calorie malnutrition. Table Nº 1 shows the range in weight and ages of the children fed each protein under study.

TABLE Nº 1

AGE AND WEIGHT OF CHILDREN

Case	Age months	Weight kg	Case	Age months	Weight kg
	Milk			Vegetable Mixture 9	
PC-82	25	9.92	PC-82	26	10.23
PC-86	36	10.35	PC-86	37	10.55
PC-91	69	13.98	PC-91	71	13.69
PC-97	51	18.26	PC-97	47	17.46
	Eggs			Vegetable Mixture 14	
PC-152	34	13.25	PC-147	36	14.55
PC-153	33	13.30	PC-149	34	15.40
PC-159	25	9.77	EE-24	52	13.98
PC-160	56	15.98	EE-26	48	11.94
	Vegetable Mixture 15				
PC-148	31	11.70			
PC-151	29	13.80			
PC-152	29	11.89			
PC-153	28	11.57			

In general terms, the study plan was as follows: protein intake was decreased from approximately 3.5 g/kg/day to 0, in steps of 0.5 g/kg/day in most cases. All the protein ingested was derived from the food proteins used in the study. Each level of protein was tested for a minimum of 6 days or a maximum of 9, following a 4-day adaptation period. The caloric intake remained constant throughout the experiments, at a level of 90 or 100 calories/kg/day. These were provided both by the food itself and by pure carbohydrates. In all cases, 20% of the calories were derived from hydrogenated vegetable oil. Water intake was also kept constant throughout each experiment, and a vitamin[6] and mineral supplement[7] was administered. A representative example of the composition of one of the diets used in a 3-day period was: protein under test X[8] g; sugar, 110 g; Dextro-Malto, 40 g; margarine 30 g; salt 1 g, and water, 866 ml. The quantities of the ingredients were changed according to protein intake and in order to keep the intake of calories constant as indicated above. The total weight of each diet preparation was kept at 1,200 grams using 400 g per day, offered in three servings.

Food, feces, and urine were analyzed every three days for total nitrogen content. Urine was collected in dark bottles which contained 1 ml of concentrated acetic acid. The children were fed three times a day, and strict records were kept both of food intake and fecal and urine output.

RESULTS

The protein quality of the various mixtures fed to the children, as tested in rats, is shown in Table Nº 2. The values are similar to those previously reported. Table Nº 3 summarizes the results obtained with milk and whole egg protein, while Table Nº 4 presents the results obtained with Mixtures 9, 14, and 15, as tested in children. Unless otherwise indicated, each value represents an average of a maximum of 15 observations and a minimum of 4.

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6 Protavit, Parke-Davis and Co., Detroit, Michigan, U. S. A.

7 0.61 g of mineral mixture/kg body weight/day. Mineral Mixture: 44 g KCl; 7 g Na_2HPO_4; 5 g $CaCO_3$; 5 g $MgSO_4 \cdot 7H_2O$. Children also received 100 mg $FeSO_4$ per day.

8 X represents a variable amount of the protein under test, which depends on the calculated intake.

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TABLE Nº 2
PROTEIN EFFICIENCY RATIO OF PROTEINS

Food	Protein in diet %	Average weight gain g	PER*
Skimmilk	12.7	133 ± 25	2.56 ± 0.30
Whole egg	12.8	138 ± 24	2.90 ± 0.27
Vegetable Mixture 9	13.8	113 ± 13	1.93 ± 0.13
Vegetable Mixture 14	14.2	148 ± 28	2.24 ± 0.23
Vegetable Mixture 15	14.6	141 ± 17	2.20 ± 0.20
Casein	11.2	132 ± 20	2.88 ± 0.20

* PER = Protein efficiency ratio.

