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EFFECT OF DIFFERENT FOOD SOURCES ON URINARY NITROGEN LEVELS IN HUMAN SUBJECTS

ISHT PREET KAUR* AND S. BAJAJ**

(Department of Foods and Nutrition, Punjab Agricultural University, Ludhiana)

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Introduction

Total urinary excretion of nitrogen for the day can be used as an index of dietary nitrogen utilization in mammals. Wide variations have been shown for total urinary nitrogen excretion depending on the type of diets being consumed in different countries. Mongia *et al*[1] reported higher urinary nitrogen excretion in non-vegetarian subjects as compared to lacto-vegetarians and mixed diet eaters. Urea is the major end product of protein metabolism. Its increase or decrease with the level of dietary protein intake has been known for many years. It is also reported that with increased protein intake, urea nitrogen accounts for a greater part of the total nitrogen excreted[2-4]. Uric acid levels in urine have been known to be affected by the quality as well as source of protein in the diet[5,6]. The average per capita protein consumption in developing countries, like India, is far below than that of industrialized countries of the West. To what extent such medium protein diets based largely on vegetable protein sources affect nitrogen excretion has not been studied to an appreciable extent. The present investigation was, therefore, undertaken with a view to compare the effect of different protein sources on total urinary N, urea N and other end products of protein metabolism in human subjects.

Materials and Methods

The effect of different food sources on nitrogen metabolism was studied using primarily the survey technique as the basis for constructing the experimental diets which were fed to young adult Punjabi girls. Nine young adult girls in the age group of 19-23 years were selected and medically examined for blood and stool tests to ensure normal health and freedom from infections and infestations of the intestines. The heights, weights and haemoglobin (Hb) levels of the subjects were within normal limits when compared with the Indian Council of Medical Research (ICMR) standards.

Four experimental diets viz cereal (C), cereal-pulse (CP), cereal-pulse-vegetable (CPV) and non-vegetarian (NV) diets representing the dietary pattern of low, low middle, middle and upper middle socio-economic groups, respectively were formulated. Ingredient composition of the experimental diets is shown in Table I. Cereal (C) diet was planned so that about 82 per cent of total protein was from cereals. In cereal-pulse (CP)

* Govt. College for Women, Patiala - 147 001

** National Institute of Public Cooperation and Child Development, Delhi

TABLE I Ingredient Composition of the Experimental Diets

Food item	Diets			
	Cereal g	Cereal-pulse g	Cereal-pulse-vegetable g	Non-vegetarian g
Cereals	282.5	262	233.3	195
Pulses	—	90.5	62.5	—
Milk	90	90	90	90
Hydrogenated fat + butter	42.5	45	40	53
Meat (chicken)	—	—	—	65
Egg	—	—	—	100
Sugar & Jaggery	55	36.2	37.5	42.5
Vegetables				
Bottlegourd or	100	—	—	—
pumpkin	100	—	—	—
Tomato	20	—	30	48
Peas and	—	—	25)	—
Cabbage or	—	—	55))*	—
Cauliflower and	—	—	40)	—
Potatoes	100	—	40)	200
Onion	40	—	30	50
Radish	—	—	40	—
Carrot	—	—	40	—
Fruits				
Banana or	100	50	80)*	100
Chickoo	—	—	100)*	—
Apple	—	50	—	—
Lemon	10	10	10	—

* Change in two days menu.

TABLE II Composition (d.b.) of Experimental Diets

Diet	Protein (Nx6 25) %	Fat %	Crude fibre %	Energy Kcal/100g
Cereal (C)	9.5	11.8	2.0	450
Cereal-pulse (CP)	12.3	12.3	2.2	452
Cereal-pulse-vegetables (CPV)	10.9	12 0	2.7	455
Non-vegetarian (NV)	14.7	19.7	1.8	513

diet, cereals and pulses provide about 9 and 34 per cent, respectively of the otal protein. In cereal - pulse - vegetable (CPV) diet, per cent protein derived from cereal, pulse and vegetable was about 58, 25 and 10, respectively. Non-vegetarian (NV) diet was planned in such a way that more than half of the total daily protein that is about 55 per cent was supplied in the form of animal protein.