TABLE Nº 3
NITROGEN BALANCE RESULTS OF CHILDREN FED DECREASING LEVELS OF PROTEIN FROM MILK AND WHOLE EGG POWDER

Period	No. of observations	Nitrogen Intake	Fecal	Urine	Absorbed	Retained
		mg/kg/day				
			Milk protein			
1	11	477 ± 7.5*	65 ± 6.8*	313 ± 13.7*	412 ± 11.6*	99 ± 10.3
2	4	386 ± 5.6	45 ± 3.0	258 ± 11.0	342 ± 4.8	83 ± 11.3
3	15	303 ± 6.4	48 ± 3.8	185 ± 7.7	255 ± 6.9	69 ± 10.4
4	8	225 ± 3.4	42 ± 4.1	132 ± 3.6	184 ± 5.9	52 ± 5.5
5	13	164 ± 2.7	36 ± 2.8	74 ± 4.8	128 ± 4.2	54 ± 6.3
6	4	95 ± 1.2	29 ± 1.0	50 ± 3.0	66 ± 1.1	15 ± 3.5
7	6	0	23 ± 1.7	46 ± 4.2	--	--
			Whole egg protein			
1	10	385 ± 7.9	42 ± 3.3	262 ± 3.4	343 ± 6.0	80 ± 5.9
2	10	328 ± 4.6	40 ± 2.6	214 ± 6.7	288 ± 5.2	75 ± 6.5
3	10	239 ± 5.5	27 ± 1.9	142 ± 4.1	212 ± 5.6	69 ± 6.4
4	10	160 ± 6.1	25 ± 1.4	93 ± 6.2	134 ± 6.2	41 ± 6.6
5	9	76 ± 4.4	19 ± 2.5	63 ± 3.7	57 ± 6.0	-6 ± 7.2
6**	4	37	23	54	14	-40
7	9	0	17 ± 3.2	59 ± 3.0	--	--

* Standard Error.

** Only two children, 2 balances each.

TABLE Nº 4

NITROGEN BALANCE RESULTS OF CHILDREN FED DECREASING LEVELS OF PROTEIN FROM INCAP VEGETABLE MIXTURES 9, 14 AND 15

Period	No. of observations	Intake	Nitrogen Fecal	Nitrogen Urine	Absorbed	Retained
			mg/kg/day			
			Vegetable Mixture 9			
1	12	457 ± 10.7*	134 ± 4.9*	243 ± 12.0*	322 ± 13.8*	80 ± 6.8*
2	6	388 ± 7.3	110 ± 9.9	201 ± 7.3	277 ± 10.2	77 ± 10.4
3	14	315 ± 4.7	88 ± 2.8	162 ± 4.8	227 ± 5.8	66 ± 4.8
4	8	251 ± 4.4	81 ± 5.5	127 ± 3.3	170 ± 6.1	43 ± 4.9
5	12	174 ± 4.0	57 ± 5.5	91 ± 2.8	117 ± 5.9	25 ± 5.8
6	6	92 ± 6.5	37 ± 1.8	51 ± 3.7	55 ± 5.0	+4 ± 7.4
7	10	0	23 ± 1.5	43 ± 3.7	--	--
			Vegetable Mixture 14			
1	4	435 ± 13.7	102 ± 12.1	182 ± 16.8	333 ± 16.6	151 ± 16.5
2	5	347 ± 12.6	85 ± 15.1	168 ± 18.2	262 ± 11.7	94 ± 15.5
3	12	297 ± 8.1	61 ± 5.1	148 ± 9.1	236 ± 11.4	88 ± 13.3
4	6	230 ± 4.2	69 ± 2.8	96 ± 8.1	161 ± 5.4	65 ± 11.4
5	11	155 ± 4.8	39 ± 2.9	81 ± 2.9	117 ± 5.2	34 ± 4.7
6	10	90 ± 2.2	32 ± 1.5	60 ± 1.8	58 ± 2.2	-2 ± 2.4
7	12	0	22 ± 1.5	54 ± 3.8	--	--
			Vegetable Mixture 15			
1	12	473 ± 5.5	106 ± 5.9	258 ± 15.6	368 ± 7.5	108 ± 15.0
2	15	398 ± 3.8	89 ± 6.1	216 ± 8.5	309 ± 7.4	93 ± 6.7
3	11	311 ± 7.8	75 ± 3.9	179 ± 9.6	237 ± 9.1	58 ± 5.7
4	11	234 ± 4.3	54 ± 2.8	131 ± 4.9	178 ± 3.8	48 ± 3.6
5	12	168 ± 3.7	48 ± 2.5	96 ± 5.4	120 ± 4.1	24 ± 5.9
6	12	103 ± 2.3	38 ± 1.9	70 ± 4.1	65 ± 3.0	-5 ± 3.0
7	10	0	29 ± 4.5	64 ± 8.9	--	--

* Standard Error.

In all cases, as nitrogen intake decreased, nitrogen retention also decreased on an absolute basis. Although when expressed as percentage of nitrogen intake, nitrogen balance also decreased; this reduction became evident only at very low levels of protein intake. Nitrogen absorbed expressed as mg/kg/day also diminished when nitrogen intake was lowered, decreasing only slightly, however, when expressed in terms of the nitrogen ingested.

The relationship between nitrogen intake and nitrogen balance at all levels of intake, can be clearly appreciated in Fig 1. In all cases there is a linear relationship between nitrogen intake and nitrogen balance, particularly at low nitrogen intakes. This relationship tends to reach a plateau as nitrogen intake increases.

The correlation coefficients and the regression equations between nitrogen intake and nitrogen balance, as well as between nitrogen absorbed and nitrogen retained, are presented in Table Nº 5. Only nitrogen intake values below 160 mg were used to calculate the regression lines. The coefficient of nitrogen intake or absorbed are indices of protein quality. The relationship between nitrogen absorbed and nitrogen retained, as developed by Allison (1), is known as the nitrogen balance index. Figures in Table Nº 5 indicate that the protein quality of skimmilk and whole egg is similar, followed by that of Mixtures 14, 15 and 9, in that order. The regression coefficients for nitrogen absorbed to nitrogen retained were 0.69 and 0.64 for milk and whole egg, and 0.63, 0.53 and 0.50 for Mixtures 14, 15 and 9, respectively.

For the purpose of determining the level of nitrogen intake at which larger differences in nitrogen retention were obtained, the nitrogen balance values observed at intakes between 360 and 81 mg of nitrogen, grouped as shown in Table Nº 6, were statiscally studied. The analysis indicates that at intakes between 156 and 251 mg of nitrogen/kg of body weight/day, highly significant differences in nitrogen retention values can be detected among the different protein foods.

Table Nº 7 summarizes the data concerning the endogenous fecal and urinary nitrogen excretion of 20 children. These values were obtained when the subjets were fed nitrogen free diets for 6 to 9 days after a 4-day adaptation period. The diets and protein levels consumed before feeding the nitrogen-free diet contained the various proteins under study. Intakes of nitrogen before feeding the nitrogen-free diets varied from 37 to 103 mg nitrogen/kg/day for all children. Endogenous fecal nitrogen; averaged 24 mg, and urinary nitrogen, 57 mg. No difference was observed between the values obtained during the first 3-day period of nitrogen-free diet feeding, and the third balance period. This was true for both types of nitrogen.

Using the endogenous fecal and urinary nitrogen values, relationships between N intake and apparent and true N digestibility, were calculated (Table Nº 8). As the data reveal, apparent nitrogen digestibility decreased as N intake diminished. True protein digestibility, on the other hand, remained constant or tended to increase.

Table Nº 8 also shows the relationship between nitrogen intake and biological value. In all cases, as N intake decreased, the biological value increased. Proteins of better quality, such as milk and eggs, had the lowest biological value at the highest intake, and the value increased further as nitrogen intake decreased.

DISCUSSION

In recent years great emphasis has been placed on the utilization of vegetable protein sources to supplement the poor protein-quality diets of small children. These vegetable proteins have been tested either by themselves or as mixtures prepared with other foods. The test most commonly used has been the nitrogen balance method. The results of the present investigation show that the nitrogen balance method can be a very useful tool in evaluating protein quality at low levels of protein intake, and can rank protein foods as well as protein efficiency ratio can in experimental animals.

The results clearly reveal that differences in protein quality tend to disappear as nitrogen intake increases; this is also true in the case of the customary PER assay for protein quality, as performed in rats. It is common to see that most of these new protein sources are tested at high levels of intake, with the conclusion that their protein value is equal to that of the better balanced proteins such as milk and eggs. In the present study, maximum differences in quality became evident when the intake of nitrogen was less than 250 and greater than 80 mg of nitrogen. In the case of the protein efficiency ratio, on many occasions it has been indicated that the largest differences became apparent at a level of 10% of protein in the diet. Good correlations were found between the PER of the proteins tested and the nitrogen balance results reported herein.

The findings of the present investigation also showed that it is not appropriate to report nitrogen balance results as percentage of the nitrogen intake, since this method of expressing them tends to decrease differences in protein quality even further. Although not presented in the tables, nitrogen balance expressed as percentage of nitrogen intake, showed relatively little variation for a wide range of nitrogen intake values.

Using the endogenous fecal value to correct the fecal nitrogen at different levels of N intake, it was calculated that fecal nitrogen represents less than 10% of the N intake for the animal proteins, and about 20% for the vegetable proteins. The biological values calculated are similar to those reported by other workers (2). The trend observed was the expected one, since as protein intake increases, the efficiency of its utilization decreases. The same trend is observed in other measurements of protein quality, such as PER or NPU (7). This emphasizes again that all methods used for protein quality evaluation measure the efficiency of essential amino acid utilization directly related to the amounts and proportions found in food proteins. Similarly, when the methods are applied to various species under well-controlled and standardized conditions, they tend to rank the various proteins in the same order.