The standard methods of preparation of meals[7] were followed except for a few modifications. The food ingredients were weighed and measured before and after cooking. The subjects were fed five times a day: breakfast, mid-morning tea, lunch, evening tea and dinner. The subjects in each group were fed the same amount of diets in the laboratory under author's direct supervision and complete intake was ensured. Each diet was fed to a group of nine subjects for a period of 18 days, of which first 14 days served as adjustment period and the last four days as balance period in each human balance study.

Representative samples of food of each group were collected for the last four days of each feeding trial. The food samples were homogenized and dried in a shot air oven at 50 ± 1 °C.

TABLE III Daily Food, Protein and Calorie Intake (d.b.) of Subjects Given Experimental Diets

Diet	Food g	Protein g	Energy Kacl
Cereal (C)	425.5	40.3	1914
Cereal-pulse (CP)	424.4	52.3	1918
Cereal-pulse-vegetable (CPV)	417.8	45.7	1900
Non-vegetarian (NV)	355.0	52.2	1820

TABLE IV Essential Amio-acid Contents (EAA) and Chemical Scores of Dietary Proteins Cousumed by the Human Subjects

Amino acid/ chemical score	Diet group				
	Cereal	Cereal-pulse	Cereal-pulse-vegetable	Non-vegetarian	Provisional pattern in egg protein
Total EAA, mg/g N	1982	2230	2066	2679	2250
Lysine, mg/g N	196	283	269	342	340
Chemical score, %	57.6	83.2	79.1	100.6	–
Tryptophan, mg/g N	70	87	61	67	60
Chemical score, %	116 7	145.0	101.7	111.7	—
Methionine + cystine, mg/g N	242	191	238	269	220
Chemical score, %	110 0	86.8	108.2	122.3	—

* FAO reference protein (FAO/WHO, 1973)

Twenty four hour urine samples of the subjects were collected for the last four days of each feeding trial.

Moisture, crude fat, crude fibre and nitrogen of the diets were determined by AOAC methods[8]. Calorific value of diets were determined using Ballistic Bomb Calorimeter.

Urine samples of subjects were analysed for urea, uric acid and creatinine by methods of Wootton[9], Folin and Folin and Wu[10] respectively.

Significance of difference between mean values of protein, urea, uric acid and creatinine were determined by the two way analysis of variance[11]. Correlation coefficients between protein intake and urea N and urea N/creatinine ratio were also calculated.

Results and Discussion

Protein, fat, crude fibre and calorific values of the four experimental diets (dry basis) are shown in Table II. The results showed that the NV diet contained about one and half times the protein content of the C diet due to the presence of egg, meat and less cereals, being typical of such diets. In the case of C diet, it was not possible to increase the protein content because of inability of the subjects to consume large quantities of exclusively cereal based diet. Coincidently, the NV diet had about twice the fat content of C, CP and CPV diets and it contained less fibre than the rest of the test diets. The NV diet contained about 14 per cent more calories than C, CP and CPV diets which were almost iso-caloric.

The data on daily food, protein and calorie intake (d.b.) of subjects (Table III) indicates that daily intake of food was minimum in case of NV diet while it was almost same for the other three diets. The protein intake of C diet group was the lowest and of NV diet group was the highest. The calorie intake of all diet groups was almost the same except for NV diet group where it was slightly lower.

The NV diet contained sufficient amounts of lysine, tryptophan and sulphur containing amino acids as indicated by the respective chemical scores shown in Table IV. Lysine was the limiting amino acid in C, CP and CPV diets. However CP diet was marginally deficient in sulphur containing amino acids.

Data regarding the effect of feeding various experimental diets on urea, uric acid, creatinine and total urinary N levels and urea N/creatinine ratios in urine are summarized in Table V The analysis of variance of urea N values revealed that differences in the mean values were statistically significant ($P<0.01$) Improvement in lysine score of diet was associated with increased load of urea N in urine. The results indicated that urea N in urine was affected by the quantity and quality of protein in the diet. A significant correlation was obtained between urea N levels and quantity of protein in the diet ($r\pm 0.96$). Lysine score also correlated well with urinary urea N levels ($r\pm 0.99$). It appeared that utilisation of nitrogen was better with lower level of protein intake since more nitrogen gets incorporated into body protein and therefore decreased urea excretion as in case of subjects fed with cereal diet. Increased levels of urinary urea N have been observed with increased protein intake previously reported by Cheng *et al* [12] and Iyengar