TABLE Nº 5

CORRELATIONS AND REGRESSION EQUATIONS BETWEEN NITROGEN INTAKE (NI) AND NITROGEN RETETION (NR), AND BETWEEN NITROGEN ABSORBED (NA) AND NITROGEN RETAINED

Protein source	Correlation coefficients	Regression equations
Milk	NI vrs NR = 0.66	NR =-35.7 + 0.55 NI
	NA vrs NR = 0.80	NR =-33.0 + 0.69 NA
Vegetable Mixture 9	NI vrs NR = 0.56	NR =-23.8 + 0.28 NI
	NA vrs NR = 0.81	NR =-30.3 + 0.50 NA
Vegetable Mixture 14	NI vrs NR = 0.89	NR =-52.0 + 0.55 NI
	NA vrs NR = 0.94	NR =-39.3 + 0.63 NA
Vegetable Mixture 15	NI vrs NR = 0.75	NR =-55.4 + 0.48 NI
	NA vrs NR = 0.73	NR =-39.5 + 0.53 NA
Whole egg	NI vrs NR = 0.85	NR =-52.5 + 0.59 NI
	NA vrs NR = 0.87	NR =-43.3 + 0.64 NA

TABLE Nº 6
AVERAGE NITROGEN RETENTION AT SEVERAL LEVELS OF NITROGEN INTAKE

Nitrogen intake mg/kg/day	310-360	230-251	156-174	81-114
Protein food	Nitrogen retention mg/kg/day			
Milk	62	50	54	8
Vegetable Mixture 9	64	40	26	-4
Vegetable Mixture 14	86	65	38	0
Vegetable Mixture 15	59	51	24	-5
Soybean protein textured food	82	49	9	-10
Egg	80	80	49	--
F	1.51	5.85**	11.06**	1.84
L.S.D.* (.01)	45	22	21	20
No. of observations	31	31	32	22

* Least significant difference.
** Highly significant.

TABLE Nº 7
ENDOGENOUS FECAL AND URINARY NITROGEN OF TWENTY CHILDREN

	Average	S.D.*	Range
Age, months	39	-	22-72
Weight, kg	13.04	2.48	9.02-18.60
Height, cm	77.7	7.4	66.00-92.6
Endogenous fecal nitrogen mg/kg/day	24	6	9-36
Endogenous urinary nitrogen, mg/kg/day	57	15	32-80

* Standard Deviation.

TABLE Nº 8

TRUE PROTEIN DIGESTIBILITY AND BIOLOGICAL VALUE AT VARIOUS LEVELS OF PROTEIN INTAKE

Milk			Egg			Vegetable Mixtures 9			Vegetable Mixtures 14			Vegetable Mixtures 15		
NI mg/kg/day	TPD %	BV	NI mg/kg/day	TPD %	BV	NI mg/kg/day	TPD %	BV	NI mg/kg/day	TPD %	BV	NI mg/kg/day	TPD %	BV
568	89.4	38.6	432	92.8	41.6	563	70.3	51.0	435	81.6	63.9	473	84.6	54.7
473	91.1	36.9	370	95.7	42.6	469	77.4	41.9	360	81.4	55.3	398	85.2	57.5
386	94.1	41.4	334	93.7	50.2	397	74.0	50.3	297	88.2	62.2	310	85.8	59.4
315	90.1	45.4	251	96.4	65.0	324	79.6	50.4	230	79.6	77.0	235	88.1	72.5
283	9[illegible].9	53.2	168	95.2	78.1	243	79.0	55.2	158	91.8	78.6	168	88.7	82.5
231	90.9	55.4	94	97.9	96.7	174	80.4	65.7	90	91.1	92.7	103	91.3	100.0
163	92.0	80.6				89	86.5	80.5						
94	95.7	84.4												

NI = Nitrogen intake; TPD = True protein digestibility; BV = Biological value.