TABLE V Urinary Urea N, Uric Acid N, Creatinine N, Total Urinary N Levels and Urea N/Creatinine Ratios of Human Subjects Fed Experimental Diets for 18 Days

	Diet group			
	Cereals C	Cereal-pulse CP	Cereal-pulse-vegetable CPV	Non-vegetarian NV
Urine urea N,g/day	2.90 ± 0.53	4.12 ± 0.59	3.64 ± 0.46	4.66 ± 0.48
Urine uric acid N, g/day	0.11 ± 0.02	0.12 ± 0.03	0.12 ± 0.02	0.14 ± 0.02
Urine creatinine N, g/day	0.36 ± 0.06	0.36 ± 0.03	0.35 ± 0.06	0.36 ± 0.03
Total urinary N*, g/day	3.36 ± 0.57	4.59 ± 0.61	4.12 ± 0.50	5.15 ± 0.50
Urine urea N/creatinine ratio	3.02 ± 0.44	4.26 ± 0.50	3.87 ± 0.43	4.87 ± 0.31

* Total urinary N=Urinary urea N + Urinary uric acid N + Urinary creatinine N

and Rao[13]. The results further showed that urea metabolism was affected by the source of protein in the diet as well. Although the protein content of CP and NV diets was about the same, urea N values were found to be significantly higher for the subjects consuming NV diet which had higher lysine score than those fed with CP diet. The findings on the effect of source of protein on urea levels were some what contrary to the results of others who suggested an inverse correlation between protein quality and urea N levels[14]. Possibly that was the result of feeding individual proteins rather than proteins in the mixed diets which varied in quantity as well as source of protein, as has been done by the present Author. Mixed diets are more complex but represent an actual state of the dietary regimen on which various socio-economic groups subsist. Besides, mixed diets based on cereals contain fibre which also affects absorption and metabolism of protein[15].

Urinary uric acid N values were higer in subjects fed with NV diet probably due to the presence of purines in the meat. The difference in the mean values of urinary uric acid of different groups were found to be satistically significant ($P<0.01$). There are literature reports to the contrary that level of nitrogenous components other than urea remain stable with change in protein intake[2,12]. But there are also reports regarding the increased uric acid excretion in humans with the increased protein intake.[16,17]

The mean urinary creatinine N values were more or less constant and showed no significant differences when the results were examined statistically. Earlier studies with different levels of protein intake from different sources of protein showed that creatinine excretion was independent of protein quantity and quality[2,12,18].

The differences in the mean values of total urinary N were statistically significant ($P<0.01$). The results indicated that N excretion was influenced by the quantity and quality of protein in the diet. Ramamurti[19] reported higher urinary N excretion among American and European males as compared to those of the Indians. Total urinary N excretion was lower in lacto-vegetarian subjects as compared to non-vegetarian and mixed diet eaters[1].

Analysis of variance of urinary urea N/creatinine ratio data showed that differences in mean values were statistically significant ($P<0.01$). These results suggested that urea N/creatinine ratio appeared to be a meaningful index of protein metabolism with a bearing on quantity and quality of protein intake. It may be pointed out that the experimental subjects in the present investigation had better nutritional background and their period of adjustment to different diets was kept longer than most of the investigators have followed in such studies on human subjects. Martins[20] reported a direct relation between the urea N/creatinine ratio among the members of a family and adequacy of protein in the family diet. In the present investigation, the ratio of urea N/creatinine was found to be higher in subjects consuming more of better quality protein.

Summary and Conclusions

The present study was undertaken to find out the effect of different food sources on N excretion in human subjects. The results of feeding four experimental diets viz C, CP, CPV and NV diets to young adult human subjects for a period of 18 days have shown that urea excretion was affected more by differences in quantity and quality of protein in the diet than uric acid and creatinine which did not depend on dietary protein *per se*. Urea N and total urinary N levels were found to increase with increased intake of better quality dietary protein. Urea N, total urinary N and urea N/creatinine ratio seem to be good indicators of quantity and quality of ingested protein and can be used in field studies.

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