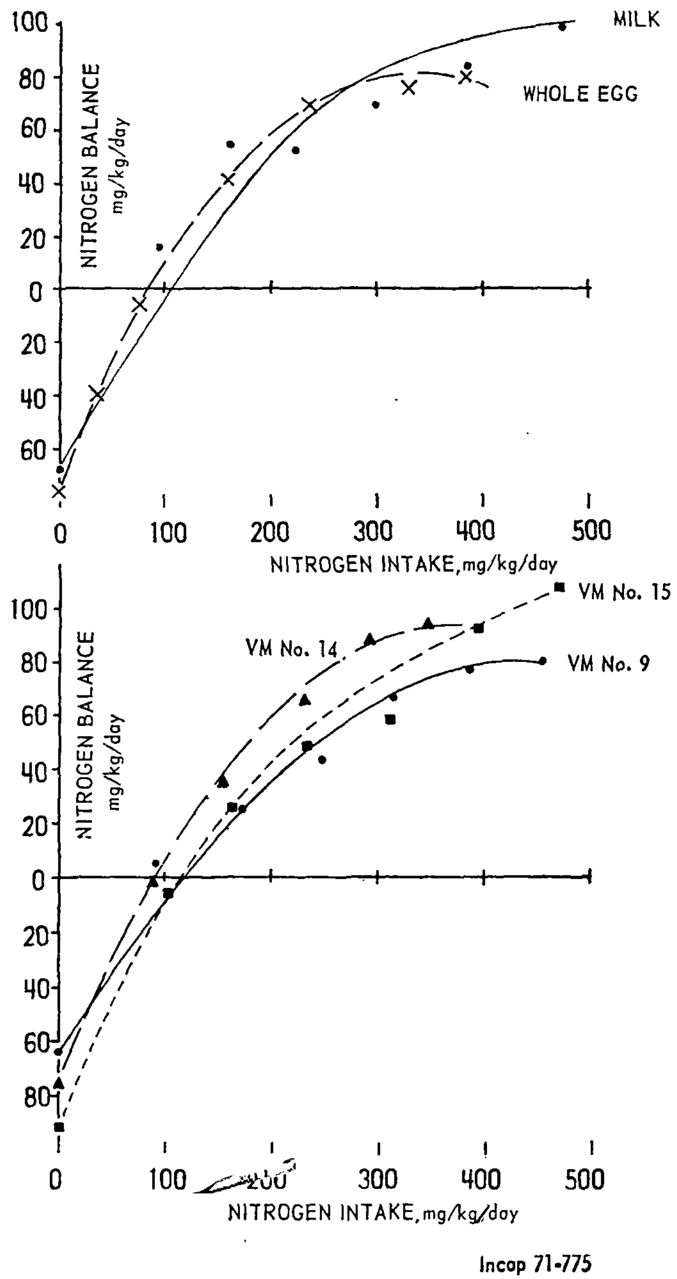

Fig. 1.—Relationship between nitrogen intake and nitrogen retained of children fed different protein sources.

RESUMEN

Excreción urinaria y fecal de nitrógeno endógeno de niños y valor nutritivo de diversas proteínas de origen animal y vegetal para consumo humano.

Se dan a conocer los resultados de la evaluación biológica, en niños, de teínas de origen vegetal y animal. Se proporciona también información sobre la excreción fecal y urinaria de nitrógeno endógeno de niños comprendidos entre las edades de 25 a 71 meses. Los valores de retención de nitrógeno fueron similares a los obtenidos con la administración de proteína de leche y huevo cuando las diferentes proteínas vegetables se les administraron a ingestas de 250 mg de nitrógeno y más. En los casos en que la ingesta de nitrógeno osciló entre 150 y 250 mg, se obtuvo mejor sensibilidad para distinguir diferencias entre la calidad nutricional de las diversas proteínas. En todos los casos, la representación gráfica de la relación entre ingesta de nitrógeno y balance nitrogenado dio una relación linear a niveles bajos de ingesta de nitrógeno. La curva del N retenido tendió a estabilizarse a medida que la ingesta de N aumentaba. El nitrógeno endógeno fecal promedió 24 mg, y el urinario, 57 mg/kg/día. La fuente de proteína administrada previo al período de alimentación libre de nitrógeno no tuvo ningún efecto sobre la excreción del nitrógeno endógeno. El nitrógeno fecal representa cerca de 20% de la ingesta de nitrógeno después de corregirlo por los valores de nitrógeno endógeno fecal cuando la proteína fue de origen vegetal, y 10% cuando se derivó de una fuente animal. En el caso de todas las fuentes de proteína, se observó que a medida que la ingesta de nitrógeno decrecía, el valor biológico aumentaba. Las proteínas de mejor calidad acusaron el valor biológico más bajo a niveles más altos de ingesta de nitrógeno, y el valor aumentó más aún conforme la ingesta decrecía. La proteína de huevo tuvo el valor nutritivo más alto, seguido de la leche. Los alimentos de origen vegetal, ricos en proteína, tuvieron un valor proteínico similar entre ellos, pero ligeramente inferior al de la proteína de la leche o del huevo.

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